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INSIDE

Review of the 50th **ERA-EDTA** Congress
Istanbul, Turkey

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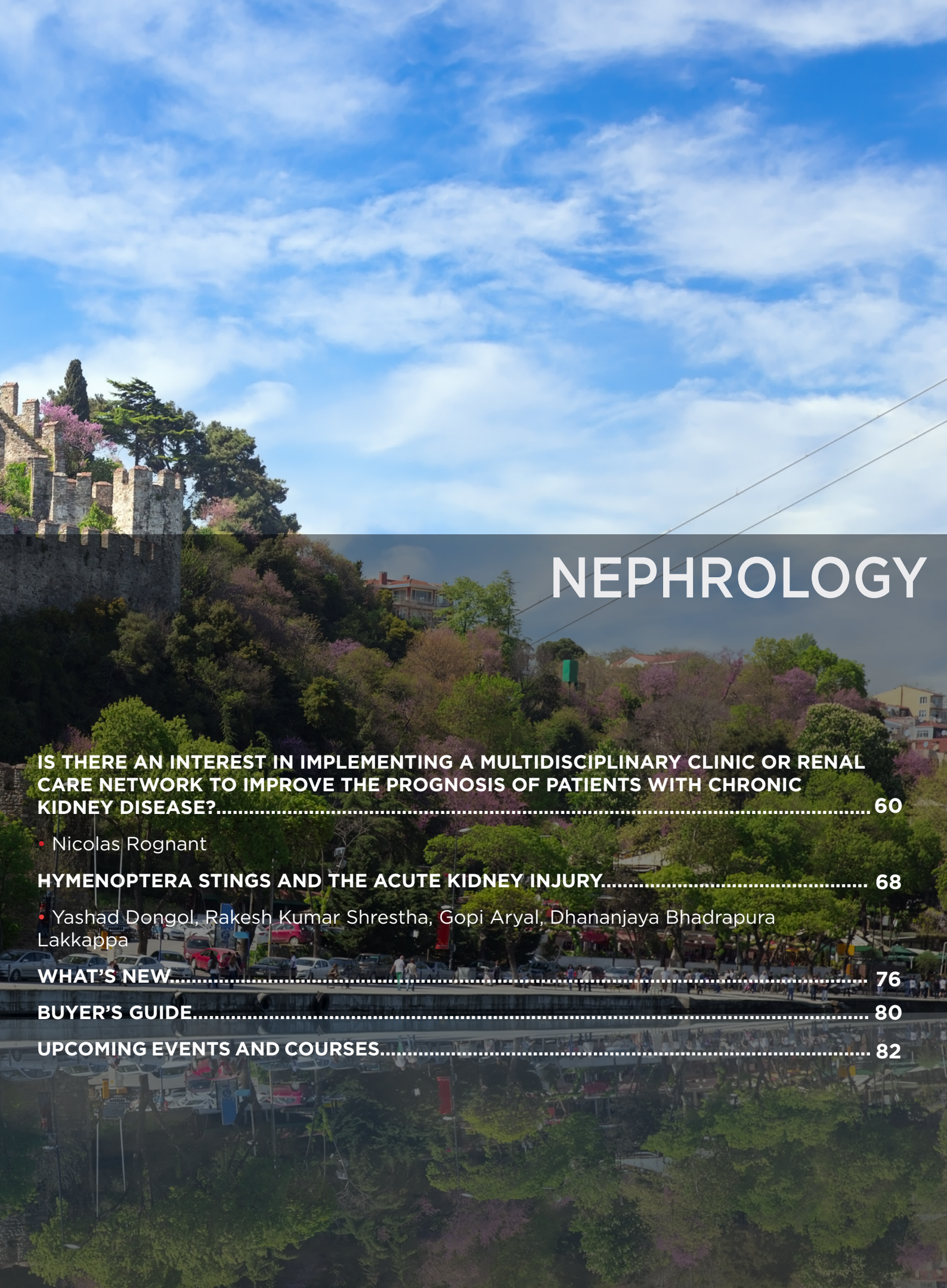

NEPHROLOGY

Editorial Panel

NEPHROLOGY

EMJ GORELY NEW MEDIA

Welcome

Kelly-Ann Lazarus, Editor

I would like to offer a very warm welcome to this first issue of *European Medical Journal – Nephrology*. This edition includes a stimulating mix of articles, breaking news and our comprehensive review of the 50th ERA-EDTA Annual Congress, the most important nephrology event of the calendar year.

This year's meeting of the European Renal Association – European Dialysis and Transplantation Association, held in Istanbul, Turkey, marked The Golden Year Celebrations of ERA-EDTA with an outstanding scientific programme. The Congress occupied the Istanbul Congress Center with the attendance of over 7,600 delegates and the presentation of 2,400 abstracts, submitted from all over the globe.

Please see our Congress Review section on page 8 for an in-depth review, with concise news coverage and insight into key developments presented in Istanbul. This review aims to provide a concise breakdown of the most significant presentations and seminars, particularly for those unable to attend the Congress, aiming to keep all clinicians informed of new data, technology and the status of on-going research and trials.

It is essential that physicians are kept up-to-date and EMJ are fully committed to advancing learning, knowledge and research worldwide. Our open-access policy increases the breadth of doctors, nurses and clinicians that can be reached and can benefit from the information which we provide.

We are proud to present papers from key opinion leaders in the field of nephrology, which stand together with our congress review to promote discussion and inquiry between practising physicians nationwide. These review articles, theoretical discussions and original research, are fully peer-reviewed to ensure the highest quality.

I sincerely hope that this issue of *European Medical Journal – Nephrology* reaches many of the medical community, providing a go-to reference of this year's topical issues within the field of nephrology.

Kelly-Ann Lazarus, **Editor**

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An integrated approach to the growing burden of kidney disease.

The International Federation of Kidney Foundations (IFKF) is one of the major multidisciplinary umbrella organisations in the kidney world that brings together health authorities, medical and healthcare professionals with volunteers and patients in local foundations, all of whom share a vision to improve the outcome for patients and people at risk from kidney disease. Through our members activities in local communities, we make a valuable contribution to the global improvement of kidney health.

This multidisciplinary approach encourages integrated prevention and treatment initiatives and brings effective patient care within reach. It is a proven recipe for a more effective and efficient approach to cost effective health care solutions.

IFKF's mission is to help develop local kidney foundations, not-for-profit organisations and individuals, enabling and empowering them to improve health, well-being and the quality of life for all individuals under threat from kidney disease.

How we will succeed in this mission:

- Through encouraging a worldwide multidisciplinary community, developing close contacts with all stake-holders.
- Creating a forum for debate and learning, through Annual and Regional Meetings.
- Developing a Global Voice in the World Kidney Day campaign.
- Encouraging the development of Early Detection of disease, through the SeeKD® screening programme.
- Developing the professionals, through Educational Programmes for healthcare professionals.
- Encouraging Peer support for developing organisations by promoting the Kidney Foundations Partner Programme (KFPP).
- Help raise awareness of the global kidney disease challenge, by developing central promotional material and by supporting a global website at: www.ifkf.org, with member specific content and support.

IFKF International Federation of Kidney Foundations
improving kidney health worldwide

IFKF: leading worldwide kidney health organisation for integrated detection, prevention and patient care.

Foreword

Professor Norbert Lameire

Chairman

European Kidney Health Alliance, Belgium

Dear Colleagues,

There is no lack of high quality scientific and clinical information that can be accessed for the practicing nephrologist. A great number of well-recognised nephrology journals are published and nephrological topics are also widely recovered in the more general medical journals of basic science, internal medicine, and surgery. However, with the arrival of open access publishing, new and often easier and more modern ways of obtaining theoretical and practical information has become available, without extra charge for the reader.

It is a my great pleasure to introduce this issue of the *European Medical Journal* devoted to topics in nephrology, some of which are still quite controversial, despite intensive research over the last years. This open access journal aims at a free and wide dissemination of valuable knowledge in nephrology to a global readership. *EMJ – Nephrology* encourages the submission of up-to-date clinical and therapeutic developments in all aspects of nephrology. All papers are peer reviewed by a board of international experts, guaranteeing the accuracy of the information.

The present issue covers a broad spectrum of interesting topics including new aspects of bone and mineral metabolism, diabetic nephropathy, some 'exotic' causes of acute kidney injury, and kidney transplantation. In addition, new aspects of organisation of renal health care and their potential positive aspects on patient outcome are discussed.

I wish all readers of the journal an interesting 'journey in nephrology' in reading and appreciating this issue.

Prof Norbert Lameire

University Hospital Ghent, Editor in Chief of the Acta Clinica Belgica, *Journal of the Belgian Society of Internal Medicine*, *Clinical Biology and Clinical Chemistry*, Emeritus Professor of Medicine at the Medical Faculty of the Ghent University, Chairman of the European Kidney Health Alliance, Belgium.

ERA-EDTA ANNUAL CONGRESS 2013

ISTANBUL CONGRESS CENTER, ISTANBUL, TURKEY

18TH-21ST MAY 2013

Welcome to the *European Medical Journal* review of the 50th Annual **ERA-EDTA** Congress of Nephrology

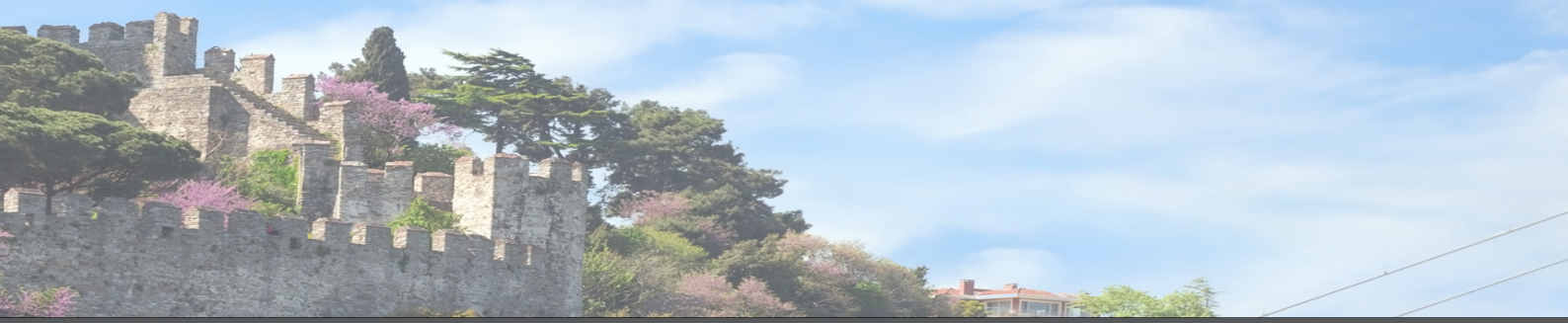
EMJ EUROPEAN MEDICAL JOURNAL

Welcome to the European Medical Journal Review Of ERA-EDTA Annual Congress 2013

SINCE its creation 50 years ago, The European Renal Association – European Dialysis and Transplant Association (ERA-EDTA) has, in supporting basic and clinical research in the fields of nephrology, steadily evolved into one of the fastest growing medical associations, with this year's Congress held in Istanbul, Turkey between 18th and 21st May 2013, boasting over 6,400 members and an attendance of over 7,600 delegates.

Istanbul, the only city in the world to stand within two different continents, Europe and Asia, was a hub for some of the most important empires in history: Roman, Byzantine, Latin, and Ottoman. Now a diverse and culturally-rich city, the imaginative and artistic atmosphere surrounding the architecture made the perfect setting for the conference, held inside the Istanbul Congress Center. The Congress' primary focus leaned towards the three topics of haemodialysis, clinical nephrology, and chronic kidney disease, with a total of 2,400 abstracts submitted, and participant interest ranging from anaemia to hypertension.

Keen to encourage interdisciplinary research and communication of knowledge, while aiming to promote teamwork between specialists, a science fair project was initiated for the first time in ERA-EDTA's history. With the first day dedicated to the presentations from the seven working groups founded for the purpose of promoting research,and education in specific scientific fields, CME courses later offered important updates in such topical conditions as hereditary renal disease, cardiovascular complications, transplantation,



and paediatric nephrology, all closely linked with advances in modern nephrology and the epidemiology of chronic kidney disease.

Paediatrics was a dedicated topic, as areas from prevention and early detection of urinary abnormalities, to diagnosis and management of severe cases using the latest therapeutic tools to prevent any progression towards the loss of renal function, were covered throughout the Congress, in the form of symposia, mini-lectures, free communications, and master classes.

Though the number of end stage renal diseases requiring renal replacement therapy via dialysis or renal transplantation is about 20 times less frequent in children than adults, Professor Rosanna Coppo, Chair of the ERA-EDTA Scientific Committee and President of the European Society for Paediatric Nephrology (ESPN), summarised the need for such new reported research as studies on cardiac damage in children with chronic kidney disease by dubbing a child diagnosis as a “family tragedy”.

Successfully covering beneficial advancements in continuously changing areas – including announcing the completion of the European Validation Study of the Oxford Classification of IgA Nephrology (VALIGA) study, the biggest database for glomerular disease research ever assembled – ERA-EDTA truly were successful in setting up a purposeful, memorable, and meaningful Congress, leaving an eager anticipation for the 51st Congress in Amsterdam in 2014.

UTIs in children can no longer be ignored

THE long-term effects of urinary tract infections (UTIs) can result in growth retardation, hypertension, renal scarring, and renal failure. Therefore, it has become a condition that requires special attention, especially during childhood.

Since UTI is a disease with a high incidence in children, occurring in 3-5% of girls and 1% of boys, it is critical to detect the underlying risk factors and to prevent and treat the infections. It is also a condition in which recurrences are common. Risk of recurrence, following 1 year after the first infection, is 60-80% for girls and 30% for boys.

Furthermore, a recurrent urinary tract infection (RUTI) can increase permanent risk of renal injury. The risk factors of RUTI in uncircumcised boys are: urethral instrumentation, voiding dysfunction, constipation, urinary tract abnormalities, obstruction (PUV, UP and UV stricture etc.), and vesicoureteral reflux (VUR) (Grade III-V). VUR is a congenital defect of the urinary tract, resulting in the reflux of the urine from the bladder to the ureter and sometimes to the renal pelvis, the calyxes and the collecting system. While circumcision during the first year of life reduces the UI incidence, the recurrence risk for infection increases by 60% in the case of bladder dysfunction (neurogenic or non-neurogenic).

According to the 2006 annual report of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS), reflux nephropathy is identified to be the cause of chronic renal failure (CRF) in 536 (8.4%) of 6405 children, whereas in Turkey, VUR nephropathy is the major cause of CRF in children with 32.4%.

Due to the long-term health risks throughout UI and renal scarring, it is critical to properly assess the symptoms early in diagnosis and treatment. Various treatment methods are applied to prevent renal scarring, long-term anti-microbial prophylaxis and surgical intervention.

The risk of kidney scarring in children is the primary factor, with high grade VUR 4-6 times more likely to occur than children with low grade VUR, and it is 8-10 times more likely than children without VUR. Voiding disorders are also a risk factor for UI and their recurrence and may lead to the development of VUR. It may also delay the reflux recovery process and end up in permanent kidney injury.

Potential lipid lowering therapies

THE German Diabetes and Dialysis Study carried out an investigation called 4D (Die Deutsche Diabetes Dialyse-Studie) to investigate the effectiveness of high-density lipoprotein (HDL) cholesterol, in 1,255 dialysis patients with diabetes.

The aim was to investigate the usefulness of HDL cholesterol and the HDL protein

components apolipoprotein A1, A2 and C3 in serum of HD patients. The research revealed that there is no association between HDL, apolipoprotein A1 and C3 with cardiovascular events (such as cardiac death, myocardial infarction, stroke), but a high concentration of apolipoprotein A2 were linked with lower cardiovascular risk.

A follow-up investigation with the 1,255 patients from the 4D study examined the link between intestinal cholesterol absorption and cardiovascular events. The placebo group (636 patients) received no drugs while the remaining patients received daily dosage of 20 mg atorvastatin. The drug is a product from the statin group, known as cholesterol synthesis enzyme (CSE) inhibitors. The results had shown that high cholesterol absorption was predictive of experiencing a cardiovascular event in both groups. Further analysis to test the efficiency of cholesterol absorption was also done. The results for the placebo group show that there was no association between the efficiency of absorption and the risk of cardiovascular event, while the atorvastatin group had shown that there is a link between absorption and risk of cardiovascular event.

Though it is widely accepted that lowering cholesterol, either by dietary or pharmacological means, is key to preventing cardiovascular disease, particularly applying to patients who are non-dialysis dependent with kidney disease, it has not been established to prescribe pharmacological cholesterol-lowering therapy to HD patients.

The study concluded that atorvastatin therapy was poor after noting an increase in cholesterol absorption efficiency when investigating cardiovascular endpoints, adding investigations into statin therapy may be beneficial to patients on dialysis through the measurement of cholesterol absorption.

Atrasentan reduces albuminuria in diabetic patients

FOR non-dialysis dependent CKD patients, controlling albiminuria levels is the overall goal to lower the cardiovascular risk and delay the dependence on dialysis.

An investigation presented at ERA-EDTA 2013 used atrasentan to reduce albuminuria in type 2 diabetic patients. Atrasentan is a selective endothelin-A receptor antagonist used as an add-on to optimal renin angiotensin-aldosterone system (RAAS) blockage. Two multinational, double-blind, randomised, placebo-controlled parameters were used in an investigation involving 211 patients. Other parameters also include RAAS inhibition over 12 weeks, placebo for the control group and atrasentan either 0.75 mg/day or 1.25 mg/day. More than half of the patients that were treated with the drug had experienced a 30% reduction in albuminuria. There are also a considerable reduction in total and LDL cholesterol concerning active treatment groups. No significant differences with volume-associated adverse effects, such as peripherial oedema or heart failure were found.

Proteinuria is a marker and a promoter of renal disease since protein loss correlates to the level of severity. It is also considered a marker for cardiovascular mortality. By reversing proteinuria, there is a significantly positive correlation with improved renal and cardiovascular prognosis.

Atrasentan is considered to be a promising therapy, in addition to RAAS blockade, for reducing albuminuria in diabetic patients and hence for slowing the progression of nephropathy. However, it is still necessary to gather long-term data.

PRIME study reveals positive results for anaemic dialysis patients

OVER 60% of patients with chronic kidney disease (CKD) have an iron deficiency, and frequently suffer from anaemia as the kidneys can no longer synthesise sufficient erythropoietin. One of the main causes of the advanced stages of CKD is a blockade of the release of storage iron in the body; furthermore, dialysis patients experience regular blood and iron loss during treatment, which often aggravates the anaemia and results in a deterioration of physical performance and quality of life.

The results from a new placebo-controlled, double-blind PRIME study, investigating the effects of Soluble Ferric Pyrophosphate (SFP) against erythropoietin stimulating agents (ESAs) have been revealed and have shown that SFP is effective at maintaining iron balance and maximising iron delivery.

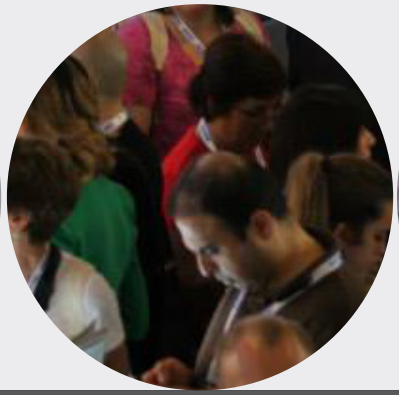
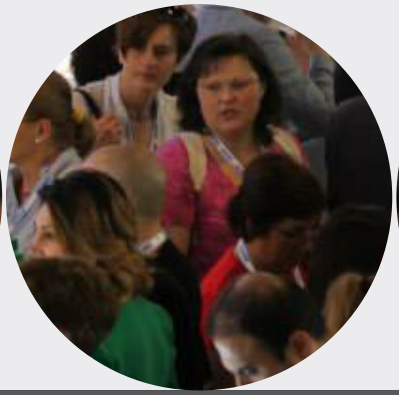
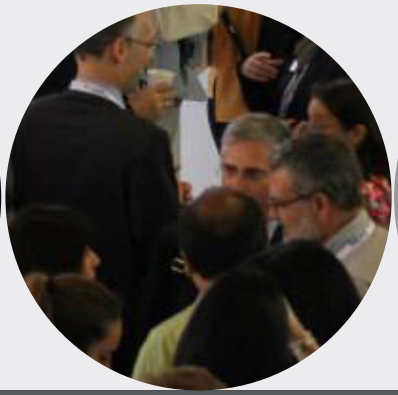
It has been found that the reduction in haemoglobin (Hb) is accompanied by a reduction in transferrin saturation (TSAT; transferrin is the iron transport protein) and in ferritin (storage iron). Mortality in CKD patients with TSAT of <15% and ferritin levels of <100 ng/ml is increased by 50% compared with patients who have values above these cut-off levels. Therapy to increase Hb levels involves administration of an erythropoiesis-stimulating agent (ESA) as well as iron substitution.

However, ESA therapy is relatively expensive, and is also associated with an increased risk of stroke and malignancy. Also, Kidney Disease Improving Global Outcomes (KDIGO) guidelines state that ESA should only be used in persistent anaemia because ESA therapy is hardly effective in severe iron deficiency.

SFP, designed to prevent and treat iron deficiency anaemia in ESRD patients, was created for the use in dialysis patients as it avoids storage in the liver and travels directly to the bone marrow, delivering iron in a physiologic manner.

SFP was found to be particularly stable, preventing any toxic, anaphylactic and inflammatory potential, and iron-induced liver damage, contrary to intravenous iron which has allergic and life-threatening anaphylactic reactions.

The results of the study have shown that slow, continuous delivery through dialysis is safer and more effective in maintaining the optimal iron balance. The current ESA dose can be reduced by 35%, which increases patient safety and lowers costs, and Hb levels will remain stable.

Next generation bi-osmosis water treatment system revealed

A NEXT-GENERATION bi-osmosis system for treating water for haemodialysis was unveiled at the 50th ERA-EDTA Congress in Istanbul on Saturday.

Designed by Culligan®, the Culligan RO² utilises two separate reverse osmosis systems, operating in tandem, which effectively treats the required water twice. Controlled by a simple touch screen interface, 90-99% of salts and other contaminants in the water are removed in the first pass, while a further 90-99% of the remaining residual contaminants are extracted in the second.

Making use of certain semi-permeable membranes' ability to separate water from substances dissolved in them, reverse osmosis is a physical type of procedure that does not require the use of any chemical regenerants.

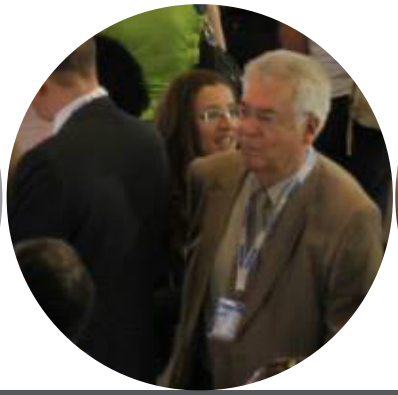
> "Our state-of-the-art equipment is designed and manufactured to reliably produce water of the highest possible standards."
>
> *-Chris Freeman, Marketing Director, Culligan EMEA.*

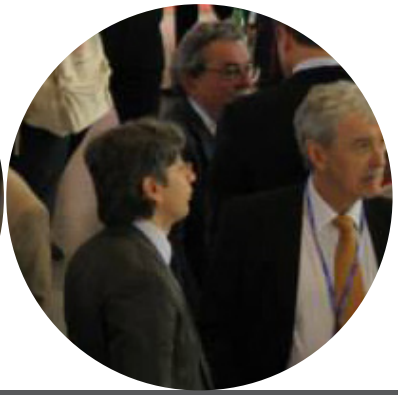
"A person in need of dialysis has his or her blood in close contact with huge amounts of water – approximately 150 litres per session – three times a week. Our state-of-the-art equipment is designed and manufactured to reliably produce water of the highest possible standards. That is why hundreds of Culligan's water treatment units for haemodialysis are installed in Europe, the Mediterranean Basin and the Middle East," Marketing Director EMEA of Culligan International, Chris Freeman, said.

The unit contains a programmable logic controller, allowing simple integration to either place into existing hospital systems, build management systems, or monitor alarms and operating parameters. And in the event of one osmosis system failing, the machine can operate on a single reverse osmosis system while still producing dialysis-quality water.

Though able to set flow rates at up to 3,600 litres per hour, through a modular design with a range of flow rates and by recycling and retreating as much water as possible, the Culligan RO² potentially reduces utility costs while meeting environmental commitments.

Revealed at the Congress, the technology comes from the first company to introduce RO technology to patients and leading the way in bi-osmosis treatment, the system is medical device certified according to Class IIb Certified, and CE marked.





Working Groups strive towards exciting future

AMONGST the many working groups of ERA-EDTA 2013, two in particular, the Immunonephrology Working Group (IWG) and the European Renal and Cardiovascular Medicine Working Group (EURECA-m), announced exciting plans to revolutionise the nephrology world.

The IWG, established in 2009 in order to encourage teaching, research, communication, and education in the field of immune system dysregulation, recently announced several newly launched projects, including Kidney Connect, a shared European platform for excellence in nephrology and podocyte research. This arrives alongside a CKD bio research network, and a registry for post-transplantation recurrent glomerulonephritus.

Represented by Professor Rosanna Coppo at the Congress, the group, which consists of 518 members, also announced its recent completion of the European Validation Study of the Oxford Classification of IgA Nephrology (VALIGA) study, the biggest database ever assembled for glomerular disease research in the world. 1,147 patients from 55 centres in 13 European countries were enrolled, and a combination of renal biopsy tissue, clinical signs, and laboratory tests were thoroughly analysed.

Meanwhile, with 30% of all deaths worldwide attributed to cardiovascular disease, Professor Gerard London of EURECA-m, announced an update on the on-going Lung Water by Ultrasound-guided Treatment (LUST) to Prevent the Death and Cardiovascular Events at High Risk End Stage Renal Disease Patients study, stating actual patient enrolment. The research aims to test if a treatment policy involving ultrafiltration intensification and guided by the measurement of ultrasound B lines, vertical narrow-based lines arising from the pleural line to the edge of the ultrasound screen, may improve the clinical outcomes in haemodialysis patients.

With 25 centres selected based on the validity of their infrastructure, 7 French, 5 Italian, 2 German, Polish, Spanish, and Greek, and 1 British, Romanian, Israeli, Slovenian, and Turkish units, the study hopes to delve deeper into the relationship between the renal and cardiovascular system, otherwise known as cardiorenal syndrome (CRS), defined as one failing system often precipitates the failure of the other.

With these exciting plans part of on-going improvements in the world of nephrology, what emerged as categorical importance was the aim to extend the reach of nephrology to new areas of treatment, while extending the availability of nephrology knowledge to a much wider audience, through the online community.

Studies have shown that PTH lowering will help sHPT patients

OFTEN, more than 90% of patients with chronic kidney disease (CKD) also develop secondary hyperparathyroidism (sHPT); it has now come to light that an elevated level of parathyroid hormone (PTH) is a common characteristic of this condition. Moreover, some patients with sHPT also develop bone disease (osteodystrophy), and new evidence has suggested that sHPT combined with PTH is associated with cardiovascular disease and increased mortality.

Vitamin D is most commonly used to treat sHPT, as it helps to lower PTH levels, however it

does increase the levels of serum calcium and serum phosphorus, which is often associated with the risk of vascular calcification.

Further alternate treatments have been used for PTH lowering. An allosteric modulator of the calcium-sensing receptor (CaSR), cinacalcet, has been favoured in the Phase III EVOLVE study, as it increases the sensitivity of the calcium receptors in the parathyroid glands and inhibits the excessive production of PTH.

The EVOLVE study, a large multinational, prospective, randomised controlled double-blind trial of cinacalcet versus placebo in haemodialysis patients with moderate to severe hyperparathyroidism has revealed a trend towards lower mortality following treatment with cinacalcet plus low-dose vitamin D, in comparison with vitamin D therapy alone. After adjustment for age and for actual exposure to study medication, it was concluded that the results of the randomised study can be transported to a broader population.

Another alternative is paricalitol, a selective vitamin D which lowers PTH without significantly influencing serum calcium and serum phosphorus. The 3 year European COSMOS study (Current management of secondary hyperparathyroidism: A multicenter observational study), observing 6,273 patients with HD, assessed the usefulness of PTH-lowering products in terms of mortality. It concluded that many patients benefited significantly from all PTH-lowering treatments.

A study was also conducted to investigate the effect of oral paricalcitol on left-ventricular mass after 52 weeks of therapy. The study also looked at biochemical parameters of chronic kidney disease – mineral bone disorder (CKD-MBD). Although the results did not show any significant difference in terms of left-ventricular mass index, there was a significant improvement in the biochemical parameters of CKD-MBD.

Sevelamer has proved its worth in phosphate binding

AS A RESULT of chronic kidney disease (CKD), many patients often develop hyperphosphatemia, which usually occurs when there is an abnormally elevated level of phosphate in the blood and the phosphate balance can no longer be maintained, in some cases leading to skin and/or bond problems and increasing mortality. As this becomes a frequent occurrence, there is a necessity for a phosphate-binding substance to come into the fold so that phosphate levels can be controlled.

To combat the electrolyte disturbance, sevelamer has been developed, a calcium-free phosphate binder (PB) and non-absorbed poly(allylamine hydrochloride) polymer. A number of studies have highlighted the advantages that stem from using calcium-free therapies, for example, sevelamer attenuates the progression of vascular calcification. This effect may also be attributed to a variety of vasoprotective and pleiotropic effects, such as cholesterol lowering.

A study focused on patients with diabetic nephropathy has shown a further effect of therapy with potential vasoprotective implications. Consequently, inflammatory markers and antioxidant levels also improved during treatment with the calcium-free PB. There was also a reduction in markers for the progression of diabetic nephropathy and cardiovascular risk, while the phosphate-lowering effect remained constant.

Antibacterial gel fails to be as effective as nasal mupironcin therapy

A HONEY-CONTAINING antibacterial wound gel has failed to be superior to mupirocin application for the prophylaxis of peritoneal dialysis (PD) catheter-related infections, according to research presented at ERA-EDTA 2013.

Comparing Comvita Medihoney™ with conventional prophylaxis using nasal mupirocin ointment during the multinational, multicentre, randomised and controlled open-label study, there were no other real differences found between the two treatment groups in terms of mupirocin-resistant staphylococcal strains, the frequency of serious adverse effects, or the number of fatalities in the groups.

The peritoneum, the thin membrane that surrounds the outside of the organs in the abdomen, is used as a 'natural' dialysis membrane in PD, with the dialysate fluid running from a sterile bag via silicone catheter into the abdominal cavity, exchanged following several hours. Though infections resulting from this tend to be easily-treated, multiple occurrences where the peritoneum becomes damaged, or in circumstantial life-threatening cases, prophylaxis is often advised.

The only noteworthy difference was in diabetic patients, with the increased number of catheter-associated infections revealing an inferiority concerning the honey-containing wound gel. Though it is well-known that some regulatory-approved wound gels which contain raw honey are now available to help treat drug-resistant strains of bacteria, resulting from the sweet food's remarkable ability to spark antibacterial activity without inducing resistance, much scientific research is currently being carried out, with recent emphasis on fighting infections in wounds.

THE EVOLVING WORLD OF CHRONIC KIDNEY DISEASE MINERAL BONE DISORDER

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ABSTRACT

Chronic kidney disease - mineral and bone disorder (CKD-MBD) is associated with a significant morbidity and mortality. *In vitro* and animal models suggest that phosphorous, calcium, parathyroid hormone, and vitamin D abnormalities, mediate the cardiovascular and bone diseases that characterise CKD-MBD and increase the risk of death. Currently, mineral abnormalities are corrected through phosphorous restriction, phosphate binders, calcimimetics and vitamin D administration. Nonetheless, data in humans that support the use of these compounds are still scarce, mainly based on observational studies. Thus, a considerable number of doubts and questions still challenge clinicians dealing with CKD patients and mineral metabolism imbalances. We herein critically review clinical evidence that support the use of different drugs in CKD-MBD.

Keywords: CKD-MBD, dialysis, outcome, management, needs.

INTRODUCTION

Calcium, phosphate, vitamin D and parathyroid hormone (PTH) have been repeatedly recognised as predictors of outcome in chronic kidney disease (CKD).[1-4] Though the mechanisms are still poorly understood, numerous studies suggest that mineral homeostasis abnormalities are associated with bone and cardiovascular (CV) diseases that portend a poor survival.[5] Hence, biochemical, CV, and bone abnormalities are now considered part of the multifaceted CKD-MBD syndrome (Figure 1). [5]

In spite of convincing preclinical data linking mineral metabolism imbalances to cardiovascular and bone diseases, clinical evidence is still far from conclusive[4,6] and a considerable number of doubts and questions still challenge clinicians dealing with CKD patients and mineral metabolism imbalances. CKD-MBD is currently treated with nutritional interventions, native and active vitamin D phosphate binders, and calcimimetics administration (Figure 2). The aim of this review is to critically evaluate and summarise available evidence as well as highlight

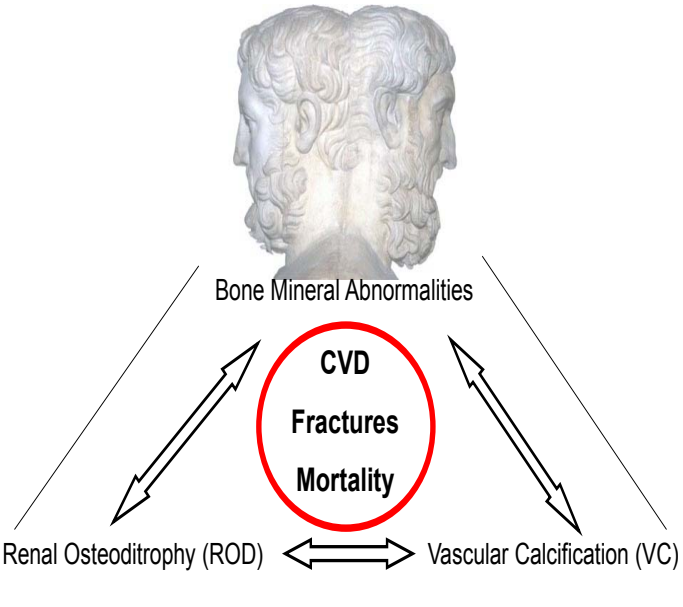

Figure 1. CKD-MBD a multifaceted syndrome characterised by serum parameters abnormalities, bone and cardiovascular marker of disease and associated with poor outcome.

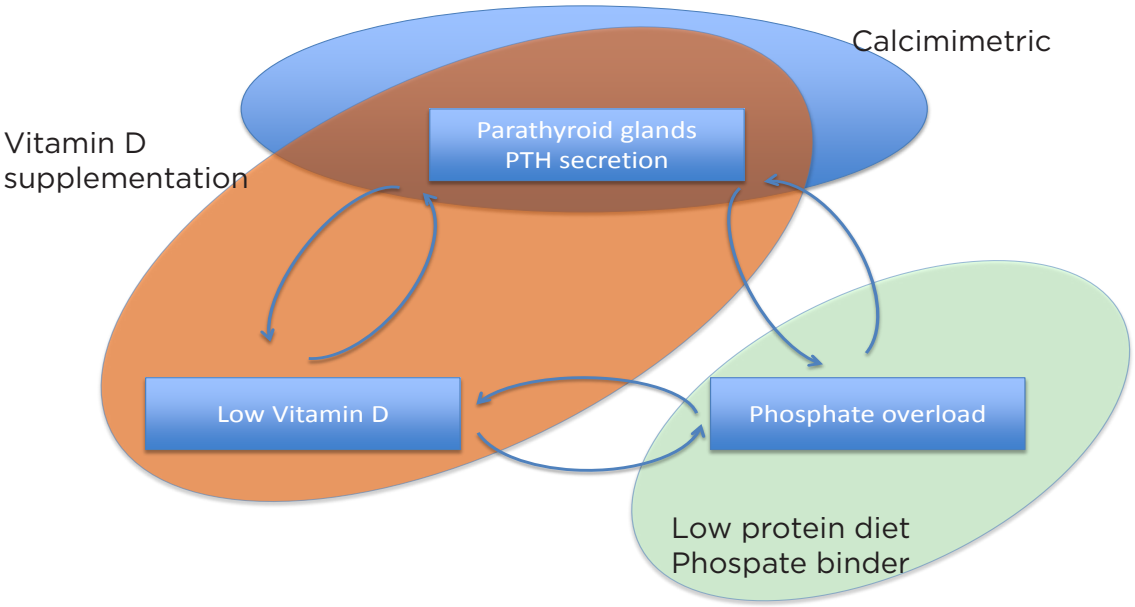

Figure 2. CKD-MBD pathophysiology is characterised by phosphate overload, PTH hypersecretion and vitamin D depletion. Our armamentarium is composed by low protein diet and phosphate binder (light green circle) to lower phosphate overload; different forms of vitamin D (orange circle) to overcome vitamin D deficiency and inhibit PTH production and secretion; calcimimentics (light blue circle) to reduce PTH secretion

the numerous unanswered clinical questions on CKD-MBD management.

Diet: Facts, Promises and Expectations

Hyperphosphatemia control is perceived by nephrologists as one of the most relevant targets to achieve in CKD.[4] Indeed, numerous studies have reported a close association between serum phosphorus levels and the risk of death in both subjects from the general population[7,8] as well as subjects with varying degrees of renal function impairment.[1-4] Furthermore, a large body of evidence suggests a direct link between phosphorous and the cardiovascular and bone systems.[5] Thus, it is commonly accepted that phosphorus is a uraemic toxin, and current guidelines on mineral metabolism management recommend maintaining it within the range of normality.[9-10]

As kidney function declines, urinary phosphate excretion becomes insufficient and eventually hyperphosphataemia ensues if the phosphate daily intake remains constant.[11] It is estimated that the daily phosphate intake in a standard diet in Western countries is about 1500 mg/day.[11,12] Considering that faecal excretion is about 600 mg/day of which about 200 mg/day are secreted by the intestine, the amount of phosphorous absorbed by the gastrointestinal tract may approach 1100 mg/day (Figure 3).[11,12] To maintain phosphorous homeostasis and keep serum levels within the range of normality, renal excretion should match the daily intake at the expense of increasing the tubular workload of each functional nephron.[13] Notably, the average phosphate level in the general population varies according to sex and menopausal status[14,15] and data suggest an increased risk of unfavourable outcomes for phosphorous levels within the range of normality[8,15] further corroborating the notion that serum phosphorus may not adequately reflect phosphorous balance.

Two different strategies to lower phosphorous intake are available: low phosphate diet and phosphate binders. A low phosphorous intake can be achieved via protein restriction and quality selection.[5] Indeed, Moe et al.[16] showed that a vegetarian rather than a meat-based diet significantly reduces serum phosphorous and the phosphaturic factor fibroblast growth factor 23 (FGF23). Notably, these differences were independent of the circadian serum and urine phosphorous changes, suggesting that phosphorous contained in the vegetarian diet is less adsorbable in the gastrointestinal tract which is possibly due to the phosphate binding to phytate.[16]

Cooking method and food additives are two other factors that significantly affect phosphorous intake.[17-22] Cupisti and coworkers[18] reported that 20-30 minutes boiling significantly reduce (30-50%) phosphorous burden at the expense of a minimal reduction of the protein content (9-17%).

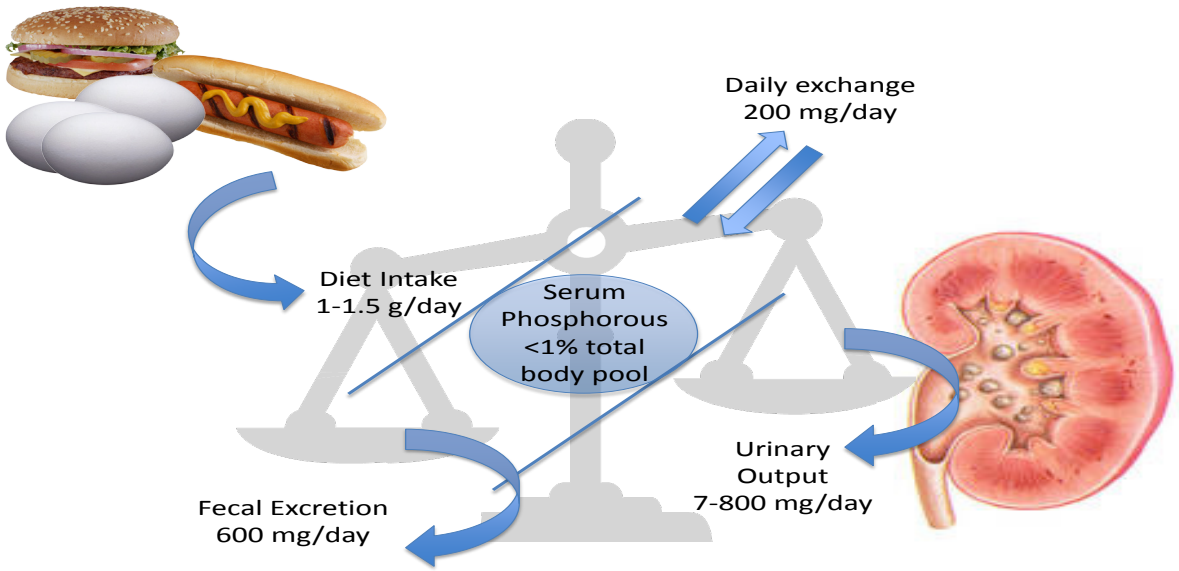

Figure 3. Phosphorous balance is the net results of intake (diet), quota exchanged with bones and output (urine, faeces).

Food additives are another source of phosphorous in prepared meals. A recent survey of best-selling processed groceries concluded that phosphorus additive-containing foods averaged 67 mg phosphorus/100 g more than matched non-additive-containing foods (about 736 mg more phosphorus per day compared with meals consisting of only additive-free foods).[23] Phosphorous-based additives (phosphoric acid, tetrasodium pyrophosphate, tricalcium phosphate, disodium phosphate, monopotassium phosphate, etc.) are used to enhance taste and consistency of different foods such as baked goods (baking powder, cakes, frozen dough, etc.), beverages (colas, chocolate milk, buttermilk, fruit juices, sport drinks, canned milk, soy beverages), cereals, dairy, meat and egg products, fruit and vegetables, and pasta and noodles.

Inorganic phosphorous contained in food additives is highly bioavailable and adsorbed in the gastrointestinal tract to a greater extent than the organic phosphorous. It is estimated that as much as 90% versus 60% of the ingested inorganic (food additives) and organic (vegetable and meat protein) phosphorous is absorbed, respectively.[21,22]

Though the mechanisms are still unclear, accumulating evidence suggests the high serum levels of phosphorous are associated with increased levels of FGF23 that in turn, have been independently associated with a significant risk of endothelial dysfunction,[24] left ventricular hypertrophy,[25] CKD progression and all-cause mortality.[26] In the absence of a randomised controlled clinical trial (RCT), it is unclear whether elevated serum phosphorous or FGF23 mediates the toxicity[1,26] or, alternatively, both factors contribute to the organ damage and poor survival in CKD-MBD.[27]

A balanced nutritional program should control both serum phosphorous and FGF23. Di Iorio et al.[28] showed that a very low protein diet (0.3 g/kg of ideal body weight per day) supplemented with alpha-chetoanalogues and essential aminoacids significantly reduces FGF23 and phosphoremia. In 32 CKD subjects randomised to cross-over sequential treatments with either standard low protein diet (60-70 g of protein/day) or very low protein diet (25-30 g of protein/day), they reported a significant 33.5%, 12% and 34% reduction of FGF23, serum and urinary phosphorous levels associated with very low protein diet (VLPD), respectively.[28] Of note, the two diet regimens did not differ only in the total protein intake but also in the animal/vegetal protein ratio (VLPD regimen based on vegetable protein only) and phosphorous content (350-420 mg/day versus 600-700 in VLPD and standard diet, respectively).[28] Other groups have confirmed that phosphorous restriction with or without phosphate binders, is effective in controlling FGF23.[29,30]

Low phosphate and protein diet has also been associated with proteinuria and CKD progression reduction.[19,31,32] In a seminal paper by Brunori at al.,[32] it was demonstrated that life expectancy among old patients with end-stage renal disease (ESRD) was similar if patients were randomised to VLPD and conservative treatment or haemodialysis.

The most important drawback of low protein and phosphorous diet is the potential for malnutrition.[33] Indeed, a balanced nutritional program should be tailored to each individual and should provide the patient with the right amount of calories and nutrients.[34] In this regard, an observational study suggests that protein malnutrition maybe more detrimental than phosphorous intake and that the ideal nutritional regime should provide enough protein with minimal phosphorous burden.[33]

Future RCT studies should investigate the safety and the impact of low protein and VLPD on long-term survival and CKD progression, in both CKD patients not receiving and receiving dialysis. In consideration of the substantial increase of the mean age of dialysis patients, it is to be established if the recommended protein intake by current guidelines is still adequate in light of the considerable number of patients with increased levels of serum phosphorous.[35] Finally, a pharmacoeconomic analysis should evaluate the cost burden connected to aproteic foods, chetoanalogue or essential aminoacid supplements.

Phosphate Binders: Facts, Promises and Expectations

Phosphate binders are another strategy for reducing phosphate intake. These compounds share the property to bind phosphorous in the intestinal lumen, prevent its absorption and increase the faecal excretion. Various drugs are now available on the market with this indication.[36,37] For ease, these compounds can be divided into two different groups: calcium-based phosphate binders (calcium carbonate and calcium acetate) and calcium-free phosphate binders (aluminium hydroxide, lanthanum carbonate, magnesium carbonate, sevelamer hydrochloride, and sevelamer carbonate). Alternatively, these compounds can be divided into absorbable (calcium-based binders, aluminium hydroxide, lanthanum carbonate, magnesium carbonate) and not absorbable (sevelamer hydrochloride, and sevelamer carbonate) in the gastrointestinal tract. Though all these compounds might have different affinity for phosphorous in the gastrointesinal tract and different doses have to be administered,[38] clinical studies suggest that they all effectively lower serum phosphorous.[36,39,40] Nonetheless, due to the different adsorbability in the gastrointestinal tract, the safety profile of these compounds can be profoundly different. Indeed, the prolonged use of aluminum-based phosphate binder is not indicated due to its accumulation and toxicity.[41]

The debate on calcium-containing versus calcium-free phosphate binders has characterised the last decade.[36,42] Preclinical data suggest that both phosphorous and calcium can actively induce vascular calcification,[43-45] a marker of vascular disease[46] and a risk factor for arterial stiffness[47] and mortality.[46,48] A seminal paper by Cozzolino and coworkers[49] demonstrated that the use of sevelamer was associated with a similar phosphate control but lower extraosseous calcification than calcium-based phosphate binder. Observational data suggest that excessive calcium intake may result in a positive calcium balance that in turn has been associated with arterial stiffness and vascular calcification,[50,51] adynamic bone disease[52,53] and, in some but not all studies, excessive mortality.[35,54]

RCTs have also yielded somehow conflicting results. To date, three studies have tested the impact of calcium-free and calcium-containing phosphate binders on vascular calcification, CKD progression and all-cause mortality in moderate CKD.[55-57] In the first study ever published on this topic, Russo and coworkers[55] observed a significant reduction of coronary calcification (CAC) progression among patients with CKD stage 3-4 treated with sevelamer as compared to patients treated with calcium carbonate or low-protein diet.[55] Considering that the dose of both binders was based on a similar reduction in urinary phosphate excretion (i.e. phosphate binding equivalency), it is plausible that the different impact of sevelamer and calcium carbonate on vascular calcification is due to the excessive calcium load in the calcium carbonate-treated arm. Indeed, recent evidence suggests that a calcium intake greater than that usually ingested in a normal Western country diet (about 800 mg/day) can induce a positive calcium balance in moderate CKD.[58] However, it is also possible that the additive effects of sevelamer on FGF23, fetuin-A, lipids, C-reactive protein, and uric acid[59,60] may account for some of these results. Block and coworkers[56] recently failed to confirm the beneficial effect of non-calcium-containing phosphate binders (sevelamer carbonate, lanthanum carbonate) on vasculature. Though the study was designed to address the phosphate lowering efficacy of calcium and non-calcium-containing phosphate binders versus placebo in mild to moderate CKD, authors report among treated patients on a worrisome increase in CAC, measured as secondary endpoint.[56] However, it is unclear whether calcium or non-calcium-containing phosphate binders drive this result. The limited statistical power of the study further limits the interpretation of this finding.[56]

A third RCT designed to test the impact of sevelamer versus calcium carbonate on hard outcomes (all-cause mortality and CKD progression) in mild to moderate CKD patients (mean creatinine clearance 30 ml/min) with hyperphosphatemia supports the notion that non-calcium-containing phosphate binders may be associated with a more favourable renal and life survival rate.[57] In this study, a significant CAC progression attenuation was also noted.[57] Although sevelamer-treated patients showed a higher CAC prevalence and burden at baseline (prevalence of CAC 62.6% versus 47.6%; P=0.02; median CAC score: 122 AU [IQR, 0–180] versus 0 AU [IQR, 0–215]; P=0.01 in the sevelamer and calcium carbonate group respectively), at study completion a significantly lower risk of CAC progression or *de novo* onset (12.8% in sevelamer-treated patients and 81.8% in calcium carbonate–treated patients) was noted.[57]

Other studies in ESRD patients new to[48] or on maintenance dialysis[61] have also investigated the differential impact of calcium salts and calcium-free phosphate binders on vascular calcification or hard outcome.[48,62] Though the majority of these trials point toward a harmful potential of calcium-containing phosphate binders, metanalyses have repeatedly failed to confirm this hypothesis.[39,63,64] A recent study by Di Iorio et al.[65] unfolds an almost 10-fold reduction of CV and all-cause mortality associated with sevelamer versus calcium carbonate in a large cohort (N=466) of patients new to dialysis.

Though these data suggest a different effect of calcium-free phosphate binders on the cardiovascular system and survival, no study has ever tested whether serum phosphorous-lowering is associated with a survival benefit. In light of the many adaptive mechanisms to hyperphosphataemia such as increased PTH and FGF23 that can modulate phosphorous toxicity and the potential calcium toxicity,[66,67] future studies should address when to start in the course of CKD and to what serum phosphorus target should we aim when prescribing phosphate binders. Finally, cost-effectiveness analyses of these compounds are needed in light of the expanding epidemiology of CKD.[68]

Native Vitamin D: Facts, Promises and Expectations

Native vitamin D has received growing interest in the last ten years. Every year, hundreds of manuscripts on native vitamin D associations with a variety of diseases such as osteoporosis,[69] hypertension,[70] cardiovascular disease,[71,72] insulin resistance,[73] infections,[74] cancer[75] and mortality[76] are published. Similarly, nephrologists have traditionally linked native vitamin D deficiency to CKD progression,[77] secondary hyperparathyroidism (SHPT)[78] and survival[79] in renal patients. The widespread association between native vitamin D and unfavourable outcomes in the general population, as well as in selected diseased sub-cohorts, together with the emerging knowledge of the extra-renal activation of native vitamin D, support the hypothesis that vitamin D deficiency is an etiologic factor rather than a mere biomarker of frailty.[80]

The term 'native Vitamin D' refers to the 25 hydroxlate vitamin D (25(OH)D) forms. Vitamin D precursors ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are synthesised by the UV radiation in yeast and in animals starting from ergosterol and 7-dehydrocholesterol, respectively.[81] In turn, vitamin D precursors are hydroxylated in the liver to form 25(OH)D2 and 25(OH)D3, respectively.[81] These are the substrates that are subsequently activated to 1-25$(OH)_2$D (calcitriol) by the renal and, to a lesser extent, by the extra-renal 1 alpha hydroxylase.[82] Of note, humans do not synthesise vitamin D2[81] and almost 80% of vitamin D is obtained by UVB irradiation with only a minor contribution of diet intake.[82]

Though it is commonly prescribed as a supplement, we currently ignore what is the desirable level of 25(OH)D.[69,83] It is commonly accepted that levels of 25(OH)D above 30 ng/ml, between 21 and 29 ng/ml and below 20 ng/ml define vitamin D sufficiency, insufficiency and deficiency, respectively.[82]

Native vitamin D deficiency is highly prevalent in the general population as well as in CKD and is almost ubiquitous in dialysis patients (greater than 80%).[84] Three drugs are currently available for vitamin D supplementation (ergocalciferol, cholecalciferol and calcifediol) based on the precursor from which they are originated. A few subtle pharmacologic differences have been described.[85,86] Several studies observed that ergocalciferol is less potent than cholecalciferol in restoring 25(OH)D levels,[86] possibly due to a stronger affinity of cholecalciferol to the vitamin D binding protein.[86] Moreover, the activated form of vitamin D (1,25OHD – calcitriol), originated from cholecalciferol, induces a sustained activation of the vitamin D receptor (VDR) due to a higher affinity of its catabolite 1-24-25(OH)D3 to the VDR than the ergocalciferol-derived catabolite 1-24-25(OH)D2.[85] Thus, it is commonly accepted that

50.000 IU of ergocalciferol are pharmacologically equivalent to 5-15.000 IU of cholecalciferol.[87] However, whether or not these two forms of vitamin D may have different clinical implications is still unknown. Two RCTs are currently recruiting patients to compare the effect of vitamin D2 versus Vitamin D3 on mineral metabolism in CKD stage 2-5 (NCT01633853, NCT01173848) to shed light on which 25(OH)D form is better suited in CKD. Current evidence suggests a potential role for 25(OH)D as PTH lowering agent. Indeed, a recent meta-analysis by Kandula and colleagues[88] concludes, based on the available observational studies, that 25(OH) D compared to placebo reduces PTH levels in CKD (about 25 pg/ml) as well as in ESRD (about 60 pg/ml) patients. However, the heterogeneity of the studies precludes speculation on what could be the best 25(OH)D regimen in CKD. Whether 25(OH)D can be used instead of VDR activator for PTH suppression in CKD is still under debate, though preliminary data suggest that paricalcitol and doxercalciferol induce a stronger PTH reduction compared to ergocalciferol and cholecalciferol in CKD 3-4[89,90] and ESRD patients,[86] respectively. Similarly, data concerning PTH reduction by the co-administration of native and active vitamin D are still inadequate, mainly based on observational and retrospective studies.[91-93] Further evidence is advocated before recommending the implementation of this combined approach.

In spite of the many pleiotropic effects described in the past decades and the substantial increase in the risk of death associated with low 25(OH) D levels,[75] only a few studies have investigated the impact of native vitamin D on surrogate endpoints such as renal osetodystrophy, vascular calcification, proteinuria, LVH or survival. However, numerous RCTs are currently investigating the effect of native vitamin D on left ventricular hypertrophy (NCT01323712), insulin resistance (NCT00893451), erythropoietin dosing (NCT01395823), proteinuria (NCT01426724), immunity (NCT00892099), ateriovenous fistulae maturation (NCT00912782) and physical and cognitive performance (NCT00511225, NCT01229878) to shed light on the potentials of this treatment. Finally, the NUTRIVITA study is actively randomising dialysis patients to 25(OH)D versus placebo treatment to test the effect of 25(OH)D on survival, fatal myocardial infarction, and non-fatal stroke (NCT01457001).

Due to the scarce data available, current guidelines on mineral metabolism management,[10] suggest 25(OH)D deficiency replenishment as the first step to correct SHPT in CKD stage 3-5,[10] whereas no suggestion is provided for dialysis patients. These statements are 'not graded' and based on expert opinion rather than on evidence.[10] A considerable ongoing and future effort is needed to clarify the impact of 25(OH)D administration to CKD and dialysis patients.

Vitamin D Analogues: Facts, Promises and Expectations

Repeated observational data described an independent association between PTH levels and unfavourable outcomes in CKD stage 3-5[94,95] as well as in ESRD.[2,3] However, no RCTs have yet proven that an active reduction of PTH values improves such patient-centred hard outcomes as hospitalisations, cardiovascular events, CKD progression, and survival. Thus, the optimal PTH target is still uncertain in CKD as well as in ESRD subjects. KDIGO guidelines provide a low-grade suggestion to maintain PTH levels into the range of normality in CKD stage 3-5 and between two and nine-times the normal range in ESRD.[10]

The reduction of calcitriol levels, together with hypocalcemia and hyperphosphataemia, are the leading causes of increased PTH levels. Thus, KDIGO guidelines suggest the use of vitamin D in case of increased PTH values and its tailoring in case of PTH over-correction, hypercalcemia or hyperphosphataemia.[10] The risks related to high doses of vitamin D are mainly due to phosphate and calcium overload that possibly contribute to the low achievement rate of calcium and phosphate recommended targets[96] and to a poor survival in dialysis patients.[3] However, selective vitamin D receptor activator (VDRA), with a stronger effect on PTH and a lesser impact on calcium and phosphate load, may improve the global achievement of serum PTH, calcium and phosphate targets reducing the vitamin D toxicity.[97-99]

In recent years industries have provided multiple synthetic vitamin D2 (paricalcitol and doxercalciferol) and vitamin D3 analogues (alfacalcidol, falecalcitriol and maxacalcitol). However, comparison data of different vitamin D analogues on mineral metabolism control, surrogate and patient-centred outcomes are currently still scarce.

Several studies suggest that VDRAs are superior to placebo and calcitriol in controlling PTH, calcium and phosphate, but the few available head-to-head comparisons between VDRAs led to heterogeneous

and inconclusive results. Alfacalcidol was similar to calcitriol in suppressing PTH values with equal change in phosphate and calcium levels,[100,101] however recent data by Hansen et al.[102] did not observe significant differences between alfacalcidol and paricalcitol on similar targets. Joist et al.[103] observed that paricalcitol at very high doses suppressed PTH with lower elevation of phosphate and calcium levels compared to doxercalciferol. However, Fadem and coworkers[104] could not detect any difference in PTH, calcium and phosphorous control when haemodialysis patients were switched from intravenous paricalcitol to doxercalciferol. No clinical data comparing doxercalciferol with alfacalcidol in humans are currently available.

More recently, a growing interest for vitamin D pleiotropic effects, related to the widespread regulation of the human genome played by VDR activation, has been observed. Albuminuria, left ventricular hypertrophy (LVH) and cardiac remodelling have all been tested as potential targets of vitamin D analogues. The activation of VDR can regulate the expression of several genes involved in glomerular and myocardial inflammation as renin,[105] TGF-beta,[106] antioxidant molecules,[107] NFκB and RANTES.[108] The VITAL study, a randomised placebo controlled trial in diabetic CKD patients, documented a dose dependent trend toward reduction of albuminuria when paricalcitol was added to RAAS inhibitors.[109] Though the PRIMO study failed to demonstrate a significant LVH reduction,[110] a post-hoc analysis documented a lower increase of brain natriuretic peptide and left atrial index in diabetic CKD patients receiving paricalcitol on top of ACE-I or ARBs compared to placebo.[111] Interestingly, paricalcitol was associated with lower risk of hospitalisation in those patients with more severe LVH.[110] However, no RCT has tested the effect of different forms of vitamin D or VDRA on hard patient-centred outcomes.

Numerous, albeit not all, observational studies suggest potential benefits beyond mineral metabolism control linked to VDRA use on hospitalisation, cardiovascular events, and mortality. Kalantar-Zadeh and coworkers[3] reported a 14% reduction in all-cause hospitalisation among patients receiving paricalcitol compared to those treated with calcitriol in a large cohort of 58,058 haemodialysis patients.[3] Paricalcitol[112-114] and doxercalciferol[114] use were both associated with lower mortality risk compared to calcitriol in other large series of patients on chronic haemodialysis. Recently published results from the Italian FARO survey[115] unexpectedly showed a better survival in dialysis patients receiving vitamin D also in the presence of PTH ≤150 pg/ml. However, the Dialysis Outcome and Practice Pattern Study (DOPPS) investigators failed to report on vitamin D improved survival after adjustment for confounders and different practice patterns.[116] Hence, these encouraging observational data have to be confirmed in RCTs prior to orient stronger recommendations on vitamin analogues prescription.

Future studies should shed definitive light on whether the use of VDRAs improve survival in CKD and ESRD as well as surrogate outcomes such as albuminuria and LVH. Finally, in consideration of the growing number of CKD patients and the high-cost burden connected to CKD management, future studies should also verify the cost-effectiveness of the use of VDRA in different stages of CKD.

Cinacalcet: Facts, Promises and Expectations

The existing body of evidence suggests that cinacalcet effectively lowers serum PTH, phosphorous, and calcium levels in ESRD modulating the parathyroid calcium sensing receptor affinity to serum calcium.[6,117-125] Phase two and three studies show that, on average a 40-50% (250-350 pg/ml) serum PTH, a 5-8% (0.5-0.8 mg/dl) serum calcium and a 5-10% (0.2-1.0 mg/dl) serum phosphorous reduction is expected when cinacalcet is administered.[6,117-125] It is conceivable that the calcium-PTH setpoint shift and the metabolic change in bone metabolism induced by this drug explain these results.[126,127]

Whether calcimimetics are superior to VDRAs in controlling CKD-MBD is another unanswered question. Two large RCTs, the ACHIEVE[119] and the IMPACT[128] study investigated this issue in haemodialysis patients. The first study[119] concluded for a better PTH control with cinacalcet, while the second study[128] showed a better PTH control among patients treated with intravenous paricalcitol. However, some major differences in the two study designs may account for some of the discrepant results: 1) in the ACHIEVE study both paricalcitol and doxercalciferol were allowed as VDRAs, while paricalcitol was the only VDRA administered in the D arm of the IMPACT study; 2) cinacalcet was admitted as a rescue therapy for hypercalcemia during VDRA treatment in the IMPACT study, whereas it was not allowed in the ACHIEVE study; 3) treatment algorithms for cinacalcet or VDRA dose modulation were different in the two trials. In light of these study

design differences it is unclear whether one of these two approaches is superior, though answering this question might be of limited clinical utility in light of the different pharmacological profile of calcimimetic and VDRAs.

The presence of calcium-sensing receptors in different tissues other than the parathyroid glands, could explain the positive impact of cinacalcet on the bones and vasculature detected in numerous preclinical data.[129] *In vitro* and animal evidence suggest that a reduction of functional calcium-sensing receptors is associated with vascular calcification,[129,130] blood pressure,[131] proteinuria,[132] CKD progression,[132] arterial stiffness and endothelial dysfunction improvement.[133] Large cohort prospective studies show that calcium-sensing receptor modulation is associated with favourable clinically meaningful outcomes. Cunningham and coworkers[134] showed a significant reduction in the risk of cardiovascular disease, bone fracture, parathyroidectomy incidence, and a parallel improvement in the general health perception among dialysis patients with secondary hyperparathyroidism. Block et al.[135] documented a substantial risk reduction in all-cause and cardiovascular mortality associated with cinacalcet in a large cohort of 25,292 chronic haemodialysis patients independent of several confounders.

However, the clinical impact of cinacalcet on hard outcome is far from being established in light of the recently published results of the ADVANCE[124] and EVOLVE[6] trials. The ADVANCE trial was conducted to investigate whether cinacalcet in combination with low dose of vitamin D (<6 mcg paricalcitol equivalent/ week) versus flexible doses of vitamin D attenuates coronary, aorta, and cardiac valves calcification progression in a cohort of 360 prevalent haemodialysis patients. After a relatively short period of follow-up of 12 months, a trend toward CAC reduction in the cinacalcet arm (Agatston CAC scores % change: 24% (95% confidence interval: -22%, 119%) and 31% (- 9%, 179%), in the cinacalcet and flexible vitamin D group, respectively, P=0.073) was noted. Notably the trend was consistent across all CV sites investigated for vascular calcification.[124] Furthermore, the large dose of calcium-containing phosphate binders and vitamin D administered in the calcimimetic arm may contribute to explain these results.[136] Finally, the EVOLVE trial was designed to test the survival benefit of cinacalcet hypothesised by observational data in haemodialysis patients. At study completion, a statistically non-significant trend toward reduction (relative hazard in the cinacalcet group vs. the placebo group, 0.93; 95% confidence interval, 0.85 to 1.02; P=0.11) of the composite endpoint (time until death, myocardial

Treatment	Type of evidence	Head-to-head comparisons between drugs of the same class	Mineral metabolism control	Tissue marker of organ damage	Survival data	Pharmacoeconomic evaluation
Low phosphate diet	Observational studies	NA	YES	NO	NO	NA
	RCTs	NA	YES	NO	NO	NO
Phosphate binders	Observational studies	YES	YES	YES	YES	NA
	RCTs	YES	YES	YES	YES	NO
Native vitamin D	Observational studies	YES	YES	YES	YES	NA
	RCTs	NO	NO	NO	NO	NO
Activated forms of vitamin D (VDRA)	Observational studies	YES	YES	YES	YES	NA
	RCTs	NO	YES	YES	NO	NO
Cinacalcet	Observational studies	YES	YES	YES	YES	NA
	RCTs	NA	YES	YES	YES Inconclusive	NO

Table 1. Available knowledge is mainly based on observational and inconclusive RCTs.

infarction, hospitalisation for unstable angina, heart failure, or a peripheral vascular event) was reported.[6] However, the lower than anticipated event rate, the high drop-in and out rate during follow-up (about 20%),[6] significantly affected the statistical power (0.54)[6] and the interpretability of this inconclusive RCT.

In essence, data support the notion that cinacalcet is a safe and effective drug to lower PTH in secondary hyperparathyroidism. Nonetheless, future research projects should indentify the ideal candidate that would likely increase survival and quality of life while on this treatment. Finally, though the use of cinacalcet in predialysis stages of CKD is not approved because of the risk of hypocalcemia, future studies should evaluate its efficacy and safety in CKD not dialysis dependent cases of secondary hyperparathyroidism, characterised by normal-high calcium and high phosphate in which vitamin D may further aggravate phosphorous and calcium balance.

CONCLUSION

Treatment of CKD-MBD is currently based largely on opinion rather than evidence, and many questions about CKD-MBD await answers. A tremendous effort has been performed in the attempt to clarify the natural history and pathogenic mechanisms that trigger CKD-MBD and modulate the astonishing risk connected to it. Nonetheless, a substantial degree of uncertainty on the clinical relevance and use of different serological and tissue biomarkers used to individualise, and titrate treatments still exists and affects patient care. Furthermore, the incompleteness (Table 1) and inconclusiveness due to various methodological flaws in the few available RCTs complicate the interpretation of the available evidence and lead to a heterogeneous use of the different drugs we have in our armamentarium.[96]

Future effort is therefore needed to elucidate mechanisms and treatment of these imbalances that, at least observational data, link to a substantial risk burden[2] in CKD patients.

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CLINICAL PROTEOMICS: THE POTENTIALITY OF URINE ANALYSIS FOR UNDERSTANDING DIABETIC NEPHROPATHY

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ABSTRACT

The incidence of diabetic nephropathy (DN) is constantly rising in parallel with the prevalence of type 2 diabetes and has been predicted to double within the next 15 years. Albuminuria is considered the earliest putative diagnostic sign of diabetic renal damage but it is poorly associated to the complex histopathological picture of glomerular and tubular damage hence, up to now, the accurate diagnosis of the DN requires renal biopsy. The identification of new biomarkers of DN is an urgent need since the proper management of the DN patients requires early and unbiased diagnosis. The Proteomics approach to the study of the human disease allows a large-scale characterisation of the protein content of a biological sample, and its application to urine may be a challenging but powerful strategy to identify new DN biomarkers. In this review we discuss the main results of a decade of proteomic studies focused on the urinary investigation of diabetic nephropathy.

Keywords: Diabetic nephropathy, urinary proteome, proteomics, urine, biomarkers.

THE PATHOPHYSIOLOGY OF DIABETES AND DIABETIC NEPHROPATHY

Diabetic nephropathy (DN) is the most common chronic kidney disease (CKD) in developed countries[1] and the most frequent cause of end-stage renal disease (ESRD) worldwide. It has been estimated that 40% of the patients undergoing renal dysfunction and that require renal replacement therapy are affected by DN.[2] DN is a severe complication of both type 2 diabetes mellitus (T2DM) and type 1 diabetes (T1D), but the incidence of nephropathy is more prevalent in T1D primarily due to the fact that, in T2DM, death as a result of cardiovascular causes is more common than death from renal failure.[3,4] The use of renin–angiotensin system inhibitors and strict glycemic control is contributing to slow the incidence of ESRD in T2DM patients.[5] However, between 2000 and 2030, the prevalence of T2DM has been predicted to increase by 20% in developed countries and about 50-70% in developing ones.[6] This will lead to an increase of the incidence of ESRD,[7,8] concomitantly with the progressively declining rate of mortality due to cardiovascular causes.[9,10]

The *primum movens* of T2DM complication is chronic hyperglycaemia, which initiates specific modifications of the electron transport proteins by advanced glycation end-products (AGEs) and alters normal metabolism by increasing production of reactive oxygen species (ROS).[11] Hyperglycaemia and increased ROS production alter cell homeostasis in endothelium and renal cells and impair endothelial nitric oxide synthase and prostacyclin synthase, that, in turn, contribute to defective angiogenesis and persistent expression of pro-inflammatory genes, also after glycaemia normalisation.[12] These factors, together with genetic background and lifestyle, may predispose a considerable number of T2DM patients to develop DN.

The pathogenesis of DN involves structural changes, including glomerular and tubular hypertrophy, with

progressive accumulation of extracellular matrix components in the glomerular mesangium and tubulointerstitium, and changes in podocytes.[13-15] According to the most recent pathologic classification of DN,[16] the severity of the glomerular lesions correlates with the progression of the DN and may allow four classes to be distinguished, namely: class I (glomerular membrane basement thickening); class II (mesangial expansion without Kimmelstiel-Wilson lesions); class III (presence of at least one glomerulus presenting Kimmelstiel-Wilson lesions) and class IV (Kimmelstiel-Wilson lesions in at least 50% of the glomeruli).

At urinary level, microalbuminuria (urine albumin excretion 30-300 mg/24h) is considered the earliest putative diagnostic sign of diabetic renal damage even if it may not correlate with the complex histopathological picture of glomerular and tubular damage in T2DM.[17] In fact, it is not always associated with the presence of Kimmelstiel-Wilson nodules when renal biopsies are examined,[18] thus representing a better predictor of cardiovascular disease than of renal damage progression.[19] Further to this, urine contains more than 60 forms or fragments of albumin,[20] which are not all recognised by the routinely immunoassay-based methods that ultimately may underestimate the correlation between the albuminuria and the renal damage. Due to the complexity of DN pathophysiology it is necessary to set up unbiased methods that can simultaneously detect new sets of biomarkers for earlier diagnosis and prognosis of DN.[21]

The development of renal damage in T2DM patients is antedated and/or accompanied by a number of molecular changes that may be now identified by a number of high-throughput strategies. These include the next generation sequencing (NGS) approaches for complete sequencing of whole genomes,[22] transcriptomes,[23] and epigenetic DNA modifications,[24] and also proteomic and metabolomics strategies for accurate measurement of the entire content of proteins and metabolites of biological samples. The aim of the present review is to provide a concise overview of the main contributions of the proteome science to the identification of a set of new urinary biomarkers that could help in achieving early diagnosis and better management of DN.

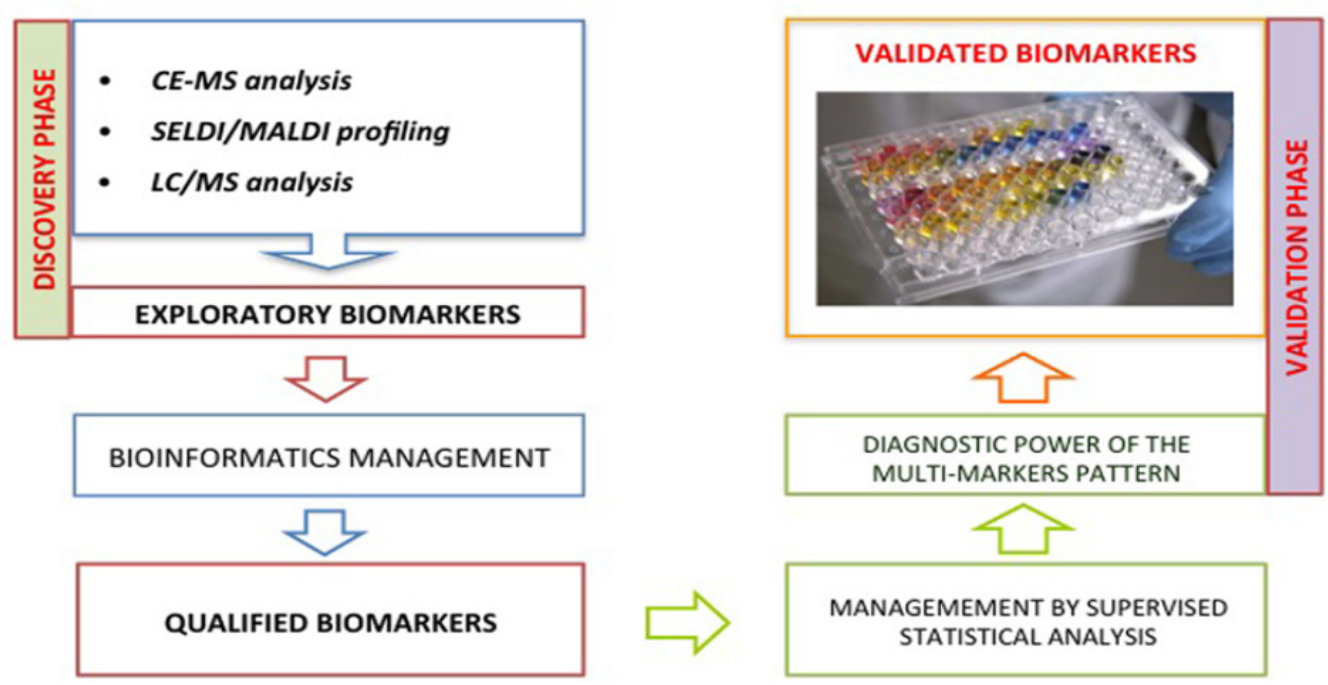

Figure 1. Workflow of the biomarker discovery strategy by hightroughput proteomic analysis.
The complex datasets generated by the high-throughput analysis may allow identification of thousands of exploratory biomarkers. The bioinformatics management is critically required to select, among the exploratory biomarkers, the disease-correlated ones (qualified biomarkers). The management of the qualified biomarkers by means of supervised statistical methods is then essential to setup new classificatory models useful for the diagnosis and prognosis of the diseases. Finally this multi-markers pattern should be validated, in multicentric cohorts of patients, by routinely immunoassays in order to verify their usefulness in clinical practice.

The Proteomic Approach To The Study of Renal Diseases

The term 'proteomics' indicates a complex and interdisciplinary matter requiring expertise spanning from chemistry to biology and bioinformatics, in order to reveal the meaning of complex protein datasets of a biological sample in physiological and pathological conditions. The completion of the human genome sequencing together with the exponential development of ionisation sources (i.e. matrix-assisted laser desorption/ionisation [MALDI][25] and electrospray ionisation [ESI])[26] and bioinformatics tools have rapidly provided new technological platforms for the analysis of complex protein datasets and the interpretation of the cross-linked relationship among the differently expressed proteins. Starting from the last decade, proteomics has been exponentially applied to nephrology leading to the identification of a number of putative biomarkers that are expected to enter shortly into the clinical practice,[27] making proteomics a science of key interest not only for researchers but also for clinicians.

The proteomics analysis of biological samples may be pursued by distinct and complementary strategies that allow separating the protein mixtures and identifying the key disease-related molecules by mass spectrometry analysis. Two-dimensional gel electrophoresis (2-DE), the most popular gel-based approach, allows double protein separation according to the isoelectric point (pI) and the molecular mass (MW)[28] and provides, for each sample, a characteristic proteomic map showing the separated proteins as protein spots or spot trains due to the presence of protein post-translational modifications (PTMs).

Comparative software analysis of the 2-DE maps between pathological samples and matching controls may allow identifying differently expressed protein spots that are excised from the gel, trypsin digested to obtain small peptides mixtures, and analysed by mass spectrometry (MALDI-TOF MS, nanoHPLC-ESI-MS/MS) to obtain the protein ID. Even if highly informative, 2-DE proteome underestimates the protein complexity of the sample since, for example, less expressed proteins, proteins having a molecular weight lower than 10 kDa and higher than 250 kDa), and transmembrane (hydrophobic) proteins are difficult to visualise. Although 2-DE is the only tool to depict protein isoforms (train spot), this approach may be laborious and expensive without providing satisfactory results. Usually, 2-DE is appropriate to study a restricted and well-characterised cohort of patients in order to identify putative disease-associated biomarkers, but they need to be further validated in larger cohorts of patients to ascertain their usefulness as disease biomarkers.

The development of a number of so-called profiling technologies has permitted high-throughput analysis of thousands of biological samples and appears to be more appropriate for clinical proteomics studies since they may combine the multicentre collection of numerous samples with their rapid analysis in order to identify a new set of biomarkers applicable to the general population. The profiling technologies include a number of complementary strategies, namely liquid chromatography (LC),[29] capillary electrophoresis (CE),[30] and thin-layer chromatography (TLC)[31] coupled to mass spectrometry (MS). These strategies can identify, in a shortened time, many putative biomarkers ready to be validated. However, the complex datasets generated by these approaches must be properly managed by means of statistical and bioinformatics tools to finally allow the recognition of reliable disease-specific biomarkers before proceeding with their validation.

Recently, the biomarker task force of the National Cancer Institute has developed the guidelines for biomarkers studies that can be extended to any kind of disease.[32] In general, a qualified biomarker must have a clear clinical significance for the disease or a consistent scientific body of evidence must support its probable implication in the pathophysiology of the disease. On the contrary, the disease-associated proteins may be defined as exploratory biomarkers. In order to select the qualified biomarkers among the exploratory, specific bioinformatics tools must be used to select functionally correlated subsets and to evaluate their diagnostic power. The use of bioinformatics software, such as String and Ingenuity, permits a search for the known interactions of any well-characterised protein, and to define a large number of potentially interacting molecules for each protein.[33] This approach may lead to an ever-expanding network of molecular correlations, thus, clinicians having a specific knowledge of the pathophysiology of the disease should always check the appropriateness of each possible interactome in order to restrict the further validation to a sub-set of disease correlated biomarkers. The lack of this essential contribution may prevent the identification of the qualified biomarkers and their use in diagnostics. After the identification, the qualified

biomarkers should be managed through supervised statistical analysis in order to verify if their combined evaluation may allow the creation of proteome-based models useful for improving the diagnostic and prognostic power of each of them.

Briefly, this kind of data analysis uses specific algorithms[34,35] that verify the best association among the identified biomarkers to recognise the pathologic phenotypes in a "training set" of control and disease samples. The optimal pattern is then validated against an independent "validation set" to confirm its diagnostic utility. The main focus of proteome analysis in nephrology is the identification of biomarkers useful for the prediction of a pathologic phenotype in still asymptomatic patients or for an early and accurate diagnosis to permit rapid and personalised renoprotective treatment. Among biological samples, urine appears the most eligible for identifying kidney biomarkers and therefore most of the clinical proteomics studies in nephrology have been focused on this biological sample. In the next paragraphs we will briefly discuss the main contribution of the urine proteomics to the understanding of DN.

The Urine Proteome: Potentialities and Pitfalls

Many published studies have discussed and emphasised the potentiality of urine as the most appropriate biological fluid for biomarkers discovery in kidney diseases.[36-42] Some of the well-known and recognisable urine characteristics include: easy, non-invasive accessibility, allowing for multiple and abundant collection; the presence of both kidney-derived (about 70%) and plasma-derived (about 30%) proteins, useful for the identification of both systemic and kidney-specific biomarkers; the lower complexity and increased stability of the urine proteome when compared to that of other biological fluids such as serum and plasma, ensuring the possibility of analysis, and also samples can be collected and subsequently stored for long periods.[43-45] However, the use of urine for proteomic analysis also has some pitfalls such as the presence of salts and other interfering agents, the higher intra and inter-subject variability,[39] and in nephropathic patients, the predominant presence of serum proteins like albumin that interfere with the recognition of the lower expressed proteins and may prevent the identification of more sensitive and specific biomarkers.

Since proteomics was firstly applied to the analysis of urine samples, it has been realised that the initial aim of any clinical proteomics study must be the definition of standardised procedures to reduce the effect of confounding factors on the reproducibility of the proteomic data. Our group and other authors have contributed to the realisation of this objective through the publication of a number of methodological works,[39,45-49] which have allowed for the planning of more accurate biomarker discovery studies in following years. The importance of this aspect is considered a central issue for the nephrology community at national, European (European Kidney and Urine Proteomics (EuroKUP) and international level (Human Kidney and Urine Proteome Project (HKUPP) through the creation of groups of study or consortia involved in the standardisation of consensus procedures for collection, storage and analysis of urine by proteomics approaches. It is expected that this attempt to spread a growing awareness of the importance of adopting standardised and comparable protocols among clinicians, nursing staff, and researchers will contribute to set clinical studies of major impact for the identification of reliable biomarkers.

Milestones In Urine Proteomics Applied To Diabetic Nephropathy

Since 2004, when Mischak and coworkers[50] described three polypeptide patterns able to recognise 'normal', 'diabetic', and 'diabetic patients with renal damage', about 15 original works dealing with the identification of urinary biomarkers of DN have been published. Even if this proof-of-concept work lacked some details on the criteria that are now considered essential for the definition of a qualified set of biomarkers (i.e. the validation in an independent test set or the bioinformatics analysis to establish the functional association between the biomarker and the disease), it has been successful in showing, for the first time, that urine proteomics could provide new important information about kidney disease in T2DM patients.

In the following years, several well-designed works, based primarily on urine screening by CE-MS and SELDI-TOF/MS, have allowed for the identification of new promising biomarkers for early diagnosis and prognosis of DN. Rossing et al.[51] applied CE-MS analysis to T1D patients, describing a panel of 65 urine biomarkers able to recognise DN with 97% sensitivity and specificity. Their results were further validated in a multicentre independent cohort[52] of T2DM patients, providing the first evidence that CE-MS urine proteome profiling may adequately identify subjects with DN in the general population. About

half of the polypeptides included in the proteomic pattern were identified as collagen fragments, thus suggesting that changes in the collagen metabolism may be closely linked to the renal damage in T2DM. Furthermore, Good and coworkers[53] reported a CE-MS based classifier including 273 urinary small peptides (namely 'Classifier273') that seem to be highly specific and sensitive for CKD, irrespective of the underlying pathology. In a very recent work, Zurbig et al.[54] demonstrated that this classifier was more specific and sensible than urine albumin excretion rate (UAER) in predicting the occurrence of the microalbuminuria in T1D and T2DM normoalbuminuric patients. These data, even if limited to a restricted number of diabetic patients, would suggest that the urine proteome might allow the identification of DN risk patients, thus permitting early onset of renoprotective treatments to slow the progression of the renal damage.

SELDI-TOF/MS analysis has also been extensively used for identifying urine biomarkers of DN. For example, Dihazi et al.[55] identified and validated among 100 differently excreted SELDI peaks, two mass peaks corresponding to B2-microglobulin and ubiquitin ribosomal fusion protein, which were selectively and differently excreted in nephropathic diabetic patients. More recently, Wu et al.[56] reported 300 differently excreted urine mass peaks among T2DM patients with normo, micro and macroalbuminuria, and described a four-peak pattern useful for recognising DN with 88% and 97% sensitivity and specificity, respectively. Interestingly, in these studies the progression of renal damage in T2DM was expressed only according to the albumin excretion rate.

Our group also performed a comparative SELDI analysis of the urine proteome,[57] taking into account a more accurate selection of the T2DM patients since only diabetic patients with biopsy-proven Kimmelstiel-Wilson lesions were included in the DN group. We confirmed the data of Dihazi, concerning the increased excretion of B2-microglobulin in DN, and found significant deregulated excretion of the ubiquitin as potential biomarkers of DN. Further, we confirmed the specificity of the identified biomarkers in an independent test set of T2DM patients having biopsy-proven non-diabetic chronic kidney disease (CKD). It is worth noting that both CE-MS and SELDI profiling are able to specifically analyse low molecular weight proteins while being ineffective to cover the medium and high size proteome.

A high-throughput approach that allows a more accurate coverage of the proteome is the so-called shotgun proteomics analysis.[29,58] In this approach, the proteins of a given biological sample are proteolytically digested into peptides and separated by bidimensional liquid chromatography prior to mass analysis (LC/MS). The ensuing peptide masses and sequences are then used to identify corresponding proteins by database search.[59] Recently, Jin et al.[60] employed the urine LC/MS analysis to search for specific DN biomarkers. Specifically, these authors used isobaric tags for relative and absolute quantitation (iTRAQ)[61] to select and quantify differentially excreted urinary proteins in pooled urine samples of microalbuminuric versus normoalbuminuric diabetic patients. This analysis allowed the recognition of 196 differentially expressed proteins, including 10 (qualified) biomarkers that were identified by bioinformatics analysis. The application of a multiparametric pattern, encompassing three of the ten qualified biomarkers, allowed identification of microalbuminuric patients with about 92% sensitivity and specificity.

It is interesting to consider that most of the urine proteomic studies have investigated only the soluble urine fraction. Indeed, recently, urinary exosomes have been receiving increasing attention as a new source of potential biomarkers.[62] Exosomes are 30-100 nm vesicles, derived from the endosomal compartment and released via fusion of multivesicular bodies with the plasma membrane.[63] They comprise of a ceramide and cholesterol-rich lipid bilayer membrane,[64] an array of membrane and cytosolic proteins,[62] and selected RNA species.[65] These vesicles are a rich source of biomarkers because they are released from every segment of the nephron, including podocytes, and are finally excreted in the urine.

Very recently, Raimondo and coworkers[66] have published an interesting proof-of-concept work on the proteomic analysis of urine exosomes in Zucker Diabetic Fatty (ZDF) rats. They profiled the urinary exosomal protein content of non-diabetic lean rats and ZDF rats with normo or microalbuminuria. By this approach, 280 differently expressed exosomal proteins were identified and categorised according to the function and subcellular localisation. They demonstrated that incipient renal disease correlated with increased cytoplasmic and cytoskeletal proteins in the urine exosomes, and that the identified proteins were mainly involved in metabolic and immunity processes. The above results demonstrate that the proteomic analysis of the urinary exosomes, together

with the analysis of the soluble urinary proteins, may fruitfully contribute to reveal the pathophysiological alterations occurring in DN progression, and to enlarge the panel of DN biomarker candidates.

CONCLUSIONS AND PERSPECTIVES

Proteomics has become one of the most powerful tools for the mass-analysis of urine samples and is yielding a decisive contribution for a better understanding DN pathophysiology. More than a decade of studies has provided significant advances in the management of urine samples to find new sensitive and specific biomarkers of DN. However, the proteomic ability to quickly analyse thousands of urinary proteins has generated the wrong belief that, in few years, novel biomarkers that are able to recognise the onset of kidney damage with 100% accuracy would have been identified. Instead, the lack of consensus protocols for collecting, processing, and analysis of the samples has led to poor reproducible results among different studies, thus making difficult their generalisation.

We are now becoming aware of the need for protocol standardisation to enlarge the collection of comparable samples in different countries, and that the bioinformatics analysis of the complex datasets represent a *conditio sine qua non* for restricting the validation of the identified biomarkers to those specifically related to the pathophysiology of renal damage in T2DM. It is expected that this new way of managing the proteomic datasets will critically favour the identification of reliable biomarkers by reducing the effect of confounding factors. Furthermore, proteins are the players of a complex game, which also includes genes, transcripts, and metabolites, each influencing the others. Indeed, in the forthcoming years, bioinformaticians will have to develop more accurate tools to correlate proteomic datasets with the corresponding genomic, transcriptomic, and metabolomic datasets in order to pursue a global characterisation of the biological systems, and to identify a multi-level panel of molecular players cooperating to the onset of the pathological phenotypes.

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TREATMENT OF ANTIBODY-MEDIATED REJECTION IN KIDNEY TRANSPLANTATION

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ABSTRACT

Antibody-mediated rejection (AMR) is a relatively rare but severe complication in kidney transplantation associated with increased risk of graft loss. Diagnosis of acute and chronic AMR is based on typical histological hallmarks, deposition of C4d in peritubular capillaries and presence of donor-specific antibodies (DSA). Many novel and attractive treatment options have become available in recent years: antibody removal and production inhibition (plasmapheresis, IVIg), B cell depletion (rituximab), plasma cell depletion and apoptosis (bortezomib), and complement activation inhibition (eculizumab). Standard therapy is based on PP and IVIg. Preliminary results with new agents are encouraging but require randomised clinical trials and long-term follow-up.

Keywords: Kidney transplantation, antibody-mediated rejection, donor-specific antibodies, management of antibody-mediated rejection, IVIg, plasmapheresis, bortezomib, rituximab, eculizumab.

INTRODUCTION

The mechanism of organ transplant rejection may be cellular (T lymphocyte-mediated) or humoral – the latter being mediated by antibodies produced in response to donor-specific antigens exposed on endothelial cells of the allograft. For a long time, transplant specialists have focused on the diagnosis and treatment of cell-mediated reactions, even though the negative effects of alloantibodies in the transplanted organ have been identified by Patel and Terasaki as early as 1969.[1] However, it was only in the last two decades that the diagnosis of antibody-mediated rejection (AMR) was rendered possible by the introduction of sensitive methods of detection of anti-human leukocyte antigen (HLA) antibodies, and most importantly, of donor-specific antibodies, using synthetic antigen assays (Luminex) and C4d detection in graft tissue as a specific marker of complement activation. In 1991, Feucht et al.[2,3] described peritubular capillary C4d deposition in renal transplants, and in 1993 postulated the association of this finding with graft loss. In 1999, Collins et al.[4] reported a correlation between humoral rejection with peritubular capillary C4d deposition and the presence of circulating anti-donor antibodies in transplant recipients. C4d is a product of systemic breakdown of C4, a classic complement activation pathway component whose biological role is unclear. C4d has more stability than other complement components because it forms a covalent complex with the surface of the endothelium and the basement membrane; the time to breakdown is about 1-3 weeks. Identification of C4d deposition using immunofluorescence or immunoperoxidase assay marks a breakthrough in histopathologic diagnosis of kidney allografts, and peritubular capillary location of deposits is considered a highly specific marker of acute and chronic humoral rejection. C4d detection is currently a standard for histopathological diagnosis of kidney allografts.[5] In the Banff classification, the term 'acute antibody-mediated rejection' appeared for the first time in 2003, and 'chronic active antibody-mediated rejection' was introduced in 2005 due to growing evidence for the role of humoral mechanisms in allograft damage. The current Banff classification adopted in 2009 includes diagnostic criteria for acute and chronic antibody mediated rejection.[6]

THE ROLE OF ALLOANTIBODIES

Anti-HLA antibodies have been identified in 1% to 60% of recipients, depending on the tested population, time from transplantation, and to a significant extent, the sensitivity and specificity of detection methods. Donor-specific antibodies (DSA) developing *de novo* after transplantation are now considered as the principal factor in the pathogenesis of graft damage. *De novo* antibodies occur in the early post-transplantation period (within the first 3 months). They indicate a risk of acute or chronic AMR.[7,8] The development of alloantibodies precedes the appearance of morphological and functional abnormalities of the graft, therefore early identification of possible AMR warrants DSA monitoring every 3 months during the first-year post-transplantation, and once a year thereafter.

Acute AMR may occur in the absence of detectable antibodies, if the antibodies are bound in the organ transplant. The incidence of acute AMR among kidney transplant recipients ranges from about 5-7% to 40-90% in non-sensitised and sensitised subjects, respectively. Acute AMR occurs most commonly as part of mixed cellular-humoral rejection (25%) and is rarely an isolated phenomenon. Chronic kidney transplant rejection manifests as slowly progressing functional deterioration that may be seen over several months, or even years. Clinical manifestations include proteinuria, hypertension and slowly progressing loss of glomerular filtration. Histopathology shows evidence of chronic transplant glomerulopathy (TG). Chronic humoral rejection is seen in 5-15% of protocol biopsies, and the onset is usually subclinical. TG has been reported in more than 40% of recipients with a history of acute AMR. Chronic transplant glomerulopathy is associated with poor outcome, which is even worse than that of interstitial fibrosis (IF)/tubular atrophy (TA).[9,10]

It is now widely considered that the principal cause of kidney transplant loss is not nephropathy, but an ongoing immunological process that can be described as chronic antibody-mediated rejection. Furthermore, it has been emphasised that modern immunosuppression regimens, which tend to minimise or discontinue calcineurin inhibitor (CNI), or glucocorticosteroids may be responsible for the development of chronic AMR. Chronic graft rejection is known to result from inadequate immunosuppression. The role of chronic humoral response in the pathogenesis of late transplant loss was confirmed in a US multicentre study (DeKAF Study – Long-term Deterioration of Kidney Allograft Function). In 173 recipients with late graft dysfunction (average of 7 years post-transplantation) who underwent graft biopsy, AMR correlates, such as C4d deposits in biopsy samples or serum DSA, were found in 57% of cases. In 2 years, the poorest outcome in terms of graft survival was seen in those patients who had both C4d and DSA, and the best in those with negative humoral reaction correlates. Signs of nephrotoxicity, if present, had no significant effect on graft survival.[11]

TREATMENT

Treatment of humoral-mediated acute graft rejection differs from that of cell-mediated rejection; it involves the elimination of circulating antibodies and suppression of antibody production by B lymphocytes or plasma cells. To date, no formal standards for the management of humoral-mediated acute graft rejection have been developed. Knowledge in this area is growing rapidly, and recent reports in the literature continue to enrich and broaden its scope. The pathogenesis of AMR forms the basis of proposed therapeutic regimens. DSA are produced by plasma cells which may be present in the pre-transplantation period or develop after transplantation from B lymphocytes (memory or naïve). T lymphocytes are necessary to initiate primary B cell-mediated response, leading to the development of plasma cells.[12,13]

Treatment Modalities in AMR Include:

- Elimination of circulating antibodies
  - Plasmapheresis (PP)
  - Immunoadsorption
- Suppression of remaining antibodies
  - IV infusions of immunoglobulins - IVIg
  - Mycophenolate mofetil (MMF)
- Blocking antibody production, B lymphocyte depletion
  - Glucocorticosteroids (GS)
  - Anti-CD20 antibody - rituximab
  - Anti-thymocyte globulin
  - Splenectomy
- Suppression of T cell response
  - Anti-thymocyte globulin
  - Mycophenolate mofetil (MMF)
  - Calcineurin inhibitors (CNI)
- Plasmocyte depletion and apoptosis
  - Proteasome inhibitor - bortezomib
- Complement inhibition
  - Anti-C5 antibody - eculizumab
  - Recombinant C1 inhibitor

State-of-the-art, promising therapies target plasma cells or the complement. Typically the treatment consists in combining several therapeutic approaches.

Plasmapheresis

Plasmapheresis is the fastest and the most efficient way to eliminate DSA; 1 volume -1.5 volume of total plasma volume is exchanged using 5% albumin or fresh frozen plasma (FFP). Plasmapheresis is performed every other day until improvement in kidney function is obtained (usually five-seven procedures). Plasmapheresis has no inhibitory effect on antibody production, therefore it is usually combined with 100 mg/kg IVIg after each PP session (up to a total of 1 g/kg body weight) and 300-400 mg/kg body weight (bw) for 1-2 days following the last PP. A combination of plasmapheresis and rituximab has been reported. Tacrolimus and MMF are recommended for primary immunosuppression due to their inhibitory effect on DSA production.

Human Immunoglobulins

The immunomodulatory activity of IgG is unknown. They are known to affect cell-mediated (T and B) immune response.

Proposed mechanisms of action of immunoglobulin:

- Anti-idiotypic antibodies neutralise circulating alloantibodies
- IVIg blocks T lymphocyte activation by interacting with the Fc receptor on antigen-presenting cells
- IVIg inhibits the activity of complement factors C3b and C4b
- IVIg inhibits cytokine secretion and activity
- IVIg inhibits the proliferation and activation of T and B lymphocytes
- IVIg inhibits epithelial cell activation
- Increase B lymphocyte apoptosis

High dose (1-2 g/kg bw) IgG should be used to achieve the desired therapeutic outcome. Non-randomised studies based on small patient populations, show combination therapy with PP+IVIg+rituximab proved more effective than IVIg alone in the treatment of acute AMR.[14]

Anti-CD20

Rituximab is a murine/human chimera, directed against the CD20 molecule located on B lymphocytes. It causes B cell lysis via antibody-dependent cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC), and prompts B cell apoptosis. The target protein for rituximab is the CD20 antigen located on immature pre-B cells and mature B lymphocytes, but not on plasma cells. Intravenous administration of rituximab leads to rapid and sustained depletion of circulating and tissue-based B lymphocytes. B lymphocyte recovery starts as late as approximately 6 months following termination of therapy, and the B cell counts return to normal within 9-12 months. Genberg et al.[15] investigated the effect of a single dose of rituximab on the B lymphocyte population in peripheral blood, kidney graft tissue and lymph nodes of 49 kidney transplant recipients. A single dose (375 mg/m^2) of rituximab was used in combination with standard triple agent immunosuppression. Total B cell depletion in peripheral blood was found in 78% of patients. At 15 months following administration of a single dose of rituximab, B lymphocytes were undetectable in peripheral blood and graft tissue (CD19 and CD20 less than 5 cells/µl). They could not be completely eliminated from the lymph nodes, but their number was significantly reduced. Rituximab is licensed for the treatment of non-Hodgkin lymphomas and post-transplant lymphoproliferative disease (PTLD). The efficacy of rituximab in the treatment of AMR was initially reported by Becker et al.,[16] who used a single dose of rituximab (375 mg/m^2) in renal transplant recipients and achieved remission in 24 patients. A number of reports in the literature support the efficacy of rituximab in the treatment of acute AMR, particularly in combination with plasmapheresis and glucocorticoid pulses. Kaposztas et al.[17] described a retrospective cohort of 54 graft recipients with AMR (the largest reported cohort to date), who were treated with a combination of PP and rituximab or PP alone. After 24 months, graft survival was significantly better in the rituximab group (90% vs. 60%). Lefaucher et al.[18] reported significantly better outcomes in terms of 36-month graft survival (92% vs. 50%) in 12 recipients treated with PP, IVIg and rituximab in comparison with a historical control group who received IVIg monotherapy [18]. The posology and duration of rituximab therapy in kidney transplant recipients have not been defined. Most reports used a single dose, but three to five doses have been described as well. Prospective randomised studies and follow-up results are lacking, and benefits of rituximab in the treatment of AMR cannot be evaluated unequivocally in the setting of concurrent polytherapies. Note should be taken of late onset, severe infectious events that may occur 3-4 months following administration of rituximab. It is recommended to take appropriate prophylactic

measures against *Pneumocystis* infection and monitor cytomegalovirus (CMV) and BK virus (BKV) replication, as well as signs of bacterial and fungal infections. The principal limitation of rituximab is the lack of effect on DSA-producing plasma cells.

Anti-Thymocyte Globulin

Anti-thymocyte globulin (ATG) is a polyclonal antibody. Its beneficial effects, in terms of suppressing AMR, involve the following mechanisms of action:

- Inhibition of T-helper lymphocytes which are necessary for B lymphocyte activation
- Complement-dependent lysis of B lymphocytes
- Suppression of B lymphocyte proliferation
- Induction of B lymphocyte apoptosis
- Inhibition of co-stimulation molecules and cytokine production

Since acute graft rejection frequently occurs via a mixed mechanism, with a predominant cellular component, ATG is often used to treat this type of rejection in combination with glucocorticosteroids (GS) and plasmapheresis.[19]

Glucocorticosteroids

Glucocorticosteroids are used as first-line therapy in acute graft rejection of any type. They are effective in T cell-mediated rejection, in mixed type rejection they act on the cell-mediated component, whereas in the humoral type they suppress B cell-mediated response by interacting with T-helper lymphocytes. Routine recommendations include pulses of methylprednisolone 250-500 mg for 3-5 days.

Mycophenolate Mofetil and Tacrolimus

MMF is an antiproliferative agent with an inhibitory effect on humoral response and antibody production. When used in combination with tacrolimus, MMF suppresses B cell-mediated response in AMR. In this context, MMF should not be co-administered with cyclosporine, as cyclosporine decreases exposure to MMF. Lederer et al.[20] showed that in kidney transplant recipients, MMF decreases the levels of anti-class I and II HLA antibodies and DSA, particularly in patients who started MMF therapy from the day of transplantation. In all cases of AMR, it is recommended to use primary immunosuppression regimens involving tacrolimus and MMF.[21]

Eculizumab

An interesting therapeutic option may consist in suppressing the complement system. Eculizumab is a humanised antibody directed against C5 complement protein, which inhibits the formation of the membrane attack complex (MAC, C5b-C9). MAC is a protein structure formed in terminal complement activation. Eculizumab induces accommodation of endothelial cells, reduces the formation of C5b-C9 (MAC) deposits in the transplanted kidney. Stegall et al.[22] reported the efficacy of eculizumab in 26 highly immunised patients with acute AMR. The incidence of AMR was significantly lower in the eculizumab group (7.7%) as compared to controls (41.2%); at 1 year, transplant glomerulopathy (TG) developed in 6.7% of patients receiving eculizumab vs. 35.7% of those who received no anti-C5 therapy. Eculizumab is not licensed for the treatment of AMR (indications include paroxysmal nocturnal haemoglobinuria and atypical haemolytic-uremic syndrome (HUS)). High cost (6,000 USD per one 300 mg vial) is another limitation for more widespread use.

Complement C1 Inhibitor

Another promising drug is the recombinant human complement C1 inhibitor (rhC1INH). It is presumed to inhibit the initial stage of complement activation via the classical pathway. The efficacy in preventing AMR has been demonstrated in chimpanzees. Phase I/II clinical trials are ongoing.[23]

Bortezomib

The largest number of recent literature reports concerning the treatment of AMR focus on bortezomib, a drug that targets plasma cells. Bortezomib is a small molecule, a tripeptide with an incorporated boron atom, which binds specifically to 26S proteasome. Bortezomib is a selective, reversible inhibitor of proteasome, an organelle containing proteases, whose role is the breakdown of proteins used throughout the cell's life cycle. Bortezomib inhibits the breakdown of pro-apoptotic factors and the cell is destroyed via the programmed cell death mechanism (apoptosis). The NFκB pathway plays a key role in the survival of memory B cells and long-lived plasma cells. NFκB pathway activation is controlled by the breakdown of its inhibitor (IκB) by the proteasome complex, and conversely, the suppression of NFκB is maintained by high levels of IκB induced by bortezomib. Bortezomib causes plasma cell depletion, thus decreasing the production of DSA. Bortezomib was

synthetised in 1995, and obtained FDA approval for the treatment of multiple myeloma in 2003. It is available as intravenous formulation. The product is 80% protein-bound, undergoes hepatic metabolism, with a half-life of 9-15 hours. The dosing is 1.3 mg/m^2/dose, four doses (day 1, 4, 7, 11). Major adverse effects include peripheral neuropathy (30% of patients), thrombocytopoenia and neutropoenia.[24] Everly et al.[25] demonstrated the efficacy of bortezomib in six kidney transplant recipients with recurrent AMR. Trivedi et al.[26] described the use of bortezomib in the protocol of tolerance induction in 11 living donor kidney graft recipients. Flechner et al.[27] used bortezomib (in combination with PP and IVIg) for the treatment of AMR in 20 recipients and obtained 85% graft survival after 10 months, 50% reduction in DSA, and significant effectiveness in the subgroup with better baseline kidney function (creatinine <30 mg/dL). Walsh et al.[28] showed better efficacy of bortezomib in early (<6 months) AMR in 13 kidney transplant recipients, as compared to 17 late AMR events; superiority manifested by a greater DSA reduction and improved morphological aspect of the graft. Waiser et al.[29] compared the outcomes of AMR therapy with bortezomib (1.3 mg/m^2 IV, day 1, 4, 8, 11) in 10 recipients with historical controls (9 patients who received a single dose of rituximab 500 mg) (all patients were given IVIg 30 g), and demonstrated a significantly higher efficacy of bortezomib at 18 months follow-up (graft loss 4/10 vs. 8/9). These preliminary results investigating the efficacy of bortezomib in the treatment of AMR are encouraging, but the outcomes of ongoing prospective randomised clinical trials are necessary to confirm them.

Splenectomy

The spleen is the largest lymphatic organ in humans and plays a major role in the production of alloantibodies. Splenectomy results in elimination of both precursor and mature DSA-producing plasma cells. The efficacy of splenectomy as rescue therapy for isolated cases of severe refractory AMR has been reported, however, due to the risk of infectious complications and the risk of surgery, it is not routinely recommended for the treatment of AMR.[30]

Chronic AMR

Risk factors for the development of chronic antibody-mediated rejection include acute AMR and pre-transplant immunisation. Hence, it is important to identify patients at high immunological risk, who are most likely to develop both acute and chronic antibody-mediated rejection. In chronic AMR, complement activation causes subclinical endothelial injury. However, slow immunological reaction leads inevitably to irreversible graft damage. Graft glomerulopathy being irreversible in the advanced stages, early detection of changes by DSA monitoring and protocol biopsies in high risk patients is justified. Theoretically, all acute AMR therapies could be useful in the treatment of chronic AMR, but there are practically no reports based on clinical trial evidence. IVIg, rituximab or bortezomib have been used in isolated cases. Therapies requiring continuous, repeated use, such as PP or eculizumab, are of limited value due to their high cost. Since TG-related changes are irreversible, the use of toxic therapies in chronic AMR cannot be justified as long as their efficacy is not confirmed in clinical trials. Preventing the development of AMR by adequate immunosuppression involving GS, tacrolimus and mycophenolate mofetil and monitoring of graft recipient is the key element.

CONCLUSION

To conclude, antibody-mediated rejection is relatively uncommon in kidney transplant recipients, but the risk of graft loss is high. Recently several promising therapies have emerged, most of them targeting B lymphocytes, plasma cells and complement (rituximab, bortezomib, eculizumab), but their efficacy should be confirmed in randomised clinical trials. Currently there is a risk of unjustified polypharmacy, severe infectious complications and high costs.[31] There is no single recommended regimen for the treatment of AMR. Many authors suggest to start with glucocorticoid pulses and primary immunosuppression involving prednisone, tacrolimus and MMF. First-line therapy consists in PP with IVIg 100 mg/kg bw (targeting 1 g/kg bw) after each PP session. If this proves ineffective, second-line therapy may involve rituximab (a single dose 375 mg/m^2) or bortezomib (four doses; 1.3 mg/m^2/dose), each dose preceded by plasmapheresis. Eculizumab or splenectomy may be considered as rescue therapy. DSA should be monitored weekly for 4-12 weeks, then once a month for 3 months. Increase in DSA levels is an indication for a repeat graft biopsy. Anti-thymocyte serum may prove effective in the presence of a steroid-resistant cellular rejection component.[32] These novel therapies cannot be used in Poland, as drugs such as eculizumab, bortezomib or rituximab are not licensed for use in transplantology.

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VASCULAR CALCIFICATION IN CHRONIC KIDNEY DISEASE: AN UPDATE

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ABSTRACT

Vascular calcification involves passive degeneration and an active process of arterial mineralisation, resembling osteogenesis. In chronic kidney disease, several proteins that physiologically control bone mineralisation, are also involved in the molecular and cellular mechanisms of the pathogenesis of vascular calcification. In fact, arterial cells grown in culture are induced to become osteogenic by inflammatory and atherogenic stimuli, such as high phosphate concentration. Mechanisms linking them must be considered in clinical decisions. Further understanding of processes causing vascular calcification may be considered for new therapeutic options for vascular disease in renal patients.

Keywords: Vascular calcifications, secondary hyperparathyroidism, phosphate, calcium.

INTRODUCTION

Patients with chronic kidney disease (CKD) develop vascular calcification (VC) much faster than the general population.[1] In particular, it has been widely demonstrated how CKD represents an independent risk factor of cardiovascular mortality and all-cause mortality. Vascular calcifications are not only the result of the mere passive process of crystal deposition, but also an actively regulated process that develops in response to physiological and pathological conditions.[2] Several risk factors play a key role in this rapid vascular ageing. They are divided into "classic" risk factors such as age, gender, dialysis vintage, inflammatory status, calcium-phosphate disorders, and diabetes,[3] and new "non-classic" risk factors such as bone-related proteins: fetuin-A (2-Heremans-Schmid glycoprotein, AHSG), matrix-carboxyglutamic acid protein (MGP), pyrophosphate, osteoprotegerin (OPG), and bone morphogenetic protein-2 (BMP-2). (Table 1) In addition, CKD promotes atherosclerosis.[6] In fact; the reduction of renal function promotes the development of an inflammatory status (increased levels of C-reactive protein) and lipid abnormalities that contribute to

INHIBITORS	PROMOTERS
Fetuin-A (2-Heremans-Schmid glycoprotein, AHSG)	OPG (Osteoprotegerin)
MGP (Matrix-GLA-Protein)	BMP 2/4 (Bone Morphogenic Protein 2/4)
Pyrophosphate	

Table 1. Inhibitors and promoters of vascular calcification.

endothelial dysfunction and vascular calcification. The prevalence and progression of vascular calcification increases dramatically once patients are on dialysis,[4] and the vascular phenotype of even young dialysis patients can be compared with that of octogenarians.[5] Vascular calcification starts developing in the early stages of CKD (stage III 25%, stage IV 35%) and is present in over 50% of patients at the time of dialysis.

PATHOPHYSIOLOGY

Ectopic vascular calcifications follow a very similar developing process to physiological bone formation. At sites of calcification, there is an up-regulated expression of mineralisation proteins, normally confined to bone and cartilage; this event induces osteo-chondrocyte-like changes in vascular smooth muscle cells (VSMCs).[7] These proteins include a number of transcription factors, such as Runx2 (Cbfa-1), Osterix, Msx2, and Sox9.[8,9] To create a microenvironment that is permissive for calcification, specialised membrane-bound bodies called matrix vesicles, serve as nucleation sites for hydroxyapatite.[9,10] VSMC-derived vesicles do not calcify until calcification inhibitors, such as Fetuin-A and MGP, are maintained in normal ranges. When calcification inhibitor levels are low, VSMCs produce mineralisation-competent vesicles that contain preformed hydroxyapatite. [10,11]

HIGH PHOSPHATE AND VASCULAR CALCIFICATION

Phosphate (P) homeostasis in normal subjects is regulated by intestinal absorption, renal excretion, and bone resorption. However, in subjects with CKD, P renal excretion is reduced. Nevertheless, P levels are maintained among normal limits by reducing P tubular resorption through increasing parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). In the same setting, reduction of intestinal phosphorous absorption happens due to the reduction of plasma levels of calcitriol.[12] At stages IV and V of CKD, dietary intake of P tends to exceed renal excretion capacity, resulting in hyperphosphataemia. Abnormalities in mineral metabolism have been claimed to be a causal factor for the development of vascular calcification in CKD patients.

Several studies have shown that high phosphorus levels stimulate the development of VC in an *in vitro* model of VSMCs.[13] *In vitro* studies have demonstrated that high phosphate concentration is responsible for VC formation through a specific activation of the core-binding factor alpha-1 (Cbfa-1), an osteoblast-specific gene that regulates the expression of several bone morphogenetic proteins.[14] In CKD, the expression of these proteins was also stimulated by uraemic patients' serum with normal serum phosphate, suggesting that uraemic milieu also has a role in CV pathogenesis.[15] Interestingly, calcified arteries from CKD patients showed an increased expression of both Cbfa-1 and osteopontin.[16] These data suggest that VC is an active process due, not only to calcium-phosphate salt deposition in artery wall, but also to a genomic regulation driven by uraemic environment and elevations in serum phosphate levels.

FETUIN – A

Human fetuin-A (AHSG, alpha2-Heremans Schmid glycoprotein, alpha2HS-glycoprotein, alpha2-HSG) is an extracellular calcium-regulatory protein acting as a potent inhibitor of calcium phosphate precipitation. It is a member of a family of four structurally-related plasma proteins containing cystatin-like protein domains. The cystatin family harbours type 1 (mainly intracellular proteins), type 2 (mainly extracellular proteins), and type 3 cystatins (plasma proteins). Cystatin domain 1 in fetuin-A is strongly negatively charged with a high affinity for calcium-rich minerals.[17]

Fetuin-A is one of the non-collagenous, most abundant proteins in bone, accounting for 25% non-collagenous proteins. Serum fetuin-A has an anti-inflammatory property; the demonstration that this protein specifically prevents neutrophils from activation by hydroxyapatite crystals, supports this issue.[18] Furthermore, anti-apoptotic activity of fetuin-A has been observed in smooth muscle cells.[19] Beside these findings, fetuin-A showed an important role in the mineralisation process.

Fetuin-A is responsible for mineral accumulation in bone from plasma, thanks to its high affinity for bone minerals, especially for nascent apatite mineral. For this reason, it is an inhibitor of *de novo* apatite formation from supersaturated mineral solutions, but it does not dissolve preformed minerals.[20] Specifically, fetuin-A binds calcium phosphate and calcium carbonate with high affinity. Haemodialysis patients with low serum AHSG levels have a major risk of CV and all-cause mortality.[21] This observation by Ketteler et al.[21] suggests that AHSG may be involved in preventing the accelerated extraskeletal calcification observed in CKD. A recent study in a

population of 115 haemodialysis patients supports this hypothesis, as VC was associated not only with increasing age and a history of cardiovascular events, but also with abnormal values of inflammatory markers, such as reduction in AHSG and albumin and an increase in C-reactive protein and fibrinogen.[22]

MATRIX GLA PROTEIN

Extracellular matrix GLA protein (MGP) is a member of the vitamin-K-dependent protein family, and it is a calcification inhibitor found in vascular and other soft tissue.[23,24] MGP promotes VSMC differentiation, antagonises BMP (BMP2 and BMP4) signalling and prevents osteochondrogenic lineage reprogramming of VSMCs. In mice, targeted deletion of the MGP gene results in rapid and complete arterial calcification, resulting in death by 6 weeks.[25] MGP is synthesised in the uncarboxylated form (ucMGP) and performs its action after vitamin K-dependent carboxylation. Without sufficient vitamin K, it remains decarboxylated and does not inhibit calcification.[26,27] The degree of γ-carboxylation required for MGP to inhibit calcification in humans is not known. Decarboxylated MGP form seems to be in high concentrations in calcified vessels, while carboxylated MGP form is more abundant in healthy vascular tissue.[28] This demonstrates that lack of functional MGP increases risk for vascular calcification. In addition to being carboxylated, MGP needs a post-translational phosphorylation, which is also thought to contribute to its functionality.[29] The phosphorylated ucMGP accumulates in a detectable amount in plasma.[30]

The role of MGP in vascular calcification has been elucidated in animal models, whereas in humans, data are conflicting. It has been suggested that the amount of ucMGP in the circulation is increased among patient populations characterised by pathologic soft-tissue calcification.[29-31] The studies that examined the association between plasma ucMGP and vascular calcification are limited to case-control comparisons or specific disease populations.[29-31] To evaluate the utility of ucMGP as a predictive marker of coronary artery calcification (CAC), it is necessary to examine a population free of clinical events.

In a randomised controlled trial with vitamin K supplementation, Shea et al.[32] found that older community-dwelling adults who adhered to phylloquinone (vitamin K1) supplementation showed less CAC progression over 3 years. The impact of MGP on regulation of calcification in humans appears to have a genetic component. An association between polymorphisms of the MGP gene and myocardial infarction has been described in low-risk individuals.[33] Furthermore, their distribution has proved to differ significantly in CKD/haemodialysis patients as compared to healthy controls, and particular alleles are associated with an increase in cardiovascular events in haemodialysis patients.[34] Potentially, the identification of polymorphisms of the MGP gene, and their association with cardiovascular morbidity, is a critical step towards the understanding of the pathogenetic mechanisms of VC in CKD and dialysis patients.

PYROPHOSPHATE

Isopentenyl Pyrophosphate (IPP), a well-known inhibitor of hydroxyapatite formation in urine produced by VSMCs, chondrocytes and osteoblasts, is an important inhibitor of vascular calcifications. Its reactive chemical nature suggests that it is a compound used to bind or deliver oxygen and phosphate at tissue level for rapid employment. Several intracellular enzymatic reactions are responsible for its production.[35] Plasma IPP is normally cleared by the kidney,[36] however, serum IPP levels in haemodialysis patients are reduced.[37] Furthermore, the calcification-inhibitory action of IPP *in vivo* is well-documented. However, its simple chemical composition and heterogeneous metabolism, as well as the local nature of its action, hinder the development of a preparation for the clinical setting.

OSTEOPROTEGERIN

Osteoprotegerin (OPG) belongs to the tumour necrosis factor receptor superfamily. It acts as a soluble decoy receptor for the receptor activator of nuclear factor-kappa B Ligand (RANKL), lying on osteoclast membrane, and inhibits its interaction with membrane-bound receptor RANK. Through this mechanism, OPG is able to inhibit osteoclasts differentiation. RANKL/OPG/RANK axis is not only involved in regulation of bone-remodelling,[38,39] but recent findings supported its role in carcinogenesis as well as central thermoregulation.[40,41]

This system has also been linked to the development of atherosclerosis and plaque destabilisation.[42,43] In observational studies, elevated circulating OPG levels have been associated with prevalence and severity of coronary artery disease, cerebrovascular disease, and peripheral vascular disease. Elevated OPG levels have also been associated with the degree

of coronary calcification in the general population as a marker of coronary atherosclerosis.[44] OPG is produced by osteoblasts but also by many different tissues and cell types, including the lung, kidney, intestine and endothelial cells. Its biological effects are still not completely understood, but it seems to be involved in apoptosis.[45]

In renal patients, increased levels have been associated with abnormal aortic calcifications in patients on dialysis[46] and with coronary artery calcifications in both dialysis and transplantation patients.[47] There is an association of serum OPG levels with all-cause and cardiovascular mortality, both in patients on dialysis[48] and in transplantation patients.[49]

BONE MORPHOGENIC PROTEINS

Bone morphogenic proteins (BMP) are secreted polypeptides, a subgroup of the transforming growth factor-beta (TGF-ß) superfamily of growth factors. BMPs were first identified in demineralised and pulverised bone powder capable of inducing ectopic endochondral bone formation in muscle.[26] Over the years, more than 15 distinct BMP family members have been identified. In 1993, Bostrom, Demer and colleagues first demonstrated the expression of BMP2 in calcified human atherosclerotic plaques, and the capacity of BMPs to direct osteogenic programming of vascular mesenchymal progenitors of the pericyte lineage. When BMP2, BMP4, and BMP6, were detected in calcified areas of atherosclerotic lesions,[50-52] it was therefore presumed that they enhanced vascular calcification, even more so when it became evident that vascular calcification is largely driven by osteogenesis in the vascular media.[53,54] However, BMP signalling is not only driving ectopic calcification but is also essential for cardiovascular development, with critical roles in the establishment of endothelial cells during vasculogenesis, the recruitment and differentiation of VSMC precursor cells, and vascular patterning.[55,56] BMP activity is important for the regulation of phenotypic plasticity, proliferation, and differentiation in VSMC.[52] BMP2 in particular has an inhibitory effect on VSMC proliferation and differentiation, whereas BMP7 promotes the VSMC phenotype transformation.[54] Furthermore, BMP inhibition, potentially in later steps, appears to be a key actor in maintaining VSMC differentiation. Many are the causes that promote increased levels of BMP. Among these, endothelial activation in response to pathogenic stimuli, such as inflammatory cytokines and shear stress, appear to play a key role in regulating serum levels of BMP.[57,58]

CONCLUSIONS

The astonishing mortality rate due to cardiovascular events in CKD has led to a great effort to identify causes and new potential strategies to improve survival in CKD. It seems that bone mineral abnormalities play a major role in inducing and sustaining cardiovascular damage in CKD. Improving understanding of cellular and molecular mechanisms of vascular calcification in CKD will give major tools to the clinicians to evaluate and choose treatments.

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'A BETTER WAY TO MEASURE CHOICES' DISCRETE CHOICE EXPERIMENT AND CONJOINT ANALYSIS STUDIES IN NEPHROLOGY: A LITERATURE REVIEW

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ABSTRACT

Discrete choice experiments (DCE) and conjoint analysis (CA) are increasingly used to address health policy issues. This is because the DCE and CA approaches have theoretical foundations in the characteristics theory of demand, which assumes goods, services, or healthcare provision, can be valued in terms of their characteristics (or attributes). As a result, such analysis is grounded in economic theory, lending theoretical validity to this approach.

With DCEs, respondents are also assumed to act in a utility-maximising manner and make choices contingent upon the levels of attributes in DCE scenarios. Therefore, choice data can be analysed using econometric methods compatible with random utility theory (RUT) or random regret minimisation (RRM) theory. This means they have additional foundations in economic theory. In contrast, analyses described as CAs are sometimes compatible with RUT or RRM, but by definition they do not have to be.

In this paper we review the CA/DCE evidence relating to nephrology. The CA/DCE approach is then compared with other approaches used to provide either quality of life information or preference information relating to nephrology. We conclude by providing an assessment of the value of undertaking CA or DCE analysis in nephrology, comparing the application of CA/DCEs in nephrology with other methodological approaches.

Keywords: Renal, nephrology, conjoint analysis, discrete choice experiments, literature review, transplantation, dialysis.

BACKGROUND

Table 1 provides an example of attributes and levels for conjoint analysis/discrete choice experiments (CA/DCE),[1,2] whilst figure 1 is a DCE question used for it. DCE and CA methods involve the characteristics theory of demand.[3] By definition, DCEs must conform to either random utility theory (RUT) or random regret minimisation (RRM).[4,5] However, sometimes CAs do not conform to RUT/RRM, so such analysis may not be rigorously grounded in economic theory. This is because although CAs do conform to Lancaster's characteristic's theory of demand, they may not conform to RUT or RRM theory, whereas DCEs do.

If attributes are significant, data analysis confers information relating to how average the respondent's utility, or willingness to pay (WTP), is affected by changes in the levels of attributes, and the impact of different attribute levels upon choice can then be quantified. This information can help decide which

patient groups ought to be prioritised for kidney transplants, when dialysis should be provided, or which modality is used.

METHODS – LITERATURE SEARCH

We conducted PubMed literature searches using keywords 'discrete choice experiment' or 'conjoint analysis' and 'renal transplant' or 'renal dialysis', reviewing DCE/CA papers identified. We also included a DCE conference abstract.[6]

RESULTS

The literature searches revealed four papers, for three studies involving DCE/CA for renal transplantation.[1,2,7,8] Other DCE/CA studies (five papers, for four studies) relate to dialysis.[8-12] Preliminary findings (conference abstract) about a dialysis DCE,[6] are also considered. A summary of some key features of these studies is provided in Table 2.

Renal Transplant DCE/CA Analyses

A Canadian renal transplant DCE[8] of 150 healthcare professionals, 169 patients, and 32 caregivers involved decisions about end-of-life care in chronic kidney disease. The DCE had six attributes, two related to transplantation: 'how should live kidneys for transplantation be obtained?' (levels of first come first served, or best match); and 'how should live kidneys for transplantation be obtained? (levels of family member, paired kidney exchange, anonymous donor, or buy a kidney). Other attributes included 'who provides comprehensive day-to-day care; when should end-of-life care discussions be started; how much information on prognosis end-of-life care issues should be provided, and how should decisions to stop dialysis be made?'. Findings suggested patients/healthcare professionals preferred detailed prognostic information, and shared to individual care planning, and co-ordinated care. For transplants they preferred 'best match' to 'first-come first served' allocation criteria, whilst donor donation by family members/friends was most preferred followed by an unknown donor, paired kidney exchange, and buying a kidney.

A USA study[7] involved a CA of 175 patients. It looked at preferences for accepting a kidney from donors at increased risk of blood-borne viral infection (DIRVI). It considered the influence of HIV infection risk, donor age, and transplant waiting time (years). Key findings were that 24% of respondents would not accept a DIRVI kidney at all, 58.9% would under some circumstances, and 17.7% always accepted DIRVI kidneys. Patients would be more likely to accept a DIRVI kidney when waiting times were longer, donors were younger, and when HIV infection risk was lower. Dialysed patients and older patients were more likely to accept a DIRVI kidney. This is a CA, not a DCE, and so results may not be compatible with RUT/RRM, and may not be as rigorously grounded in microeconomic theory.

A UK renal transplant DCE study,[1,2] assessed preferences of 908 patients, 113 healthcare professionals, 48 live donors/relatives of deceased donors, and 41 carers. Attributes and levels are shown in Table 1.

Patients highly valued transplant waiting time and quality of tissue match, prioritising those with child or adult dependents and transplanting younger adults. Those with moderate diseases affecting life expectancy were prioritised over those with severe diseases, but those with moderate diseases affecting length of life were not prioritised over those without other disease(s). Patients prioritised those with moderate rather than no disease, and moderate rather than severe disease affecting quality of life. Ethnic minority patients did not prioritise recipients with close donor-recipient tissue matches, as this disadvantages them.

Healthcare professionals valued allocation based on tissue match less than patients, but valued prioritising those with dependents more. They also valued prioritising those with no diseases rather than moderate diseases affecting life expectancy, whereas patients did not. Healthcare professionals also valued prioritising those with severe rather than moderate diseases affecting quality of life more than patients. Carers did not value prioritising those with better tissue matches or those with dependents. However, they valued prioritising those with moderate, not severe, diseases affecting life expectancy more than patients. The live donors/relatives of deceased donors group did not value prioritising kidney transplants on the basis of tissue match. However, they valued transplants to those with dependents, younger recipients, and to those with moderate rather than severe diseases affecting life expectancy, more than patients.

Results are broadly in line with current UK renal transplant policy, but another criterion (whether recipients had adult or child dependents) was also valued, and differences in ethnic minority patient preferences were highlighted.

Attribute	Description of attribute in questionnaire preamble	Levels for the attribute that appear at different levels across questionnaire questions
A) Amount of time a person has waited for a kidney transplant	Timescales for people receiving a transplant after being placed on a waiting list are likely to differ. The waiting time could be (see next column)	1) 1 month. 2) 2 years. 3) 10 years.
B) Tissue type matching – and likelihood of transplant success	This affects the likelihood of a transplant proving to be successful. Below are the up-to-date figures from UK Transplant for the survival of all transplants in the UK. There are 6 main tissue types used in matching. A perfect tissue type match is all 6 types matching; favourable is 4-5 out of 6 matching, non-favourable is less than 4 matching. If a transplant fails the patient will return to renal dialysis (see next column)	1) Non-favourable tissue match (86% average survival rate of the kidney 1 year after the transplant). 2) Favourable tissue match (89% average survival rate of the kidney 1 year after the transplant). 3) Perfect tissue type match (90% average survival rate of the kidney 1 year after the transplant).
C) How many dependents (either children or adults) recipients have	Some respondents might consider that those who have dependent children, or others who are dependent either because of their age or a physical or mental handicap, ought to be prioritised for kidney transplant. So we assume that respondents might have (see next column)	1) No dependents. 2) 1 dependent. 3) 4 dependents.
D) Recipient age	The recipient could be aged either (see next column)	1) 20 years. 2) 45 years. 3) 65 years.
E) Diseases affecting life expectancy	As well as having kidney failure, someone may have other conditions prior to kidney transplantation which affect their life expectancy. Some of the conditions which reduce life expectancy may occur in young people, and some older people may be entirely healthy apart from kidney disease. We assume these could be either (see next column)	1) None. 2) Moderate diseases (uncontrolled hypertension or obesity). 3) Severe diseases (heart attack, stroke, or diabetes with complications).
F) Other recipient illnesses	Someone with kidney failure may have conditions other than kidney failure, which are not life-threatening but do affect their quality of life. Respondents might or might not wish to allocate kidneys according to such conditions. Examples would be (see next column)	1) Healthy except for kidney disease. 2) Kidney disease with a condition that sometimes affects their activities, such as mild asthma. 3) Kidney disease with a condition that affects their activities on a daily basis, such as severe arthritis.

Table 1. Details of attributes and levels used in the UK renal transplant discrete choice experiments (DCE) preference study.

NOW PLEASE READ DESCRIPTIONS OF OPTION A AND B AND INDICATE WHO YOU THINK SHOULD BE PRIORITISED FOR A KIDNEY TRANSPLANT – PATIENT A OR PATIENT B?:

	Patient A	Patient B
Amount of time a person has waited for a transplant	1 month	2 years
Tissue type match – and likelihood of transplant success	90% average 1 year chance of transplant success	86% average 1 year chance of transplant success
How many dependents (children or adults) recipients have	4 dependents	No dependents
Recipient age	20 years	45 years
Diseases affecting life expectancy	Moderate: Uncontrolled hypertension or obesity	Severe: Heart attack, or stroke, or diabetes with complications.
Other recipient illnesses (other than kidney disease)	Mild asthma	Severe arthritis

Which patient would you choose? (tick 1 box only) Patient A ❑ Patient B ❑

Figure 1. Details of the instruction appearing over questions, followed by the first discrete choice experiments (DCE) question used in the UK renal transplant DCE preference.

Dialysis DCE/CA Analyses

The aforementioned Canadian DCE,[8] included the attribute 'how should decisions to stop dialysis be made?' Findings revealed a preference for joint, over personal, decision-making for healthcare providers, patients, and caregivers.

A USA analysis[10] of 126 patients looked at switches from conventional dialysis (three times a week) to daily haemodialysis (six treatments of 2-3 hours weekly) to improve dialysis outcomes. This study included four attributes: life expectancy (levels of 6, 8 or 10 years), quality of life on a 1 to 10 scale (levels of 5, 7 and 9), annual number of hospitalisations (levels of 3, 2, and 1), and transport time (levels of transportation time which were 3, 2, or 1 times the reference case [i.e. travel time for conventional haemodialysis]). People were more willing to switch to daily dialysis when the regimen offered increased life expectancy and better quality of life, and when there were larger decreases in hospitalisations and transport time.

Another DCE[9] about nephrology facilities in Greenland (Denmark), estimated willingness-to-pay (WTP) for dialysis amongst 206 members of the general public. Its three attributes included numbers of specialists (levels of many specialists, one specialist, or monthly visiting specialist), accommodation for patients (levels of patient hotel for Greenlandic people, patient hotel in Nuuk, or own apartment in Nuuk), and increased annual tax per person (levels of 0 DKK, 50 DKK, and 700 DKK). Findings suggested that alternatives involving treatment in Greenland were chosen in around two-thirds of cases, implying a tendency to favour treatment in Greenland even when it involves increased tax. Also WTP for patient hotel accommodation was higher than apartment accommodation, or having a permanent specialist. The authors concluded that Greenlanders' perception of self-dependence and their attitude to health services in Denmark strongly impacted upon findings.

An Australian DCE study[11,12] involved well-conducted qualitative analysis to inform the attribute and levels selected.[13-15] One of the two DCE papers published by these authors surveyed 105 patients with stage 3-5 renal disease, evaluating whether patients preferred dialysis or conservative treatment. Attributes included differences in: life expectancy, weekly hospital visits, ability to travel, hours attached to a dialysis machine, treatment timing, availability of subsidised transport, and flexibility of dialysis schedules. Findings suggested patients were more likely to choose dialysis than conservative care if dialysis increased average life expectancy, if they

Study focus and authors	Country and type of study (DCE/CA)	Sample size	Type of attributes
End-of-life care in chronic kidney disease – kidney transplant. Davison et al.[8]	Canada, Renal transplant and dialysis (DCE)	169 Patients, 150 Healthcare professionals, 32 Caregivers	a) For whom should live kidneys for transplantation be provided? b) How should live kidneys for transplantation be obtained? c) Who provides comprehensive day to day care? d) How much information on end of life issues should be provided? e) How should decisions to stop dialysis be made?
Acceptability from donors at increased risk of blood-borne viral infection. Reese et al.[7]	USA, Renal Transplant (CA)	175 Patients	a) HIV infection risk b) Donor age c) Transplant waiting time (years)
Renal transplant prioritisation by stakeholders. Clark et al.[1,2]	UK, Renal Transplant (DCE)	908 Patients 113 Healthcare professionals 48 Live donors/ relatives of deceased donors 41 carers	a) Waiting period b) Tissue type matching c) Dependents d) Recipient age e) Diseases impacting life expectancy f) Other illnesses
Willingness to switch from conventional to daily haemodialysis. Halpern et al.[10]	USA, Dialysis (DCE)	126 Patients	a) Life expectancy b) Quality of life c) Number of hospitalisations d) Transport time
Willingness to pay for dialysis. Kjaer et al.[9]	Denmark, Dialysis (DCE)	206 People	a) Numbers of specialists b) Accommodation for patients c) Increase in annual tax per person
Dialysis modality preference of patients with CKD. Morton et al.[11] Factors influencing patient choice of dialysis versus conservative care. Morton et al.[12]	Australia, Dialysis (DCE)	105 Patients 73 Caregivers	a) Life expectancy b) Weekly hospital visits c) Ability to travel d) Hours attached to a dialysis machine e)Treatment timing f) Availability of subsidised transport g) Flexibility of dialysis schedule
Preferences for home-based haemodialysis or peritoneal dialysis or hospital-based haemodialysis. Higgins et al.[6]	UK, Dialysis (DCE)	663 Patients	a) Frequency of dialysis b) Quality of dialysis c) Type of care d) Timing of dialysis e) Dialysis costs to NHS

Table 2. Salient features of discrete choice experiments/conjoint analysis renal transplant and dialysis studies included in review

could dialyse during the day or evening not just the day, and if subsidised transport was available. Patients were less likely to choose dialysis if more visits to hospital were required, or with more restrictions on patient travel.

The other DCE published by these authors reports from the same study, but also reports results for 73 caregivers. Findings suggest home-based dialysis (either peritoneal or haemodialysis) was chosen by patients in 65% of cases, whilst the in-centre dialysis option was chosen in 35% of cases, and conservative care in 10% of cases. For caregivers, figures were 72%, 25%, or 3% respectively. Patients and caregivers preferred longer to shorter hours of dialysis, but were less likely to choose nocturnal than daytime dialysis. Patients would trade-off 23 months of life expectancy with home-based dialysis to decrease their travel restrictions, amongst caregivers the figure was 17 months.

The UK dialysis DCE[6] involved comparison of preferences for different modes of dialysis amongst 636 patients, comparing home-based peritoneal dialysis, unit-based haemodialysis, and home-based haemodialysis. Attribute levels, which included frequency of dialysis, quality of dialysis, level of care (self care, shared care, or professional care), timing of dialysis, and the costs to the NHS of dialysis varied across the labelled choices in line with feasible ranges for the modes of dialysis. Findings suggested that compared to dialysis unit haemodialysis, home peritoneal dialysis was valued positively, whilst home haemodialysis was valued negatively. Increasing dialysis frequency was negatively valued, but positively valued if it raised dialysis quality. More flexible dialysis timing was positively valued, whilst cost of dialysis provision to the NHS was statistically significant, but not highly valued.

DISCUSSION

DCE/CA may be more rigorous than other methodologies. For example, if the alternative is a series of questions about how a respondent ranks options independently on a scale,[16-18] or involves preference ranking exercises[19] or choice scenarios.[20,21] This is because DCE/CA provide a robust method for measuring the preferences of stakeholders in dialysis and transplantation compatible with the characteristics theory of demand.

Other approaches to evaluation in nephrology involve assessment of health-related quality of life.[22-28] This may involve the use of validated quality of life instruments.[29] However, such approaches have the disadvantage that they do not establish what patients prefer, which CA/DCE analysis does. Some analyses have tried to establish revealed preferences i.e. by looking at the characteristics of patients who do or do not switch dialysis therapy.[30,31] However, if such switches are driven by preferences, it is unclear whether they are renal physicians or patient preferences. In contrast, because DCEs can be used to compare the preferences of different stakeholder groups, differences in preferences between groups can be ascertained.[1,2,8,11]

Some other studies have tried to establish how respondents stated preferences for dialysis are related to patient characteristics,[17,32] and one study has looked at how nephrologist preferences for dialysis vary according to their characteristics.[33] Moreover, there are preference studies that do not involve DCE/CA methods, e.g. relating to establishing priority criteria for renal transplants.[21,34,35] However, a limitation of these types of studies is that they may fail to take into account the fact that preferences vary according to the characteristics of the different modes of dialysis, or may fail to robustly establish trade-offs between conflicting allocation criteria for kidney transplants because preferences are not related to attributes. In contrast, CA/DCE preference studies can establish how preferences are related to attributes of dialysis provision[6,9,10] or different priority criteria for transplants.[1,2,8]

Also, whilst there can be a useful role for using qualitative analysis to establish preferences,[13,14] there may be a very strong case for using such analysis to design DCEs[11] rather than to replace DCE analysis. When the authors of this paper have been involved in producing DCE questionnaires, both in relation to our transplant DCE,[1,2] and also our dialysis DCE,[6] we have been very keen to conduct rigorous qualitative analysis to determine the attributes and levels for the questionnaires before conducting the final DCE analysis. This adds enormous value over more traditional questionnaire approaches which may exclude this design step, and instead chooses attributes based upon researcher's preference. Moreover, if analyses are DCEs not CAs, the analysis will also be strongly grounded in RUT or RRM, as well as the characteristics theory of demand, so measured preferences should equate to respondents' actual preferences. One exception to this may be if an attempt is made to establish WTP.[36] A particular problem is that if WTP is calculated when healthcare provision is free at the point of use, choices posed

may lack realism. So valuations for the monetary attribute may be subject to hypothetical bias, biasing estimates of WTP. This is why, when we conducted our renal transplant DCE[1,2] we did not incorporate a monetary attribute. Moreover, for our dialysis DCE we did not present results in terms of WTP, instead valuing changes in other attributes versus increased dialysis frequency.[6] One analysis reviewed here[9] which calculated WTP may be biased because of a range of problems associated with calculating WTP using DCEs.[36] These include whether estimated WTP obtained via DCEs may be sensitive to factors including: the range specified for the monetary attribute, or the presence or absence of payment per se;[37] or the presence of a positive cost, rather than the level of cost indicated by the monetary attribute;[38] or the placement of the monetary attribute in the list of attributes.[39] Other evidence suggests that the way attributes are 'framed' may impact upon estimated WTP.[40]

CONCLUSIONS

CAs and DCEs have the disadvantage of being more expensive to design, deliver and analyse than simple questionnaires, but extra expense may be justified if results are more meaningful/robust. Moreover, in renal transplantation, DCE results endorse factors such as waiting time and tissue type match as criteria for organ allocation, and raise important questions about whether recipient co-morbidity and recipient social responsibilities should be included in organ allocation systems,[1,2] and about ethnic minority preferences. For dialysis, DCEs have yielded important information about when dialysis is indicated, and about preferences for different modes of dialysis.

It is difficult to provide an overview of all the potential additional applications of DCEs in nephrology, as DCEs could be used to address a wide range of additional nephrology research questions. However, if more studies are undertaken, in the interests of methodological rigour, they should be DCE analyses (not CA analyses which are incompatible with RUT/RRM). We would also point out that it is important to use rigorous qualitative analysis in the first instance in a pilot analysis, in order to establish what the attributes and levels specified in the DCE questionnaires are, before a DCE is conducted.

One possible future application of DCEs might be to establish healthcare professionals/patients' preferences relating to different characteristics for patient education in relation to dialysis provision. Also, there are other areas of self-management and therapy choices in nephrology, especially over non-dialysis care of the frail elderly, where being able to ask patients, professionals, and carers DCE-type questions would be useful.

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IS THERE AN INTEREST IN IMPLEMENTING A MULTIDISCIPLINARY CLINIC OR RENAL CARE NETWORK TO IMPROVE THE PROGNOSIS OF PATIENTS WITH CHRONIC KIDNEY DISEASE?

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ABSTRACT

Chronic kidney disease (CKD) is highly prevalent in several countries and is associated with the incidence of end-stage renal disease (ESRD) and also with premature morbidity and mortality, especially from cardiovascular origin. However, efficient treatments have existed for two decades but have not led to major decrease in either ESRD incidence or premature death of CKD patients. Some authors suggested that the deliverance of suboptimal care can explain, at least partly, these disappointing findings. Several observational studies support this idea by showing that some recommended medications are under-prescribed in CKD patients, and that some patients are sometimes insufficiently monitored for clinical and biological parameters. Therefore, new models of renal care deliverance have been developed, trying to optimise patient treatment with the hope that it could positively impact their outcomes. In this article, we will focus on the multidisciplinary clinic and the renal care network models and we will review the results of the main studies that sought to test the impact of these new structures on patient's prognosis. Although most of these studies are observational, they predominantly show a positive effect on renal prognosis and also survival. However, the only one randomised clinical trial with long-term follow-up failed to find any positive effect despite increased cost. Therefore, more evidence, based on results of randomised clinical trials, is needed before a wide implementation of this kind of program.

Keywords: Chronic kidney disease, quality of care, multidisciplinary care model, renal care network.

INTRODUCTION

Chronic kidney disease (CKD) is an increasingly prevalent progressive disease that ultimately leads to the requirement of chronic renal replacement therapy (RRT). This treatment is associated with high morbidity and mortality and a significant reduction in the quality of life for patients. Furthermore, this is an expensive care that has to be supported by the health care system. Therefore, the prevention of the progression to end-stage renal disease (ESRD) and of the premature deaths of CKD patients is of paramount importance and has become a public health issue.[1] Clinical studies have been led to find an efficient treatment to improve CKD patient prognosis.[2,3] Nevertheless, there has been no significant decrease in the incidence of ESRD in developed countries over the last 10 years.[4,5] Moreover, even if the mortality rate of non-dialysis CKD patients tends to decrease, it remains higher comparatively to non-CKD patients in the USA.[4] Although several factors may be involved, some authors emphasise the role played by suboptimal care delivered to CKD patients.[6] The inappropriate coordination of care, the lack of communication between the healthcare professionals (HP), and a certain degree of therapeutic inertia might explain the deliverance of suboptimal care.[6,7] This has led nephrologists to question how to provide multidisciplinary care in an efficient way to CKD patients. In this process, two different models have emerged: the multidisciplinary clinic (MDC) and the

dedicated renal care network (RCN). In this review, we will focus first on the recent Kidney Diseases Improving Global Outcomes (KDIGO) guidelines[8] about non-dialysis CKD management and then on the results of studies that sought to evaluate the quality of care received by CKD patients. We also focus on other studies which have found a link between this quality of care and patient prognosis. Finally, we will talk about the results of studies that aimed to test the impact of MDC or RCN on CKD patient outcomes.

What Do the KDIGO Guidelines Tell Us About Nephrologist Referral and the Model of Care?

The most recent clinical guidelines about the evaluation and management of CKD came from the KDIGO foundation and were published in January 2013.[8] An entire chapter has been dedicated to the clinical situations justifying a specialist consultation. Additionally, in this chapter some recommendations have been made about the model of care that should be used to optimally treat patients with progressive CKD. Amongst the situations that should lead to a nephrologist consultation we find the occurrence of acute kidney injury or of a rapid decrease of glomerular filtration rate (GFR), the progressive nature of CKD or the existence of a persisting proteinuria or haematuria. Regarding the level of estimated GFR (eGFR) which justifies specialist referral, irrespective of the presence of other factors, the work group advises an eGFR of less than 30 ml/min per 1.73 m^2. The level of evidence assigned to these guidelines is moderate. Moreover, the work group stresses the importance of evaluating the risk of requiring renal replacement therapy in the next 12 months for patients with progressive CKD, and in cases of a risk above 10-20%, they recommend referral of the patients. The level of evidence is moderate for this recommendation and relies on several studies and two meta-analyses. The benefits of timely referral of patients for preparing RRT are based on mortality (the 1 year mortality was decreased by more than 50%), morbidity (shorter length of stays in the hospital) and on the cost of the care. Finally, the work group also suggest that patients with progressive CKD should be managed in multidisciplinary care framework (level of evidence moderate) with access to dietary counselling, education, and psychological and social care. However, they did not suggest any particular model that would be the most suitable for reaching care appropriateness and state that the implementation of this structure may be customised to specific circumstances. As a conclusion, it can be said that these recommendations clearly express the clinical situations requiring a nephrologist referral, emphasise the crucial matter of the timely referral of patients with progressive CKD, and underline, for the first time, the importance of multidisciplinary care to reach the clinical targets enabling to ameliorate patient outcomes.

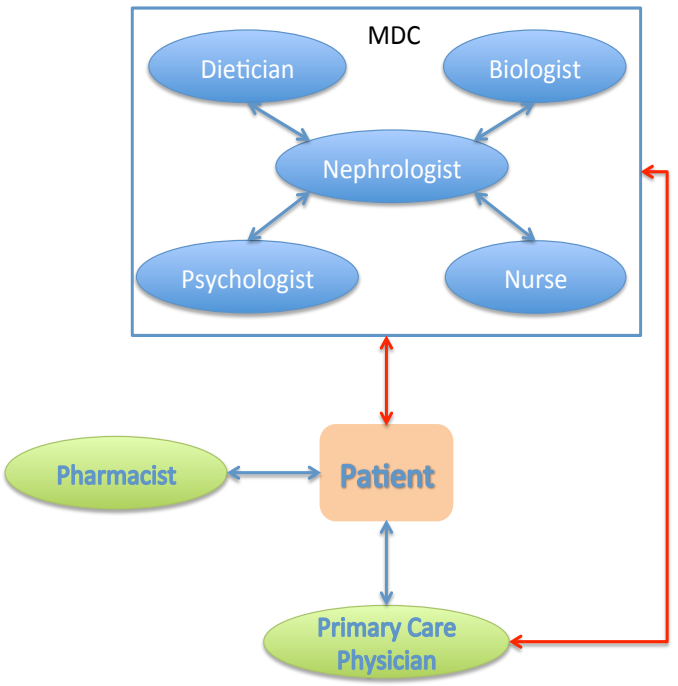

Figure 1. Multidisciplinary care model for chronic kidney disease patients. Arrows represent the interaction between the healthcare professionals and the patient.

Care of CKD Patients Could Be Optimised

Several studies showed that care for CKD patients could be optimised. Kausz et al.[9] showed, in an observational study of 600 patients with average eGFR of 22.2 ml/min/1.73 m^2, that both the frequency of biological parameters monitoring and of recommended therapeutic agents prescription were low in CKD patients. These findings were associated with a low proportion of patients achieving targets for the control of anaemia and calcium/phosphate disorders. In another large sample of American patients with moderate to severe CKD, Nissenson et al.[10] found that the prevalence of patients with an angiotensin-converting-enzyme inhibitor (ACEI) prescription ranged from 5% to 59% (although the proportion of CKD patients in USA with hypertension is 74% according to the US renal data system (USRDS) report).[5] Interestingly, there were no differences in ACEI prescription rates between patients who visited a nephrologist and

those who did not. Finally, they also found a low prevalence of Erythropoietin (EPO) prescription (7.4%) despite 36.2% of patients with haematocrit below 33%.[10] When we focus on the cardioprotective treatments prescription in CKD patients, the rate of prescription of this kind of treatments is also low. In a Canadian study, which included 304 patients with creatinine clearance <75 ml/min, the authors focused on the prescription rate of cardioprotective treatments. They found that cardiovascular disease (CVD) history was present in 38.5% of the patients and that several cardiovascular risk factors were highly prevalent (hypertension 80%, diabetes 37.5%, hyperlipidemia 43.4%). Nevertheless, they also found a low prescription rate of aspirin (45.3%), renin-angiotensin system (RAS) blocker (63.2%) and beta-blocker (50.4%). Moreover, among patients with known hyperlipidaemia, statin was prescribed in only 49%.[11] Taken together, these results suggest that the care of CKD patients could be improved at several levels: monitoring of biological parameters, renoprotective and cardioprotective treatments, and treatment of the metabolic complications of CKD.

Beside data about the care of the general population of the CKD patients, some investigators have studied specific populations at increased risk of adverse outcomes. Kausz et al.[12] studied the issue of care adequacy in a subset population of American patients aged over 67 years. In this study, the authors found that a low proportion of diabetic patients with CKD had been tested for HbA1c assay or fundus examination during the two year period before dialysis started (75% and 60% respectively). In addition, the proportion of CKD patients having been properly tested for anaemia condition (iron studies and research of occult blood in stool) and parathyroid hormone (PTH) was low (less than 50% and 15%, respectively), with an even lower proportion in the population of patients subsequently treated by haemodialysis. Lastly, the results of this study show that CKD patients were less frequently screened for cancer and were also less likely to receive some immunisations, despite a more frequent monitoring for hyperlipidaemia and heart conditions.

Several studies have also focused on the care delivered to CKD patients with heart failure (HF), a population that raises particular concern due to a growing prevalence of HF in American CKD patients, and a high mortality rate.[5] Furthermore, this population is also of interest because the prescription of some medications is recommended by both the guidelines for treatment of CKD and those for HF treatment (leading to expectation of a higher prescription rate of these treatments). In 2004, Ezekowitz et al.[13] analysed data from the Canadian prospective cohort APPROACH (6,427 patients with HF and with coronary artery disease ascertained by angiographic sutdy) in which 39% of the patients had creatinine clearance below 60 ml/min. The comparison of three groups categorised according to the level of eGFR (>60 ml/min, between 30 and 60 and <30 ml/min) showed that the proportion of patients receiving aspirin or other antiplatelet agents, beta-blockers, RAS blockers and statins lowered as renal function decreased. In patients with eGFR below 30 ml/min, the prescription rates of aspirin, beta-blockers, and RAS blockers were respectively 67%, 52% and 52%. In another Canadian cohort of patients with CKD and HF, McAlister et al.[14] also showed that the CKD patients were less frequently treated with certain therapeutic agents like ACEI, beta-blockers and spironolactone while they were receiving diuretics more often. The rate of prescription of RAS blockers and beta-blockers were respectively 75% and 34% in patients with CKD stage 4 or 5 while it was 92% and 57% in patients with eGFR >90 ml/min.[14] Taken together, these findings suggest that the prescription rate of some pivotal drugs for HF and CKD treatment, like ACEI, are quite low. In addition, other recommended types of drugs are probably under-prescribed as well. Therefore, it is likely that patients with both CKD and HF are more prone to suboptimal treatments. Thus, there is an opportunity for improvement that could possibly lead to less morbidity and mortality in these patients.

Impact of the Quality of Care on Patient Outcomes

The first step in order to improve outcomes of patients with advanced CKD seems to be the referral to a nephrology consultation. This is especially justified in the 1 to 2 year period before starting dialysis, when patients request adequate preparation for dialysis treatment. It is well known that late referral of patients leads to start acutely RRT, which is largely detrimental for early outcomes in dialysis.[6] The importance of the nephrologist visit has been underlined by two large observational and retrospective studies from the DOPPS that showed a 35% decrease of patient mortality during the first 4 months of dialysis treatment in patient groups that have been followed by a nephrologist[15] and a 43% decrease of mortality in the first year.[16] In patients with less severe CKD, Jones et al.[17] showed that nephrology referral allowed a decrease in the renal disease progression

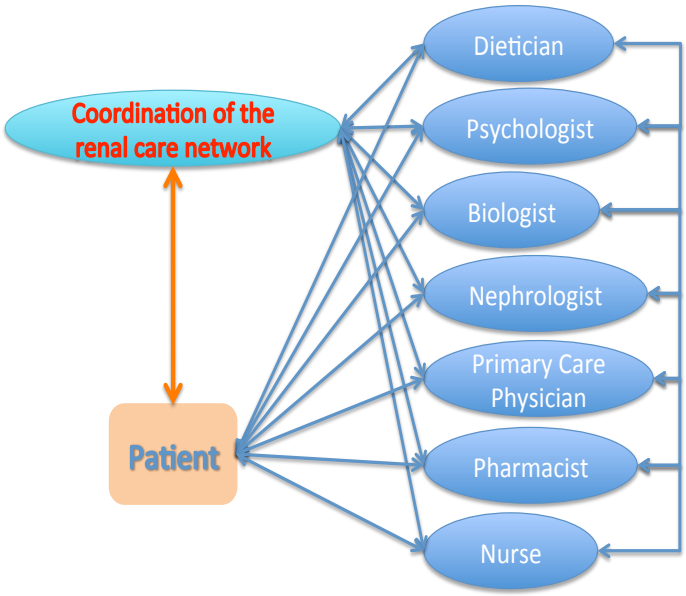

Figure 2. Renal care network model for chronic kidney disease patients. We can note the existence of a structure dedicated to the care coordination and the manifold interactions between the healthcare professionals and the patient.

and that this effect was associated with significant improvement in patient survival over a 1 year period. However, if we refer to some results of previously cited studies about CKD patient care, some further improvement seems possible, even in the case of a nephrologist follow-up.

In France, Thilly et al.[18] conducted the AVENIR study, which aimed specifically to study the impact of pre-dialysis care on early patient outcomes after dialysis start. In one published report, they showed that physicians failed to reach recommended blood pressure and proteinuria level in a high proportion of CKD patients in the year before starting dialysis (respectively 75% and 85%) and that this result was not modified when the patients had been followed-up by a nephrologist. Although there was more intensive treatment of patients when a nephrologist followed them, the lack of differences can be explained by a trend to preferentially address the most severe patients to a nephrologist. In another study, this team aimed to describe the proportion of patients reaching the clinical target for the control of the metabolic complications linked to CKD. The clinical objectives were achieved in a variable proportion of cases, sometimes low (16.7% to 72.4% depending on parameters considered) and 12.2% of the patients have been considered as receiving low quality care. In addition, the authors showed a low prescription rate of some medications (vitamin D, bicarbonates, statins) that has probably contributed to the previous findings. Even more interesting was their observation that, after adjustment, the quality of care and the number of nephrology consultations were positively related.[19] Finally, they have recently shown that the quality of care was also positively related to the patient's 1 year survival on dialysis.[20] Taken together, these results show the impact of the care provided by the nephrologist (that could theoretically lead to better survival) and suggest that the quality of care could be related to the number of nephrology consultations. Interestingly, this research group also found that the quality of care and the time since referral to a nephrologist can independently impact the quality of life (QoL) of patients at dialysis onset.[21] This is an important finding because it is known that the QoL of patients with severe CKD is greatly impaired[22] and that few interventions are available to improve it.

Two Models of Multidisciplinary and Coordinated Care to Improve the Prognostic of CKD Patients

The first model is generally called multidisciplinary clinic (MDC), but also can be named 'low-clearance clinic'. In this model, every kind of HP involved in the treatment of CKD patients is in the same place (nephrologists, dieticians, nurses, pharmacist etc.). MDC is commonly intended for patients with advanced CKD that require enhanced monitoring with quite frequent consultations. Because of its multidisciplinary nature, this type of structure allows a theoretically appropriate coordination of the different treatments, leading to optimal care for patients (Figure 1). In theory, MDC can also provide educational sessions that allow enhancement of patient understanding of their disease in order to motivate them and help to improve QoL. The second model is called renal care network (RCN), which is a care network dedicated to the treatment of CKD patients. Compared to the MDC, which centralises care in the same location, RCN allows more 'flared' care, favouring the maintenance of ambulatory links with HP located outside of the hospital. The communication and coordination between HP are provided by the availability of electronically-shared medical records and a dedicated coordination staff (Figure 2).[23] As in the MDC, RCN can deliver education to the patients.

Several investigators in different countries have tested the clinical efficacy of MDC and RCN on the outcomes of CKD patients (Table 1). The studies

Table 1. Studies that have assessed the effect of multidisciplinary clinic (MDC) or renal care network (RCN) on outcomes of patients with chronic kidney disease (CKD).

	CKD Severity	Study type	Country	Number of patients included	Intervention	Main endpoint	Length of follow up	Result	Others
Rognant et al.	Predialysis	Observational (2013)	France	160	Dedicated renal care network	Composite: CV mortality or events	One year before and after dialysis start	Negative	Positive effect on eGFR decrease and hospitalisation days per patient
Curtis et al.	Predialysis	Observational (2005)	Canada/ Italy	288	MDC	All cause mortality	Median follow up of 14 months	Positive	Positive effect on the control of biological parameters
Goldstein et al.	Predialysis	Observational (2004)	Canada	87	MDC	All cause-mortality	3 years	Positive	Reduced hospitalisations days and positive effect on the control of biological parameters
Hemmelgarn et al.	CKD	Observational (2007)	Canada	374 (patients >66 years)	MDC	All cause mortality	3.5 years	Positive	No effect on hospitalisations
Hotu et al.	CKD	Randomised clinical trial (2010)	New Zealand	65 (diabetic patients)	Community-based medical care	BP control	One year	Positive (for systolic BP)	Larger proteinuria decrease and better prevention of LV mass increase
Harris et al.	CKD	Randomised clinical trial (1998)	USA	437	MDC	eGFR decrease and all cause mortality	5 years	Negative	More frequent consultations and significant cost increase
Wu et al.	MRC	Observational (2009)	Taiwan	573	MDC	ESRD incidence, all-cause mortality, hospitalisations	One year	Positive	Positive effect on the control of biological parameters
Bayliss et al.	MRC	Observational (2011)	USA	2002	MDC	eGFR decrease	Mean follow up of 2 years	Positive	No effect on the control of BP, HbA1C and lipids

are mainly observational and retrospective and, so far, just two randomised controlled trials (RCT) have been reported in the field.[24,25] Moreover, some studies included patients with severe CKD and were interested in the outcomes of patients in the early dialysis period, though others included patients with less advanced CKD and evaluated mainly the effect on CKD progression (Table 1).[24,26-31] The first study by Harris et al.[24] was a RCT including 437 patients with mean eGFR of 34 ml/min. The authors found no differences between the two groups after a follow-up of 5 years, even with an important increase of the cost of patient care due to more frequent consultations. The second, by Hotu et al.[25] was positive for progression of CKD in diabetic patients but the intervention, although close to the RCN model, was pretty atypical and the follow-up was of 1 year only.

Other studies were observational, with some including prospective follow-up. The follow-up duration was generally short, leading some authors to question the long-term benefit of the MDC.[32] However, it may be noted that even an unsustainable effect is not without interest for the patients, particularly for care delivered in the predialysis period. It seems likely that some studies have looked at the impact of optimised care of the patients during this period because of their potentially important impact on patients' subsequent outcomes.[28,29,31]

The studies were mainly positive for primary outcome, including one with the longest follow-up, which showed a 50% decrease of all-cause mortality in Canadian patients aged 66 years or more and presenting mainly stage 4 CKD.[30] Three studies showed a positive effect on mortality during early dialysis period[28,29,31] while another showed a positive effect on CKD progression in patients with less severe CKD.[27] An interesting study is the one by Jones et al.[33] that tested the effect of a RCN without nephrology consultation (but with remote management by a nephrologist) on the outcomes of CKD patients with a less severe health condition otherwise. The authors showed that around 30% of CKD patients referred to the nephrology department can be managed appropriately on a period of three years, with a hazard ratio for death reduced by 36% in the RCN group (however, largely explained by the initial selection of patients).[33] Finally, our recent study is, to our knowledge, the only one that tested the impact of a true RCN on patient outcomes during the early dialysis period. Although the main outcome was not different between the two groups, we found a positive effect on several secondary endpoints, including CKD progression and the rate of hospitalisation per patient during the 1 year before and the year after dialysis started.[23]

LIMITATIONS

Although the results of most of these studies are positive, it should be considered that there are some limitations that prevent unequivocal conclusions about the effect of MDC and RCN. In addition to the short follow-up of most of these studies, just two studies are RCT and the evidence brought only by observational studies is weaker because of the presence of potential confounders. Another clinical trial by Devins et al.[34] was not included in this review because of the intervention that was not really multidisciplinary (i.e. involving HP like dieticians, nurses, etc.) and was mainly based on psycho-educational care which is beyond the scope of this review, even if education of the patients is part of the MDC intervention.[34]

Another limit that has been mentioned by Van Biesen et al.[32] is that the patients were included in the MDC on a voluntary basis in several studies. As such, we can suppose this has created a bias in their results because these patients are probably more implicated and motivated in their care. Thus, Van Biesen et al.[32] underlined the potential effect of this bias on the impact of educational sessions: because these sessions are more likely to benefit well-motivated patients, that could have artificially increased the true effect of MDC (and therefore preclude its generalisability). Another drawback of this selection bias is the possible inclusion of patients who are more compliant about treatment and dietetic in the treated group. However, if this potential selection bias could explain a part of the positive effect associated with MDC or RCN, it is also probable that optimised care exerts a positive effect through others factors like prevention of iatrogeny and better vascular access management. Finally, another limitation is the lack of data regarding cost-effectiveness of such interventions. To our knowledge, there is no study on this issue and this should be requested before considering a large-scale implementation.

CONCLUSION

Several studies suggest that an optimisation of CKD patient treatment is needed in order to prevent renal disease progression and premature death of these patients. In addition, efficient therapeutic interventions are needed in order to improve the QoL of these patients. Although some studies suggest a possible global benefit of care when delivered in the context of MDC and/or RCN comparatively to conventional care, more robust evidence, which should come from RCT, are awaited to draw definitive conclusions about the positive effect of these models on CKD patient outcomes. Besides that, it would be interesting to try to define a critical amount of renal care exposure that would allow the avoidance of adverse patient outcomes during the early period following the dialysis start.

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HYMENOPTERA STINGS AND THE ACUTE KIDNEY INJURY

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ABSTRACT

Hymenoptera stings are a health concern. Apidae (bees), Vespidae (hornets, yellow jackets and wasps) and Formicidae (ants) are medically-important stinging insects under the order Hymenoptera. Clinical features from simple skin manifestations to severe and fatal organ injury are due to the hypersensitivity reactions and/or the toxic effects of the venom inoculated. Here we discuss on Hymenoptera stings involving apids (honey bees) and vespids (wasps, hornets and yellow jackets) and their effect on renal function and associated morphological changes in the kidney. Despite the differences in venom composition and quantity released per sting in two insect groups, both lead to similar medical consequences, such as localised normal allergic reactions, mild to severe anaphylaxis and shock and multiple organ and tissue injury leading to multiple organ failure. Acute kidney injury (AKI) is one of the unusual complications of Hymenoptera stings and has the basis of both immune-mediated and toxic effects. Evidence has proven that supportive therapy along with the standard medication is very efficient in completely restoring the kidney function without any recurrence.

Keywords: Acute kidney injury, allergic reactions, Hymenoptera stings, renal replacement therapy, toxic reactions, venom.

INTRODUCTION

Animals that produce toxins are classified as either venomous or poisonous. Venomous animals are capable of producing and delivering the toxin during a stinging or biting act whereas poisonous animals are those whose tissues, either in whole or in part are toxic.[1] About 75% of the world's animal species are arthropods, some of which have appreciable interaction with humans and are capable of causing significant medical problems.[2] Approximately 5 million snake bites, scorpion stings and anaphylactic reactions to insect stings occur worldwide annually, causing over 100,000 deaths each year, most of which happen in the tropics.[3] Hymenopterous insects, snakes and spiders are the three animal groups most often responsible for human deaths attributable to venomous animals.[1] The stinging insects are members of the order Hymenoptera of the class Insecta, of which the three medically important groups belong to families of Apidae (bees), Vespidae (wasps, hornets and yellow jackets) and Formicidae (ants).[4] Globally 13,671 people are exposed to Hymenoptera stings.[5] In England and Wales, about 10 people die each year from Hymenoptera sting anaphylaxis, in Australia 2-3 per year and in United States 40-50 per year.[6] In Nepal, the number of inpatient morbidity due to contact with Hymenoptera in the year 2010/2011 was 5.[7]

The sting becomes clinically significant if the patient has an allergy to Hymenoptera venom or if the patient is exposed to a large quantity of the venom due to mass/multiple stings. Most deaths related to Hymenoptera stings are the result of immediate hypersensitivity reactions causing anaphylaxis. However, death may also occur from severe local reactions, particularly if involving the airways with subsequent respiratory obstruction. Massive envenomation during swarm attacks can likewise cause death in non-allergic individuals.[4] A wide range of clinical sequelae involving multiple organ systems is observed during massive envenomation.[8-12] As a highly vascularised and excretory organ, the kidney is particularly vulnerable to Hymenoptera toxins.[3] In this review, we restrict our discussion to the immune-mediated and toxic effect of the vespid and the apid venom, with an emphasis on renal involvement.

APID VERSUS VESPID VENOM

Apid Venom

Honeybee venom is similar among the different Apis species with minor variations in component quantities.[13] Melittin is a major bee venom component (50% of dry weight) and the main pain-inducing compound. It consists of 26 amino acid residues[14] with only 28% of patients having specific immunoglobulin E (IgE) antibodies against it.[15] Melittin functions by altering the membrane integrity. The most important allergen in honeybee venom is phospholipase A_2 (PLA_2), which is a high molecular weight glycoprotein with 134 amino acid residues. The enzyme acts as a cytotoxin and an indirect cytolysin as it works in concert with melittin.[13,16] These two components are responsible for red-cell lysis. Once melittin has disrupted the membrane, PLA_2 cleaves bonds in the fatty acid portion of the membrane lipid bilayer. Hyaluronidase (1%-2%) is a secondary allergen and shares a 50% sequence identity with vespid venom allergen. It also disrupts the hyaluronic acid connective tissue matrix and allows the other venom components to infiltrate the tissues. Peptide 401, a mast cell degranulating protein (2%) causes mast cells to break down releasing histamine. Histamine reaction from honeybee envenomation is due to endogenous release initiated by other venom factors, as histamine is only a minor portion of bee venom.[13] However, in mass envenomations the venom histamine is sufficient enough to produce cardiovascular changes.[17] Additional bioactive molecules include acid phosphatase, apamin (a neurotoxic peptide), Api m 6 (an allergenic peptide of molecular weight 7.9kDa), dopamine and norepinephrine.[13,18] Bumblebee venom contains PLA_2, protease, hyaluronidase, acid phosphatase and several other proteins not found in honeybee venom.[19]

Vespid Venom

Vespid venoms are more variable in their composition among the species, different to that of bee venom. The important allergens in vespid venoms are phospholipases, hyaluronidase and antigen 5, with antigen 5 being the major allergen in all vespid venom.[2,20] The phospholipases (PL) characterised in vespid venoms are PLA_1, PLA_2 and PLB.[21] Vespid PLA_2 presents high haemolytic activities whereas PLA_1 is generally associated with allergic and inflammatory processes and also possesses mild to severe haemolysis. However, the potency of vespid phopholipases is variable among the species,[22,23] probably due to the greater taxonomic diversity of the vespids.[13] PLB from vespids not only have enzymatic activity but also have haemolytic activity and cardiotoxicity.[21] Vespid venom also contains active amines such as serotonin, histamine, tyramine and catecholamines. Peptides such as wasp kinins and mastoparans are unique to vespid venoms. Wasp kinins are of interest because two kinins, bradykinin and lysyl-bradykinin, occur in humans, and are generated and act locally in humans but are not stored as in venom. They are potent pain producers and increase vascular permeability. Mastoparan, a cationic tetradecapeptide discovered in wasp venom in a screening test for mast cell degranulating agents, is a major component of vespid venoms.[24,25] Besides mast cell degranulation, they are the potent stimulant of PLA_2 of both venom and victims. Mastoparans bind to phospholipids and facilitate the PLA_2-catalysed release of arachidonic acid, the precursor of prostaglandin and leukotrienes, which are mediators of adverse reactions associated with immediate hypersensitivity.[24] Mastoparan peptides of different vespid origins display haemolytic and cytotoxic activities of varying degrees, which are attributable to amphipathicity that promotes binding to membrane phospholipids.[24,26]

VENOM DOSE PER STING AND LETHALITY

Hymenoptera venom contains both species-specific components and shared components, hyaluronidase and phospholipase being the most commonly shared enzymes.[16,27,28] The amount of venom released per sting also varies among the species and even within the same species.[29] Bee stings release an

average of 50 μg up to 140 μg of venom per sting. Bumblebees release 10-31 μg of venom per sting. Vespids, in contrast to apids, inject less quantity of venom per sting: *Vespula*, *Dolichovespula* and *Polistes* stings release 1.7—3.1 μg, 2.4—5.0 μg and 4.2—17 μg of venom protein respectively.[16] Due to the variation in the composition and the quantity of the released venom, the lethal dose (LD_{50}) of venom differs amongst the insects. Renal failure or death may occur in the range of 20-200 vespid stings and 150-1000+ apid stings. The human LD_{50} for honeybee stings has been estimated to be between 500-1200 stings. Vespid venom has more deleterious effects than that of the apid venom. Mammalian toxicity tests on mice revealed that honeybee venom LD_{50} (3 mg/kg) is about equivalent to that of the larger hornet (*Vespa* spp), and three-fold less toxic than that of yellow jacket wasp (*Vespula* spp) venom.[13]

EFFECTS OF VENOM

The venom intoxication has variable effects in individuals depending upon the sensitivity of the person towards the venom and the amount of venom injected into the body. The reactions to vespid stings have been categorised as normal local reactions, large local reactions, graded systemic reactions,[27] systemic toxic reactions[12,30] and unusual reactions.[31,32] The most common clinical pattern of the Hymenoptera stings are the local reactions that resolve within a few hours or large local reactions that last longer than 24 hours, or the systemic reactions grade I—IV.[33,34] Both local and large local reactions are immunoglobulin G (IgG)-mediated type IV hypersensitivity whereas systemic reactions are immunoglobulin E (IgE)-mediated type I hypersensitivity.[27] The unusual delayed reactions are IgG and immunoglobulin M (IgM)-mediated type III hypersensitivity reactions, and includes vasculitis, central nervous system signs and symptoms such as seizures, peripheral neuropathy or radiculopathy, haemolysis, rhabdomyolysis and acute renal failure (ARF).[35] Toxic reactions are attributable to direct or indirect (e.g. immune-complex mediated tissue injury)[36] toxicity of venom, the effect of which might be localised or systemic.[36,37] Most deaths related to Hymenoptera stings are due to the immediate hypersensitivity reactions causing anaphylaxis. Such local reactions and anaphylaxis are not dose-dependent or related to number of stings. However, in non-allergic persons, massive envenomation can cause death, mainly due to the toxic reactions of the venom which are independent of immune mechanisms and are venom-volume dependent.[4] The sting reactions, allergic and/or toxic, affect multiple organs with results varying from a typical dermatologic expression to multiple organ failure.[8-12]

RENAL EFFECTS OF HYMENOPTERA VENOM

Acquired kidney injuries are generally induced by immunological, metabolic, haemodynamic, ischaemic and toxic assaults.[38] The term acute kidney injury (AKI), previously referred as acute renal failure (ARF), represents the entire spectrum of acute renal dysfunction from its earliest and mildest form to the need of renal replacement therapy.[39] The term defines either an abrupt increase in serum creatinine (to denote a reduction in glomerular filtration rate (GFR)) or an abrupt decline in urine output.[40] Hymenoptera envenomation significantly contributes towards AKI via haemodynamic alterations, ischaemic assaults, direct toxicity of venom and immunological mechanisms, which can be grouped under two categories i) immune-mediated effects and ii) toxic effects. The review of literature on AKI following Hymenoptera envenomation enlists the following different pathological findings: acute tubular necrosis (ATN), acute allergic interstitial nephritis (AIN), and acute cortical necrosis (ACN). Other renal changes documented are distal renal tubular acidosis (dRTA), proximal renal tubular acidosis (pRTA) and nephrotic syndrome (NS).

Animal studies with bee venom have demonstrated early and significant reduction in GFR and renal blood flow (RBF) which was more pronounced in cortical region than in medulla. The striking decrease in RBF caused renal ischaemia which ultimately led to the glomerular tuft retraction and mild tubular injury observed in early renal histology, which evolved to frank tubular injury after 24 hours. Grisotto et al.[17] proposed that various venom components such as melittin, PLA_2 and histamine are responsible for bee venom-induced RBF decrease through various mechanisms such as vascular endothelium damage,[41] vasoconstriction,[42] smooth muscle cell contraction,[43] increased renal renin secretion,[44] catecholamines[45] and arachidonic acid release,[46] and enhanced thromboxane B_2 production.[47] Grisotto et al.[17] had shown that bee venom produced clear dose-dependent proximal tubule (PT) toxicity and that the venom may enhance hypoxia/reperfusion injury.

Pigments (myoglobin, haemoglobin or both) are responsible for indirect venom toxicity. Three major underlying mechanisms are i) renal vasoconstriction/hypoperfusion due to fluid third

spacing during myolysis causing intravascular volume depletion or activation of endotoxin-cytokine cascade eliciting renal vasoconstriction, or scavenging nitric oxide (NO) by haemproteins, which is an important endogenous vasodilator, ii) intraluminal cast formation, and iii) haem-mediated proximal tubular toxicity.[48] Decreased GFR due to renal vasoconstriction and volume depletion, both decreases the clearance of haemprotein and increases intraluminal myohaemoglobin concentration favouring cast formation and tubular obstruction, which in turn causes luminal stasis allowing more time for proximal tubular haem reabsorption. The accumulated haemprotein confers tubular toxicity leading to necrosis and ultimately filtration failure. The haemprotein-induced kidney injury are due to i) intense renal vasoconstriction causing ischaemic tubular injury and ATP depletion via haemodynamic (in the setting of volume depletion) and non-haemodynamic (ischaemic interaction directly at the proximal tubular cell level) mechanisms, ii) direct sensitisation by endocytosed haemprotein of tubular cells to ischaemia-triggered membrane injury via PLA_2 attack, and iii) haem iron-induced oxidative stress via hydroxyl radical formation by accumulated intrarenal haem iron leading to oxidant renal damage. Besides haem-protein, other factors produced during rhabdomyolysis and haemolysis such as hyperphosphataemia and hyperuricaemia potentiates ischaemic and nephrotoxic renal damage. Release of tissue thromboplastin in rhabdomyolysis triggers disseminated intravascular coagulation (DIC), potentially causing intrarenal microthrombus formation, and thus, injury.[49,50]

Acute Tubular Necrosis

Acute tubular necrosis refers to the reversible destruction of tubular epithelial cells with acute suppression of renal function. It is the primary cause of AKI following Hymenoptera envenomation. The pathogenesis of ATN includes i) indirect toxicity of venom i.e. the deposition of pigmented casts such as myoglobin and haemoglobin, due to rhabdomyolysis and intravascular haemolysis respectively, in the renal tubules,[51,52] ii) direct toxicity of venom to tubular cells[(53)] and iii) hypotension/haemodynamic instability caused by venom toxaemia-induced cardiovascular injury and anaphylactic shock.[52,54] ATN predominated in the cortex and outer medulla and was more intense in the proximal tubules. This tubular segment is the most susceptible to the toxic effects of the venom due to greater reabsorption of substances associated with intense metabolic activity, with energy expenditure and vulnerability of the enzyme system. The toxic substances of the venom itself probably contributed to the lesion, especially melittin and phospholipases, which are cytotoxic. The ultrastructural examination of the kidney revealed intracytoplasmic structures resembling myelin figures, which might be lipid accumulations resulting from the altered metabolism of these substances due to ischaemia or to toxic aggression. Some of these structures contained mitochondria compatible with phagocytosed apoptotic corpuscles undergoing digestion or autophagic vacuoles surrounding altered organelles, suggesting that direct toxicity to these organelles may occur in the model.[55] Sandbank et al.[56] detected similar changes in proximal tubular cells in experimental studies in hornet venom suggesting a direct toxic effect of the venom on mitochondria. The immunohistochemical analysis showed the presence of myoglobin in tubular casts as well as in the more apical portions of proximal cells. Muscle actin was also detected in the tubular cells. There is also a possible role of renal ischaemia in the genesis of ATN. The ischaemic lesion of the myocardial infarction type and catecholamine release due to Hymenoptera envenomation may affect the cardiac output and secondarily cause renal ischaemia. Likewise, the action of the venom components, such as vasoactive substances, might cause renal ischaemia.[55] Proximal tubule cells have a limited glycolytic capacity and are more vulnerable to ischaemia than distal tubule cells and cells of thick ascending limb. Although thick ascending limb has high glycolytic potential, the thick ascending limb is also a site of ischaemic lesion due to the precarious oxygenation of the renal medulla.[57-59] It was also noted that haemoglobin in the intratubular casts was not detected when the venom dose was reduced to half with regard to the experiment which has intratubular haemoglobin cast findings.[55]

Acute Interstitial Nephritis

Acute interstitial nephritis (AIN) defines a pattern of renal injury usually associated with an abrupt deterioration in renal function characterised histopathologically by inflammation and edema of the renal interstitium, sparing the glomeruli and the blood vessel. AIN accounted for 15% of cases hospitalised for AKI patients which is due to the immune-mediated tubulointerstitial injury,[60] either IgE mediated or non-IgE mediated.[61] The most common etiology of AIN is drug hypersensitivity; other causes include infection, immune-mediated disease, glomerular disease and idiopathic. Zhang et

al.[62] showed that AKI in the setting of wasp stings is not confined to rhabdomyolysis and ATN only, but also acute allergic interstitial nephritis could be the mechanism. The pathology in AIN is the inflammatory cell infiltrate within the interstitum of the kidney. The inflammatory infiltrate is a mixture of T lymphocytes, monocytes and occasionally plasma cells and eosinophils. Although the exact mechanism of AIN is unclear, it is highly probable that AIN is due to immuno-allergic disequilibrium, mainly cell-mediated immunity supported by the presence of T helper and T suppressor lymphocytes among the cellular infiltrates.[63] However, in some cases, humoural mechanisms are involved with complement proteins, immunoglobulins and anti-tubular basement membrane antibodies found in the interstitium.[60,64]

Acute Cortical Necrosis

Besides the common ATN and less common AIN, acute cortical necrosis (ACN) is the more infrequent cause of AKI. Glomerulus is the most affected part of nephron in ACN, however, the cortical tubules are also adversely affected, leading to both glomerular and cortical tubular necrosis. Kumar et al.[65] reported the first case of ACN leading to AKI following a single wasp sting. The renal biopsy revealed the features of thrombotic microangiopathy. However, he failed to investigate the venom specific IgE level or tryptase level in the serum of the patient to correlate the coagulopathy with venom anaphylaxis. The thrombotic microangiopathy with patchy cortical necrosis has been reported by George et al.[66] in a patient with more than 50 wasp stings, with the clinical course of increased serum total IgE level (venom-specific not assayed), DIC, rhabdomyolysis, hepatic necrosis and acute respiratory distress syndrome. DIC is a striking clinical presentation in snake envenomation, particularly of Viperidae family. Presence of fibrin thrombi in glomerular capillaries and renal microvasculature leads to microangiopathic haemolytic anaemia, thrombocytopenia and cortical necrosis.[66] A single bee sting also unusually caused intravascular coagulation and elevated serum level of allergen-specific IgE. The role of mesothelial injury, thrombocyte and macrophage activation, release of cytokines, leukotrienes, bradykinin and platelet aggregation factor, immune complex deposition in small vessels, and complement activation, is postulated mechanism for intravascular coagulation.[37] The pathogenesis of ACN postulated include i) endothelin induced vasospasm of small vessels, ii) toxic capillary endothelial damage, iii) endotoxin-induced generalised Schwartzmann phenomenon and iv) hypercoagulable state.[67]

Nephrotic Syndrome

The immune disturbances are considered important in the pathogenesis of nephrotic syndrome (NS). It has been postulated that involvement of T lymphocytes and their cytokine secretion influences the permeability of the glomerular basement membrane with consequent development of proteinuria. Hymenoptera stings are a potential factor for the occurrence of NS.[68,69] Tauk et al.[70,71] in his review has reported NS following wasp stings with diverse renal pathological changes, which includes minimal change disease, mesangial proliferative glomerulonephritis, severe glomerular hyalinisation and a mixed pattern of mesangioproliferative glomerulonephritis and early membranous nephropathy.

Renal Tubular Acidosis

Both pRTA[72] and dRTA[34] have been documented after Hymenoptera stings. Ram et al.[53,73] had reported a case of pRTA in a patient after honeybee stings wherein the renal biopsy showed dense lymphocytic interstitial infiltrate and biochemical parameters consistent with pRTA, such as presence of hyperchloremic metabolic acidosis with normal anion gap and hypokalemia with preserved ability to acidify urine to a pH of 5.5 in a steady state along with hypophosphataemia, hypouricaemia, renal glucosuria and high urinary excretion of calcium, phosphorus and uric acid. Animal studies have shown that bee venom or melittin inhibits apical transporters of proximal tubules with evidence of increased fractional excretion of phosphorus, sodium and potassium in urine.[53,73] Han et al.[74] have further shown the involvement of oxidative stress due to bee venom and its melittin-related reactive oxygen species (ROS) generation by proximal tubular cells (PTC) in inhibition of apical transporter of PTC via PLA_2 activation. Melittin from bee venom has been suggested to activate the tissue PLA_2 that induces an increase in arachidonic acid, which attributes to free radical-induced lipid peroxidation.[74] Mastoparan has also been shown to facilitate the PLA_2 activity of both venom and victims.[24] Free radicals play an important role in the pathogenesis of tubular dysfunction, which may lead to necrosis and thus renal failure by their severe cytotoxic effects such as lipid peroxidation and protein denaturation in cell membranes, followed by the changes in membrane fluidity, enzyme properties and ion transport.[74] Likewise, Sanjay et al.[34] reported the case of

dRTA following a wasp sting. These reports try to correlate between Hymenoptera stings and renal tubular dysfunctions, however, further studies are required to elucidate the exact pathogenesis of tubular dysfunction.[34,72]

TREATMENT AND MANAGEMENT

Allergic and toxic reactions are the complications encountered during Hymenoptera stings. The complications range from normal skin reactions to anaphylaxis and multiple organ failure. There are many different species in Apidae and Vespidae family; however, their stings all lead to similar medical conditions, mostly negligible in a medical sense. Symptoms vary by victim, with individual sensitivity and the amount of venom inoculated in the body, both playing important roles. There is no specific treatment for Hymenoptera stings in general and no manufactured antivenom available for severe reaction cases.[75] Treatment consists of cold compresses and analgesics for local reactions. Acute medical therapy for systemic reactions includes standard treatment for anaphylaxis including epinephrine, H_1-receptor antagonists, corticosteroids and other supportive therapy under symptomatic treatment strategy.[76] Venom immunotherapy has also been recommended for patients who exhibit systemic reactions following and inadvertent Hymenoptera sting.[28]

Renal complications do not occur as quickly as anaphylaxis, therefore the follow-up of urine output and colour, urine analysis, blood pressure, haematocrit and renal function tests are essential post-sting standard monitoring parameters.[75] The major treatment strategy is to i) correct the hypovolemia and attend the renal ischaemia, ii) enhance the clearance of haemproteins, toxins or toxic wastes from the circulation and the kidney and iii) alleviate the direct adverse consequences of venom toxins, toxic wastes, electrolyte imbalance and haemproteins on kidneys and other organs.[48] The early pharmacological intervention incorporates the volume replacement and alkaline diuresis in order to prevent the factors that lead to AKI, such as dehydration and renal hypoperfusion, intratubular cast formation and tubular obstruction, aciduria, and free radical release.[77] Haem iron cast formation in the renal tubules is facilitated in patients with acidic urine (pH<5.6) and a high concentration of haemoglobin or myoglobin in the renal tubules, which reacts with Tamm-Horsfall protein (THP) and precipitates, forming casts. Such binding is enhanced under acidic conditions, and thus urinary alkalinisation with sodium bicarbonate is believed to be helpful in reducing cast formation.[78] The ideal regimen for alkaline diuresis in patients with haemolysis and/or rhabdomyolysis is half isotonic saline (0.45%, or 75 mmol/L sodium) to which 75 mmol/L of sodium bicarbonate is added. Furosemide is the popular choice as a diuretic agent. However, once the overt kidney injury has been established, the only reliable therapeutic intervention is extracorporeal blood purification such as intermittent haemodialysis, continuous renal replacement therapy, peritoneal dialysis and plasmapheresis (whenever indicated).[77] The treatment of established AKI is, thus, largely supportive in nature, renal replacement therapy being the cornerstone.[79] Forced alkaline diuresis can avert the need of renal replacement therapy[52] provided it is instituted early after the incident and before the progression of kidney injury, however, the diuretic therapy in AKI remains controversial despite its common use, and awaits for higher quality evidence on diuretic use in AKI.[80]

CONCLUSION

Hymenoptera stings are common medically-significant insect stings. The sting incidents are high during late summer or early fall when there is an increased outdoor activity of human beings or large numbers of vespids are attracted to the foods of humans eating outdoors. The sting or mass envenomation occurs if the insect is disturbed or their hive is interrupted.[13] The sting(s) result into diverse clinical sequelae either due to allergic reactions or due to toxic reactions. AKI is the unusual medical complication developed after Hymenoptera sting(s) with due basis of both hypersensitivity reactions and/or toxic reactions posed by the venom. Provided the timely medical intervention, along with the supportive therapy, there is an adequate evidence of complete and non-recurring recovery of kidney function.

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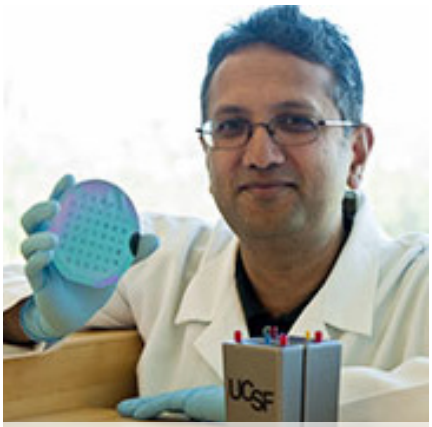
No donors needed: First steps toward solving kidney shortages

THE University of California, San Francisco, has made a significant effort to create an implantable artificial kidney for dialysis patients. The artificial kidney project aims to combine nano-scale engineering with the most recent advances in cellular biology to create an implantable device that would enable patients with chronic kidney failure to lead healthier and more productive lives, without external dialysis or immune suppressant medication.

End-stage renal disease (ESRD) affects 2 million people worldwide and is fatal unless treated, costing the nation $40 billion (£26.5 billion, €31 billion) each year for treatment. Although transplantation is the most effective treatment, donor organs are in short supply, and kidney dialysis is a short-term and costly treatment.

Shuvo Roy, the inventor of the artificial kidney and a bioengineer in the faculty of the UCSF School of Pharmacy said: "We can provide an alternative therapy and a treatment option that doesn't exist today for the vast majority of people today that are forced to rely on dialysis." Targeted for clinical trials in 2017, the artificial kidney project was selected last year as one of the first projects to undergo a more timely and collaborative review at the Food and Drug Administration (FDA). The FDA chose three renal device projects intended to deliver breakthrough medical device technologies to patients faster and more efficiently.

> "We can provide an alternative therapy and a treatment option that doesn't exist today for the vast majority of people today that are forced to rely on dialysis"
>
> *Dr Shuvo Roy, iRAD Project Director, UCSF School of Pharmacy.*



Dr Shuvo Roy

UCSF's artificial kidney project was selected for its transformative potential in treating ESRD. The artificial kidney, or implantable Renal Assist Device (iRAD) would include thousands of microscopic filters as well as a bioreactor to mimic the metabolic and water-balancing roles of a real kidney.

Using an external room-sized model developed by a team member at the University of Michigan, the combined treatment has been proven to work for the sickest patients. Dr Roy's goal is to apply silicon fabrication technology, along with specially engineered compartments for live kidney cells, to shrink that large-scale technology into a device the size of the coffee cup. The device would then be implanted in the body, allowing the patient to live a more normal life.

First-of-its-kind study created to observe kidney and gum disease link

The documented link between cardiovascular disease and gum disease has caused researchers to link periodontal disease (gum infections) with kidney disease, after it was reported that people with kidney disease and those on dialysis are more likely to have periodontal disease and other oral health problems than the general population.

"This is a very new and emerging area, and there have only been a few studies," said Doctor Vanessa Grubbs, an Assistant Professor and nephrology specialist in the UC San Francisco's School of Medicine, who is determined to advance this research as part of her commitment to preventing the chronic health problems associated with kidney disease.

Teaming up with Professors George Taylor, and Mark Ryder, from the UCSF School of Dentistry, Doctor Grubbs is launching a first-of-its-kind, randomised, controlled study to track the progression of kidney disease in patients receiving treatment for periodontal disease. All patients will have both conditions, while two-thirds will receive immediate and follow-up periodontal care, and the remaining control group will receive the dental care if it is medically necessary. By analysing unique biomarkers in blood and urine associated with kidney damage, kidney function can be measured and compared between the two groups.

Periodontal disease is an inflammatory response to persistent infection, caused by bacteria getting trapped in the gum's porous tissues. This inflamed state can affect major organs such as the heart, possibly through bacteria in the blood stream. Studies have also shown kidneys are similarly at risk, due to the fact that people with kidney disease have weakened immune systems, therefore they are more susceptible to infections. By initially treating the periodontal disease, this can hinder the progression of kidney disease and any other related systems that are susceptible.

It is noted that initial funding into dental care to include a wide range of incomes could in fact be cost-effective in the long run since it is cheaper to pay for dental care than the long-term ramifications of kidney disease.

Herbal medicine detrimental to kidneys, doctors warn

Doctors in both Britain and the Philippines have condemned herbal medicine after studies showed the use of herbs in treating diseases have become increasingly common, asserting any use may cause severe kidney disease and cancer, or damage kidneys further in cases with pre-existing conditions.

In the past there have been claims that herbal medicines could heal kidney diseases, but Doctor Glen Butuyan, a member of the Nephrology Society of the Philippines, has advised patients to avoid taking these medicines. Although they "come from plants or organic sources, these are not yet purified and some of their elements can trigger the disease." Doctor Butuyan added that in developing a medicine from a certain plant, the plant's elements are extracted, after which only the elements that can cure will be taken.

It has been suggested that traces of aristolochic acid were found in some of the herbs. Aristolochic acid binds itself to DNA, causing cancer growth "in areas where it is most concentrated, which probably explains why the cancers are most focused around the urinary tract and the kidney."

This research has come as a wake-up call to users of alternative medicine, Doctor Butuyan adding that herbal medicines are not licensed under the Food and Drugs Administration as drugs, but rather as food supplements.

Facebook becomes new way to donate a kidney

A Facebook campaign saw the number of US organ donation registrations increase by 2100% in a day, according to researchers at the John Hopkins University School of Medicine, after users were given the ability to share their organ donor status, and by creating easy links on State Department of Motor Vehicles websites to encourage participants.

This innovative organ donor project on Facebook originated after a conversation between Andrew M. Cameron, MD, PhD, a transplant surgeon, and a current Facebook employee and university classmate, Sheryl Sandberg. They both expressed concerns over the organ shortage and it was through these conversations that the idea of having an organ donor status on the Facebook timeline originated.

Ordinarily, in some states, when citizens apply for a driver's license, they will simply be asked if they wish to become an organ donor, with their approval noted on the card, with an average daily registration on the motor vehicle website of 616 nationwide. However this new campaign changed that. Already 57,451 Facebook users have updated their profiles to share their organ donor status. The first day of the campaign saw 13,012 new online registrations - a 21.2-fold increase based on the average daily registration rate.

"The short-term response was incredibly dramatic, unlike anything we had ever seen before in campaigns to increase the organ donation rate. And at the end of two weeks, the number of new organ donors was still climbing at twice the normal rate," Doctor Cameron said.

Although the number of registrations did drop after 12 days of launching the page, Doctor Cameron stated that it "had a very powerful, lasting effect. But we need to find a way to keep the conversation going." He suggests that by regaining some of the lost attention it would help the campaign to go viral.

He has spoken to Facebook officials who are discussing launching it on its mobile platform, changing its prominence on the Web version or even offering incentive, such as coupons, for people who declare they are organ donors.

"This was the first effort like this designed to mobilise people for a public health cause," Doctor Cameron added. "Now we want to build on that. Studying the response to the organ donor effect is the next step in the process of using social media for social good."

> "The short-term response was incredibly dramatic, unlike anything we had ever seen before"
>
> *Dr Andrew M. Cameron, Surgical Director, The John Hopkins Hospital.*

Omega-3 fatty acids could prevent sudden cardiac death in dialysis patients

The benefits of omega-3 fatty acids for the heart has been well-known and documented among various investigations. Researchers, however, have not studied the potential benefit for people on haemodialysis, who are among the highest-risk patients for sudden cardiac death (SCD). The 5-year survival rate for patients on haemodialysis is 35%, with the risk of death highest in the first few months of starting treatment. The most common cause of death in these patients is SCD, which accounts for about one in four deaths. These patients have several predispositions for SCD due to coexisting medical problems such as diabetes, hypertension, and uraemia.

The link between chronic kidney disease and SCD has been recognised through a study in *Kidney International*, in which an investigation identified a common trend where 100 patients died of SCD during the first year of haemodialysis while 300 patients survived within the first year of starting treatment. The blood of the patients who survived was examined and was found to contain higher levels of omega-3.

Allon N. Friedman, M.D., Associate Professor of Medicine in the Division of Nephrology at the Indiana University School of Medicine and first author of the study, said the findings are impressive enough that he believes a placebo-controlled clinical study is warranted to confirm the results.

"Because omega-3 fatty acids can be obtained from certain foods, such as fish oil, our findings also have important implications for the type of diet we recommend to patients on dialysis," Dr Friedman said.

Overall, this investigation has the potential to prevent significant deaths in addition to reaping the surplus benefits of omega-3 in maintaining a healthy body.

Doctors and patients have differing opinions on kidney failure prognosis

A recent study from Boston's Beth Israel Deaconess Medical Center has found that patients with kidney failure have distorted expectations about their own outcome compared to their doctor, who is unwilling to discuss a difficult prognosis with them.

Doctor Melissa W. Wachterman, a palliative care physician who conducted the research, interviewed 62 seriously ill patients who, based on two validated prognostic models, were all predicted 1-year mortality. However, Wachterman found that most of these patients were more optimistic about their outcomes compared to their doctors. Wachterman stated that "we may not be serving these patients as well as we could. These missed opportunities and misperceptions may actually be influencing patients' goals of care."

Wachterman has suggested that nephrologists may not want to discuss prognosis because it could diminish patients hope, adding: "Overall, 81% of patients thought they had at least a 90% chance of being alive in one year, whereas nephrologists were this optimistic for only 25% of patients."

In fact, given the hypothetical scenario where patients asked their nephrologists for an estimate of their prognosis, over half the time the doctors reported that they would tell the patient they could not give an estimate. When researchers asked patients and doctors about the possibility of kidney transplantation, Wachterman's research found: "Over a third of the time patients and nephrologists were not on the same page about whether the patient is even a transplant candidate. The patients thought they were candidates, and nephrologists said they were not."

Doctor Ellen McCarthy, an epidemiologist at BIDMC and senior author has suggested that "we might start by asking patients if they want to know their prognosis. And then look for the kinds of resources... that are available, which can add a much needed extra layer of support."

However, the study has shown that many nephrologists believed that their patients would then focus on their quality of life rather than living longer, many patients commented that they would rather "choose care focused on relieving pain and discomfort, even if it meant not living as long."

Wachterman has concluded: "In the long-term, having that information may actually help bring patients a greater sense of hope and facilitate peace and closure because it enables them to plan for what to do with the time they have left."

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FRESENIUS MEDICAL CARE
GAMBRO
GARDHEN BILANCE
GENZYME, A SANOFI COMPANY
GLOMERIA THERAPEUTICS
HEMO SAPIENS HERCO WASSERTECHNIK
IMMUNDIAGNOSTIK
INFOMED
INTERMEDT MEDIZIN & TECHNIK
JANSSEN
JIHUA MEDICAL JOLINE
KARGER
KAWASUMI LABORATORIES
KDIGO
LABOR LIMBACH
LAUER
LIKAMED

MAHAN MED MEYMEH KISH

MEDCOMP

MEDICA ITALIA

MEDICAL DEVICES CORP

MEDIKIT

MEDVISION

MEDXL

MEMBRANA

MILTENYI BIOTEC

MONE MEDICAL

NIKKISO EUROPE

NINGBO TIANYI MEDICAL

NIPRO EUROPE

NOVARTIS ONCOLOGY

NXSTAGE MEDICAL

OTSUKA PHARMACEUTICAL EUROPE

PAKUMED

PHARMACOSMOS

PHYSIDIA

RENAL ILAC

ROCHE

SANDOZ – NOVARTIS GROUP

SANOFI

SERUMWERK BERNBURG

SILVER MED

SOLUDIA MEGHREB

SUISSE MED TECHNOLOGIES

SYNLAB SERVICES

TAKEDA EUROPE

TASSINARI BILANCE

TAUROPHARM

TEVA PHARMACEUTICALS

THERMO FISHER SCIENTIFIC

TIANJIN ALPHA RENAL LIFE SCIENCE

TORAY

VIFOR FRESENIUS MEDICAL CARE RENAL PHARMA

VITAFLO INTERNATIONAL

WICHTIG

WS FAR IR MEDICAL TECH

SOCIETIES

ASN – AMERICAN SOCIETY OF NEPHROLOGY

CSN – CHINESE SOCIETY OF NEPHROLOGY

CZSN – CZECH SOCIETY OF NEPHROLOGY

EKHA – EUROPEAN KIDNEY HEALTH ALLIANCE

ERA-EDTA 2014 CONGRESS AMSTERDAM

ESOT – KIDNEY COMMITTEE

ESPN – EUROPEAN SOCIETY OF PAEDIATRIC NEPHROLOGY

FSN – DIALYSIS AND NEPHROLOGY

FRENCH SOCIETIES

HESN – HELLENIC SOCIETY OF NEPHROLOGY

HUSN – HUNGARIAN SOCIETY OF NEPHROLOGY

IFKF – INTERNATIONAL FEDERATION OF KIDNEY FOUNDATION

ISN – INTERNATIONAL SOCIETY OF NEPHROLOGY

NKF – NATIONAL KIDNEY FOUNDATION

SIN – ITALIAN SOCIETY OF NEPHROLOGY

TSN – TURKISH SOCIETY OF NEPHROLOGY

EVENTS

European Society for Artificial Organs 40th Annual Congress

September 11-14, 2013

Glasgow, Scotland

Held in the heart of Scotland, the Congress program will reflect the latest developments in addressing the challenges faced by researchers, engineers, and clinicians engaged in the broad spectrum of activities in the field of Artificial Organ research. It will cover the advances in research in the cardiovascular, liver and renal specialties, while also spending time considering the very significant challenges faced by those engaged in treating children in whom the growth process is a particular issue.

Embodying the motto of the ESAO congress: "Lab to patient – from concept to treatment", all involved are encouraged to use the Congress as a platform for presenting their latest and most promising work, for networking, and to gain valuable experience, with a day fully dedicated to young researchers and clinicians in the form of the yESAO symposia.

11th European Peritoneal Dialysis Meeting

October 11-14, 2013

Maastricht, Limburg, Netherlands

The EuroPD meeting holds to the ideal that the advancement in PD therapy should always be driven by basic and clinically applied scientific advancement, bringing together worldwide PD expertise to drive the development of the therapy, and ensure the meeting provides both knowledge to experts but also education to those new to the therapy.

The theme for the 2013 meeting will focus on how doctors, nurses, clinicians, and other partners can work together to put the patients firmly in "the driving seat of their care", supported with more choice, information and control.

Kidney Week 2013

November 5-10, 2013

Atlanta, United States

With the theme set as "Changing the Focus: Innovation and Individualisation", over 13,000 kidney professionals from across the globe will arrive to the state of Georgia for what is dubbed "The World's Premier Nephrology Meeting." Kidney Week provides participants with exciting and challenging opportunities to exchange knowledge, learn the latest scientific and medical advances, and engage in discussions with leading experts in the field.

Held in Atlanta's Georgia World Congress Center, the week comprises of 17 Early Programs during the first 2 days, and then an Annual Meeting from Thursday 7th through to Sunday 10th, where participants will receive full access to the scientific exposition, plenary, educational, and poster sessions, and industry-sponsored educational symposia.

Autoimmunity Congress Asia

November 20-22, 2013

Hong Kong, China

Tailored for researchers and physicians in the fields of immunology and rheumatology, ACA is a valuable opportunity to expand a nephrologist's professional horizon by learning about the latest discoveries and developments in the field.

A regional biennial meeting, this multidisciplinary, international gathering observes the latest developments in the diagnosis and treatment of the 81 autoimmune diseases, with this year focusing on the role of genes and the environment, including infections and epigenetics in the pathophysiology of major autoimmune diseases.

ACA is one of the several autoimmunity congresses that are chaired by Professor Yehuda Shoenfeld, whose enthusiasm and expertise have turned these gatherings into utterly unique and influential events. Plenary speakers include Trevor Marshall from Australia, Tianzhi Gang and Quianjian Lu from China, David Kaltzmann from France, and Carlo Perricone from Italy.

ISN Nexus Symposium 2014 - New Era of Drug Discovery and Clinical Trials in Kidney Disease

April 3-6, 2014

Bergamo, Italy

The Nexus Symposia aims to address the increasingly multidisciplinary approach to kidney health issues by bringing together researchers and practicing clinicians to advance science and treatment around highly targeted and specific themes of topical relevance. This meeting enables collaborations to take place, focusing on real clinical challenges and directions for future research.

Showing an eagerness to evolve and grow as an event, pharmaceutical representatives and regulatory authorities will now actively join discussions on common global issues to highlight new methods towards efficient drug discovery.

The 47th Annual Scientific Meeting of the European Society for Paediatric Nephrology

September 18-20, 2014

Porto, Portugal

The legendary warmth and hospitality of the city of Porto will welcome all the Paediatric Nephrology healthcare community. Taking place in a magnificent 19th century building, the Scientific Committee has taken great care in preparing a high quality and diverse program that will include the latest developments and ideas in basic science, transitional and clinical science, to be presented in the form of lectures, mini-lectures, symposia and courses, as well as industry-sponsored symposia.

The opening of abstract submission is the 15th December 2013, and notification of abstract acceptance is 15th April 2014. Porto is easily reached by plane from a number of European cities, with the airport directly linked to the city centre via tram.