Innovations in Nephrology

Breakthrough Technologies in Kidney Disease Care

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Masaomi Nangaku
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Foreword

Hard to describe, but easily identified; innovation is fostering the "new"—the act of effecting change into established territory and infusing it with new ideas, methods, products, and/or devices. This is quite the whittled-down definition in comparison to dozens of elaborate ones eating up unnecessary page space of dictionaries. Indeed, innovation is far from trivial, and far more impactful than a few journal entries scribbled about recent scientific breakthroughs might lead you to believe—though we personally might examine it through our academic lens, its prying tentacles reach out and grasp onto all of mankind's successes and follies without discrimination.

Nevertheless, we must ask; what specifically are the links between innovation and nephrology? Nephrology itself is a specialty organized upon its *own* major medical innovation: renal replacement therapy—the driving force behind the creation and flourishing development of the field. Nephrology was, is, and will be flush with innovation, and the field itself will probably witness profound transformation over the next few decades, where a different set of treatments and approaches will undoubtedly be available among the arsenal of therapeutic options for patients, kidney doctors, and healthcare professionals.

When the invitation came to pen a foreword for *Innovations in Nephrology*, I found myself considerably humbled. Hailing proudly from a major international publisher, the book, edited by Prof Geraldo Bezerra Silva Jr. and Prof Masaomi Nangaku, is unique in several aspects, and undoubtedly a landmark publication for the global nephrology field. The editors—both of whom academic authorities and leaders in the field, with an expansive global following—have managed to unite a fantastic and savvy team of contributors, each with their own distinct backgrounds, achievements, and credentials in the field. This wide array of contributions collected under one binding means that this book is likewise an internationally coordinated effort; renowned authors spanning over a dozen nationalities provide their concerted yet varied insight into advancements in nephrology.

Innovations in Nephrology offers an arrangement of 31 independent chapters, covering concepts and ideas ranging from artificial intelligence to caregiver robots, to the internet of things, to stem cells; the reader will traverse a variety of

revolutionary perspectives that exhibit our modern (and encouraging) reality of nephrology. From this, a panorama is meticulously crafted, presenting the broader scope of innovation—through data, painstaking amounts of research, and even business opportunities in nephrology endeavors—in a manner made palatable to the medical community at large. Alas, a roadmap to the "new."

Perhaps something of a misquote, it is said that Henry Ford—a figurehead in the innovation of the modern automobile—claimed that, had he listened to the grievances of men in the era preceding vehicular domination, he would have been in the business of breeding faster horses. Indeed, the long-term necessities of the client most frequently outweigh his short-term desires, and it is this idea that functions as the primary impetus for innovation; instead of mere product or service improvements, we must always consider that the common man rarely has the insight to envision the "radical innovations" to come, and thus we should prepare appropriately by examining his necessities though a visionary lens as captains of our respective fields. In this book, the reader will certainly not encounter any "faster horses." Instead, the past, present, and future of nephrology will be reconciled through a natural evolution of innovative concepts, technological progress, and advancements in technique from a framework of scientific development.

Above the constantly fluid stream of innovation, change, and progress this book will come to represent in the field of nephrology, its waters will one day inevitably yield into a stagnance of its own, completing the very cycle of innovation it promotes by cocooning itself into a timeless snapshot of the ideas, expectations, and (why not?) dreams of an era. After all, innovation predicates its own obsolescence.

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Contents

Chapter 1
From Hippocrates to Robotics: A Journey Through the History of Nephrology

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1.1 Introduction

The term nephrology comes from the Greek "*nephrós*" (kidney) + "*logos*" (language, word), meaning "study of the kidneys," of their physiology and diseases. The history of nephrology is entwined with the history of modern medicine [1]. It is a relatively new medical specialty, having been considered for the first time as a discipline in the 1950s, with an initial focus on the pathophysiology and electrolyte disturbance therapy [1]. In this chapter, we present some historical landmarks of nephrology, its trajectory in the world, from ancient times to the advent of robotics, highlighting some perspectives for the future, as this book will present the main innovations that have been applied to nephrology and which has the potential to be a reality in the near future.

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1.2 Hippocrates and the Kidneys

Hippocrates (460–370 BC), the "father of Medicine," had several aphorisms, including some about kidney disease [2]:

- "Colorless urine is bad."
- "Bubbles on the surface of urine indicate kidney disease and prolonged suffering."
- "Kidney and bladder diseases are difficult to cure in the elderly."

Some of these observations are important even in contemporary nephrology, such as the association between foam in urine and proteinuria [3], colorless urine as evidence of concentration or tubular defect [4], and many other impressions that would be confirmed through science development and research.

1.3 The Kidneys in the Bible

There are some descriptions of the kidneys in the Bible. The kidney is mentioned more than 30 times as a site of temperament, emotions, and vigor and as a site of punishment for faults [5]. There are also reports of rhabdomyolysis in the Bible, possibly caused by poisoning due to the consumption of birds of the *Coturnix coturnix* species (common quail) [6].

1.4 Historical Landmarks of Nephrology

Galen (131–200 AD), based on experiments, was the first to demonstrate that the kidney is a urine excreting organ. He published the treatise "*De Pulsis et urinis*," which described the variations of pulse, fever, and urine in different diseases, showing association with prognosis [7].

Maurus (thirteenth century) defined urine as the result of three consecutive digestions of bodily fluids, with the separation of the pure part from the impure one by the body. Urine would be the impure part. The analysis of urine was performed by the physician, based on color, concentration and sediment. Corbeil (also in the thirteenth century) classified the several colors of urine, which allowed the dissemination of uroscopy by European medical schools and its routine use until the eighteenth century [2].

With the development of the microscope, in the sixteenth century, with the contributions of Galileo Galilei (1564–1642), who first introduced the experimental method, and Marcello Malpighi (1628–1694), pioneer of the microscopic anatomy [8], several scientists started to analyze urine, looking for elements that cause calculi. In the seventeenth and eighteenth centuries, the description of several elements found in urinary microscopy associated with lithiasis was performed. In the eighteenth century, the first description of albumin was made in the urine of patients with edema.

The anatomical-functional bases of the kidneys were described by Malpighi, William Bowman (1812–1892) [9, 10], Carl Ludwig (1816–1895) (DAVIS 1996), and Jakob Henle (1809–1885) [11]. Robert Tigerstedt (1853–1923) discovered renin [12], which, in 1939, was evidenced to be an enzyme responsible for the production of angiotensin II [11], of which inhibition/blockade is currently one of the main bases of hypertension treatment and kidney "protection" strategies.

In 1840, Pierre Rayer (1793–1867) published the book *Traité des maladies des reins*, which correlated data from pathological anatomy, urinary alterations, and clinical manifestations [2].

Richard Bright (1789–1858), considered the "father of nephrology," also correlated clinical findings with anatomical alterations found in autopsies ("Reports of medical cases")—an association between edema, urinary, and renal alterations [13].

In the late nineteenth century, Claude Bernard (1813–1878) developed the concept of the internal environment [14]. The circulatory system is responsible for connecting the internal environment to interfaces with the external environment, specialized for the exchange of gases, which are the lungs, for the absorption of solutions, after processing the multiple components of diet, which are the intestines and, finally, an interface for the controlled excretion of solutes and solvents, comprising the kidneys. According to Claude Bernard, our physical and mental capacities would result from the renal competence to counteract the external environment through the mobilization of its regulatory mechanisms, which protect our biological integrity [14].

In the beginning of the nineteenth century, Bostock and Christison evidenced elevated levels of urea in the blood of patients with kidney disease [15]. In 1847, creatinine was discovered by Justus von Liebig, being measured for the first time by Max Jaffé (1841–1911) [16], in Germany, in 1886 [17]. George Michael Edebohls (1853–1908) performed the first surgical kidney biopsy, making an incision in the renal capsule or performing a complete bilateral decapsulation [18, 19].

1.5 Twentieth Century Landmarks in Nephrology

Thomas Addis (1881–1949) standardized the method of urine collection, cell count definition ("Addis count"), quantification of proteinuria, and measurement of serum creatinine as a marker of kidney function [20].

During the twentieth century, there was the development of kidney biopsy techniques, the use of immunofluorescence, and the classification of glomerulopathies. In the 1940s, Albert Hewett Coons and colleagues first described immunofluorescence techniques to detect pneumococcal antigens. In the 1950s, these principles were first applied to renal biopsies and demonstrated by R.C. Mellors. Another important achievement in this century was demonstrated by nephrologist Nils Alwall (1904–1986), from Sweden. He worked in the same period as Willem Kolff, aiming to build an "artificial kidney," and was the pioneer of the ultrafiltration process, also being the first to perform percutaneous kidney biopsies in the world, in

1944 [18]. The first report of percutaneous kidney biopsy, however, was published in 1950 by Antonino Pérez Ara, a physician from Cuba, but in a local journal [21].

In 1951, Homer W. Smith recognized the importance of methods for measuring renal clearance, in addition to elucidating the tubular transport capacity, secretion, and reabsorption of several substances, such as urea and creatinine. In the same decade, Marilyn Farquhar, Robert Vernier, and Robert Good published the first paper describing the implications of a new electron microscopy technique for the study of glomerular pathologies [11].

The twentieth century also saw the emergence of several techniques and technologies that persist to this day. Several studies and first performances of transplants, peritoneal dialysis, vascular accesses, arteriovenous fistulas, among others, were carried out, which will be discussed later in this chapter.

1.6 Hypertension Historical Landmarks

After discovering much of the renal microscopic anatomy and the renin-angiotensin system in 1874, Frederick Horatio Akbar Mahomed, an English physician, made the first connection between kidney disease (acute and chronic) and hypertension, observing that "before any changes in the kidney or the appearance of albumin in the urine, the first observable change is high pressure in the arterial system" [22].

In 1934, Harry Goldblatt, after formulating the hypothesis that blood flow reduction and the consequent renal ischemia could cause hypertension, developed an experimental model of hypertension. For that purpose, he designed a device to clamp the renal arteries of dogs, aiming to demonstrate the high pressure induced by ischemia. The only ones that caused an increase in blood pressure were the renal arteries [23].

In the 1970s, Brazilian physician and pharmacologist Sérgio Henrique Ferreira and colleagues identified angiotensin antagonists (ARBs) and angiotensin II-converting enzyme inhibitors (ACEi), two classes of inhibitors of the renin-angiotensin system [24]. In 1985, Taugma et al. proved for the first time that ACE inhibitors could provide renal protection [11]. Currently, it is known that these two drug classes are the main nephroprotective drugs and great allies in the treatment of hypertension.

1.7 The Development of Nephrology as a Medical Specialty in the Twentieth Century

Until the early 1950s, nephrology was not a medical specialty [25]. Shortly after World War II (1939–1945), the first national organizations interested in kidney diseases started to emerge in Europe, with emphasis on the 1948 *Societé de Pathologie Rénale*, which in 1959 was renamed *Societé de Néphrologie* [26], in France, the

Renal Association, in 1950, in the United Kingdom [27] and the *Società Italiana di Nephrologia*, in 1957 [28], in Italy, the first to use the name *Nephrology*.

The International Society of Nephrology (ISN), conceived and initially chaired by Professor Jean Hamburguer, was founded in 1960, after the I International Congress of Nephrology, held in Switzerland. The ISN focuses its efforts on advancing science, providing education and assistance in nephrology worldwide and currently has approximately 10,000 members in over 150 countries [11].

Some months before the creation of the ISN, the Brazilian Society of Nephrology (SBN, *Sociedade Brasileira de Nefrologia*) was founded on August 2, 1960 [29]. Two years later, in 1962, SBN held its first congress, in the city of Rio de Janeiro, at the National Academy of Medicine. The institution's main missions comprise: spreading scientific knowledge of nephrology, supporting professionals in the specialty and ensure universal access to renal health, and currently Brazil is the country with the highest number of nephrologists in Latin America.

1.8 Technologies Applied to Nephrology

1.8.1 Hemodialysis

The history of hemodialysis can be considered to have started in 1830, when physicist Thomas Graham verified that separating two fluids with substances dissolved in them using a cellulose membrane, an exchange between them occurred. The physicist called the phenomenon "dialysis" and the membranes "semi-permeable." In 1913, John Abel made the first "artificial kidney" in dogs without kidneys. The experiment with dogs consisted in a series of cellulose tubes dipped in saline solution, which was where the dogs' blood circulated. The problems with this experience were the fragility of the membranes, the lack of heparin, and frequent infections [30].

In 1917, during World War I, the observation of war wounded soldiers with uremia due to acute renal disease led the German physician Georg Haas to change the prototype of the "artificial kidney"—he increased the area of the membranes, managing to sterilize the components of the entire extracorporeal circuit with ethanol. In 1926, he attempted to use dialysis for the first time in human beings. This first experiment consisted in taking 1/2 l of blood from a patient with uremia and circulating it through the system tubes for 1/2 h, bathed in saline solution, and reinfusing the patient, without success. During this dialysis, Haas performed, for the first time, a temporary vascular access through a glass cannula to connect the extracorporeal circulation from the radial artery to the cubital vein [31].

In 1936, with the commercialization of cellophane, there was an improvement in dialysis, but still without much success. In 1940, still during World War II, a Dutch physician called Willem Johan Kolff, born in 1911, considered the "father of hemodialysis," made the "artificial kidney," which consisted of a tube with 40 m of cellophane, wrapped in a cylinder, which was surrounded by a tank containing a

solution. The patient's blood circulated inside the tube, and with each rotation of the cylinder, it plunged into the tank. Kolff's kidney was an innovation, as it used a blood propulsion system in the circuit (after being adapted from an automobile water pump), allowing for the first time its use in a patient undergoing continuous dialysis [32], as shown in Fig. 1.1. The first patient survived treatment in 1945 after 15 attempts [33].

The first Brazilian artificial kidney was designed and built by Dr. Tito Ribeiro de Almeida (1913–1998), at Hospital das Clínicas, School of Medicine, University of São Paulo, in 1949. The first hemodialysis session performed in Brazil took place on May 19 in the same year. This machine was used until 1954, when the first imported artificial kidney arrived in Brazil [29]. Until the mid-1950s, dialysis was still considered experimental and was carried out in approximately six hospitals around the world, with dubious results. Dialysis procedures were basically aimed at acute kidney injury patients until 1960.

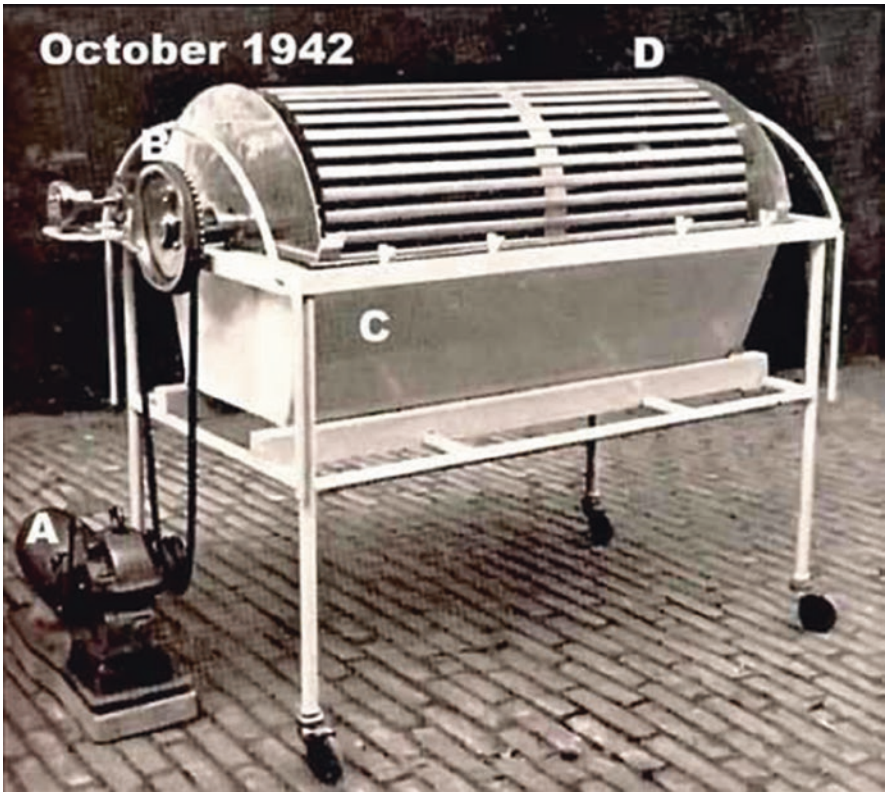

Fig. 1.1 Under German occupation of the Netherlands, in the small town of Kampen, Kolff built a horizontal rotating drum artificial kidney with a sewing machine motor (**a**), attached to a bicycle chain that turned a drum (**b**), on which circulating blood within cellophane tubing was exposed to dialysate in a 100-liter porcelain tank (**c**) with the tubing mounted on a drum built of wooden slats (**d**). Reproduced with permission from Friedman [32]. © 2009 John Wiley & Sons, Inc

In that decade, Belding Scribner, Wayne Quinton, and David Dillard at the University of Washington in Seattle (USA) developed a Teflon arteriovenous shunt, which allowed, for the first time, long-term hemodialysis for patients with chronic kidney disease (CKD) [34]. In the same decade, Brescia and colleagues were the first to describe, in 1966, the arteriovenous fistula for maintenance hemodialysis [35].

In the late 1960s, the first hollow fiber dialyzer was developed by Benjamin Lipps and John A. Sargent at Dow Chemical Company, California (USA)—a revolutionary advance. The first human dialysis test using this hollow fiber prototype dialyzer was performed in August 1967 by Richard Stewart [36, 37].

The concept of Kt/V was developed by Frank Gotch and John A. Sargent from a later analysis of the National Cooperative Dialysis Study (NCDS) published in 1985. Despite some controversy, Kt/V still stands as an important parameter for nephrologists to prescribe and monitor the adequacy of dialysis treatment [38].

Another milestone in hemodialysis was the development of erythropoiesis stimulating agents in the late 1980s. Epoetin alfa was first approved by the United States FDA in June 1989. After that, some studies and guidelines tried to establish a target for hemoglobin levels in CKD patients. In 2006, the CREATE study—conducted in Europe, Asia, and Mexico—and the CHOIR study—conducted in the United States—failed to demonstrate the advantage in achieving high hemoglobin levels [39]. A new class of drugs for CKD anemia treatment would emerge in the twenty-first century: the hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors, which had the approval for clinical use in the late 2010s [40, 41].

Dialysis treatment showed remarkable improvement in the late twentieth century and, incorporating technological innovations, left behind the status of extremely rudimentary procedure to the safety of modern machines equipped with numerous sensors, intelligent monitoring systems, performance evaluation, and automated disinfection, providing patient safety and allowing the early detection of problems.

1.8.2 Peritoneal Dialysis

The first peritoneal dialysis in human beings took place in 1923 under the supervision of George Ganter [42]. Even submitted to a procedure that used electrolyte solutions stored in glasses, coupled to rubber tubes and a needle at the end, the patient showed good blood results; however, she died after hospital discharge, making Ganter conclude that the treatment should not have been interrupted [42].

In 1965, Henry Tenckhoff improvised a flexible peritoneal dialysis catheter, using a technique that is applied, even with modifications, to this day. Moreover, this type of catheter takes its name from him, being called Tenckhoff catheter [11].

Continuous Ambulatory Peritoneal Dialysis (CAPD) was first described in 1978 by biomedical engineer Robert Popovich and by nephrologist Jack Moncrief. The

technique was tested in nine patients over 136 weeks and allowed good control of edema and protein loss, in addition to helping control the peritonitis [11, 43]. A year earlier, in 1977, Greek nephrologist Dimitrios Oreopoulos used, instead of glass containers, plastic bags in the CAPD, which resulted in a dramatic recovery of the tested patient and allowed the application of this technique at home level [11, 44].

1.8.3 Kidney Transplant

The first successful kidney transplants were described by Emerich Ullmann in March 1902. The Austrian surgeon performed an autotransplantation to a dog and a xenotransplantation from a dog to a goat. Subsequently, in 1906, the first documented attempts at kidney transplantation in humans were carried out by French surgeon Mathieu Jaboulay. The procedures, which were unsuccessful, were performed having a pig and a goat as donors [11].

The first record of a kidney transplantation from a deceased human donor was performed in 1933 by the surgeon Yurii Voronoy, in a territory that now belongs to Ukraine [45], and he was one of the first to observe possible problems due to immunological characteristics related to graft rejection [45]. On June 17, 1950, the first successful kidney transplant was performed by Richard Lawler in the USA. The compatible allograft lasted 10 months and the patient lived for an additional 5 years, possibly with residual kidney function [46]. In 1954, at the Brigham and Women's Hospital, in Boston, USA, Joseph E. Murray and John P. Merrill performed the first successful living-donor kidney transplant between twin siblings. The recipient lived for another 8 years after the procedure. In 1960, Peter Medawar and Frank Burnet won the Nobel Prize in Medicine for their pioneering in defining rejections and the study of immunosuppressants [46].

In Latin America, the first kidney transplant was in Argentina [47]. In Brazil, the era of transplants started in 1964, at Hospital dos Servidores do Estado, in Rio de Janeiro [48]. The Brazilian Association of Organ Transplants (ABTO, *Associação Brasileira de Transplantes de Órgãos*) was founded on April 14, 1987, with the purpose of promoting and stimulating the development of actions related to human organ and tissue transplants in the country, among others (ABTO). The National Transplant System (SNT, *Sistema Nacional de Transplantes*), established in 1997, is the entity responsible for controlling and monitoring the process of organ and tissue donation and transplant in Brazil and is the largest public and universal organ transplant system in the world.

Currently there are huge disparities in access to kidney transplant in the world, where the lower socioeconomic populations have the lowest chances of achieving a transplant [49]. Xenotransplantation emerges as an option for this problem, and the techniques are currently very advanced [50, 51].

1.8.4 New Technologies

From the 1980s onward, new technologies emerged to add to the practice of nephrology. In 1985, the first robotic surgery was performed in humans, and this area has shown considerable progress, especially in the 1990s. In 2000, the da Vinci robotic surgery system obtained approval by the FDA for general laparoscopic procedures and became the first operative surgical robot in the USA [52]. This method has been applied in kidney transplants, with faster donor and recipient recovery [53]. The first successful robotic assisted kidney transplantation (RAKT) was performed in France in the beginning of this century [54]. In more recent years (2018–2020), large case series have been published with successful robotic assisted kidney transplant [55].

In 1992, nephrologist Burton Rose created the "UpToDate in Nephrology and Hypertension" platform. Subsequently, he decided to expand the product and convinced leaders from other specialties to do so, renaming the program "UpToDate in Medicine," which later became simply "UpToDate." Currently, the platform has numerous updated articles on several areas of medicine and more than two million users in approximately 200 countries [56], becoming one of the most important online update platforms in medicine in the world.

Telemedicine can be considered as existing practice since the advent of "new" communications methods, such as telegraph in 1844 and telephone in 1876, providing patients with the possibility to contact their physicians in a faster and cheaper way [57]. With the growth of the internet and, consequently, of digital health, nephrology has also acquired new technologies. One of the main tools is telenephrology, which, just like telemedicine as a whole, is based on consultations and other virtual strategies. It is currently being applied in several countries with evidence of success, allowing the nephrologist to contact other specialties and patients, as well as providing effective care for conditions such as CKD [58]. There are successful experiences in telenephrology around the world [59–61], and an unprecedent increase in telemedicine application was seen with the emergence of COVID-19 pandemic [62, 63].

Another currently widely used strategy, especially with the emergence of smartphones, tablets, and other mobile devices, is applications. These can be used in many areas, and therefore, there are many health-oriented applications. Consequently, this scenario extends to nephrology. Lee et al. [64] identified 177 applications related to CKD. The most common functionalities were CKD information and CKD self-management (57%), e-consultation (25%), CKD nutrition education (24%), and estimated glomerular filtration rate (eGFR) calculators (19%) [64]. Moreover, the self-management function is common among the applications, as a study by Ong et al. [65], carried out in 2016 based on blood pressure (BP) monitoring of patients with CKD, showed a reduction in the users' systolic (SBP) and diastolic blood pressure (DBP) [65].

Another technology that promises to have an impact in healthcare sector is artificial intelligence (AI), defined as "machines that mimic cognitive functions that

humans associate with the human mind, such as learning and problem-solving," or, in a simpler definition, intelligence made by machines, with minimal human use [66]. AI is generally applied through a method called "Machine Learning," which allows the computer to learn without being programmed with algorithms. In addition, this tool already has important applications in nephrology, such as the IBM Watson system, from the Italian Society of Nephrology, which answers questions about calcimimetics based on scientific documents (https://sinitaly.org/watson/). Moreover, one can mention the PathoSpotter, created in Brazil, which contributes to the identification of glomerular pathologies and can be very useful for renal biopsy [67].

With the first steps toward creating advanced AI, as well as the advancement of telemedicine, it is possible to look at the future of nephrology with great optimism [53]. Furthermore, with the increase in the use of smartphones and their applications, social networks, research sites, among others, the increasing patient empowerment is expected over the years [58, 68].

From the 1990s onward, new discoveries and historic landmarks emerged. In 2012, John B. Gurdon and Shinya Yamanaka received the Nobel Prize in Physiology or Medicine for discovering the ability of mature cells to become pluripotent when reprogrammed (iPS cells), which has been widely applied in nephrology research [69]. Moreover, the study by Katalin Susztak [70], based on the RNA sequencing of nephron cells, identified clusters of cells that had not been discovered before, which may be essential to elucidate and treat kidney diseases. In 2010, two variants of the APOL1 protein gene coding sequence on chromosome 22 were discovered and shown to have the highest association with CKD [71]. There are currently several studies on gene editing, and one of the most exciting system of gene editing is the so-called clustered regularly interspaced short palindromic repeats (CRISPR), which can be applied in the treatment of many genetic diseases of the kidneys [72].

Other technologies are being developed in the area of nephrology, such as the implantable bioartificial kidney, which aims to simulate kidney function, as well as others, such as 3D bioprinting, kidney-on-a-chip, kidney organoids, and others [53]. These and other tools, despite demanding a wide range of studies in bioengineering, nanotechnology, and cell studies, promise to revolutionize nephrology and bring more quality of life to patients (Fig. 1.2).

Fig. 1.2 A "journey" through the history of nephrology, from Hippocrates to robotics (ancient times—2022)

1.9 Conclusion

Nephrology is a medical specialty that since ancient times challenged researchers to overcome barriers, and as science develops, technology is applied to this area of medicine. Nephrology currently has effective therapies, such as dialysis, kidney transplant, and immunosuppressant use, which have prolonged the survival kidney patients. With the development of science, the pathophysiology of kidney diseases has been better understood. The main pathological entity of the specialty, "renal failure," has been acquiring new dimensions and nomenclatures (acute kidney injury/acute kidney disease/chronic kidney disease). Treatment of acute kidney injury has improved significantly in recent years, and there has been a significant reduction in mortality of patients with this condition. Chronic kidney disease has been reaching epidemic levels worldwide, acquiring the status of a public health problem. Nephrology is a specialty closely related to public health policies, since most patients cannot afford treatment, and government should subside treatment payment. The digital era arises with several technologies applied to the healthcare sector, and promising innovative tools could provide disruptive methods for the management of kidney diseases.

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Chapter 2
Genetic Engineering of the Kidney

Constanca Figueiredo and Rainer Blasczyk

2.1 Introduction

Kidney transplantation (KTx) is currently the best treatment to increase quality of life and survival of patients suffering from end-stage kidney disease (ESKD). KTx is the most common organ transplantation in Europe. However, the large discrepancy between the number of organs available and the number of patients in need of KTx shows the necessity to develop efficient approaches that might help to prevent ESKD or prolong graft survival after KTx. Despite remarkable progresses in immunosuppressive regimens that have improved the 1-year kidney graft survival of higher than 95%, the long-term kidney graft outcome did not change. Furthermore, the severe side effects of immunosuppression such as an increased risk for malignancy and infections are a continuous and serious threat for patient survival [1, 2].

2.2 Kidney Gene Therapy

In the past years, gene therapy has gained greater acceptance based on the development of successful therapies for the treatment of B-cell cancers, neuromuscular atrophy, retinal degeneration, and viral infections [3]. In this line, several attempts have been performed to genetically engineer nearly every organ including liver, lung, and kidney. In contrast to other organs, kidney presents significant hurdles to genetic modification due to the multi-type cell composition including tubular

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epithelia, interstitial, glomerular, and vascular cells resulting in complex anatomical structures and physiological properties. In the medulla, the cells are confronted with higher osmolality, pH alterations, and hypoxia. These unique kidney features pose significant challenges when selecting the type of vector and method of administration to the kidney [4, 5]. In this chapter, we provide an overview about the late progresses and promising applications of kidney genetic engineering. In particular, a gene therapeutic approach to decrease the immunogenicity of the renal graft by silencing the expression of the major histocompatibility complex (MHC) proteins toward increasing graft survival will be presented and discussed.

2.3 Vector Systems in Kidney Gene Therapy

Mainly, three types of vectors including adenoviral vectors, adeno-associated vectors and lentiviral vectors were used as vehicle for the delivery or knockdown of specific genes. Furthermore, gene therapy vectors might be administered to the kidney by four routes: systemic, anterograde or retrograde administration, and intraparenchymal injection [4].

2.3.1 Adenoviral Vectors

Adenoviral (Ad) vectors constituted one of the first gene-delivery viral system applied to the kidney. Those vectors were derived from DNA viruses and are characterized for their capability of packing approximately 8–36 Kb and of transducing a broad variety of cell types. In earlier attempts, Ad vectors were used to transduce the liver as a strategy to secrete proteins to treat kidney diseases [4, 6]. Efficiency of genetic engineering of the kidney using Ad vectors showed to be challenging and tightly dependent on the type of animal model, dose, and route of administration. The experimental studies using Ad vectors were performed in murine models using kidneys of B6/129 mice, FGS/kist mice, Brown Norway rats, F344 rats, or Dark Agouti rats [4, 7, 8]. The vector doses administered to the animals varied between 10^8 and 10^{11} vector particles [9, 10]. One study in the pig reported the use of 10^{13} vector particles [6]. Ad vector-mediated genetic modification of the kidney to express a number of genes has been demonstrated to contribute to the prolongation of graft survival after transplantation. In particular, Ad vectors were used to deliver a variety of genes including IL-10, IDO, TGF-ß, eNOS, CTLA4Ig, and catalase [8, 11–14]. Also, RAGE shRNAs were delivered by Ad vectors to the kidney to prevent cystic disease [15]. Despite the capability of transducing renal cells, the transgene expression is transient due to the episomal localization inside the cell. Furthermore, Ad vector transduced cells are targets for innate and adaptive immune responses. Hence, Ad vector genetic modified kidneys might be dependent on immunosuppression to prevent the elimination of the transduced cells [4].

2.3.2 Adeno-Associated Viral Vectors

Since the approval of adeno-associated viral (AAV) vectors for gene therapeutic approaches to treat retinal degeneration and spinal muscular dystrophy, AAV vectors have been widely applied in the development of innovative gene therapeutic approaches for various diseases. AAVs are single-stranded DNA vectors with a small size genetic cargo of 4.5Kb. AAV studies in the kidney have been performed mainly in C57Bl/6, Bl6, and Balb/c mice and Lewis rats. The majority of the studies focus on the expression of reporter genes including GFP, LacZ, or Luciferase. Other proof-of-concept studies used AAV vectors to express therapeutic genes such as protective protein/cathepsin A (PPCA) to treat the lysosomal storage disease sialidosis, FKRP as a strategy for muscular dystrophies, or human Frataxin to treat Friedreich ataxia and hepatocyte growth factor (HGF) as a bidirectional treatment strategy to prevent renal fibrosis [16–20]. Renal transduction efficiencies with AAV vectors showed to be dependent on the serotype used. In contrast to serotype 1, 3, 4, and 5 which did not result in transgene expression, AAV2, 8, and 9 have shown some ability to promote transgene expression in the kidney. In models using AAV9 delivered by systemic administration, therapeutic efficacy was achieved despite the low transgene expression [18]. In 2018, Ikeda and colleagues showed that the incorporation of synthetic capsid proteins such as Anc80 significantly increases the kidney transduction efficiencies after systemic delivery via the mouse retro-orbital venous plexus [21]. Furthermore, the vector dose is strongly associated with the kidney transduction efficiency. AAV vector doses of less than 10^9 particles/kidney appeared not to be sufficient to enable the genetic modification of the kidney or only in rare events, in contrast to AAV doses higher than 10^{10} which have shown to contribute to detectable transgene expression [22–24]. The widespread application of AAV vectors in the gene therapy of kidney diseases may require the optimization of the vector construction such as the engineering of the capsid.

2.3.3 Lentiviral Vectors

Several studies have demonstrated the feasibility to transduce the kidney using lentiviral vectors (LV) applied through different routes of administration including systemic, retrograde, or pelvic injection. Moderate transduction rates have been achieved using LVs pseudotyped with the vesicular stomatitis virus G protein. Remarkably, relatively low doses of LVs have been used to genetically engineer the kidney. Different studies have reported the use of LV doses varying between 5×10^6 and 10^9 in mice. In particular, multiple doses applied at different time points have been associated with an increase in kidney transduction efficiencies. In those studies, different mice strains including C57BL/6, Balb/c, or FVB/N mice were used [7, 15, 25–28]. The LV-mediated genetic modification of the kidney showed

therapeutic efficacy in minimizing tubular damage triggered by ischemia reperfusion injury. Remarkably, genetic engineering of the kidney using LVs has focused on the delivery of short hairpin RNAs (shRNAs) to decrease the expression target proteins, thereby contributing to prevent disease progression. These approaches have demonstrated therapeutic efficacy in preventing diabetic nephropathy, lupus nephritis, or sepsis-induced acute kidney injury [27, 29–31]. Furthermore, we have used LVs to deliver shRNAs targeting the human leukocyte antigen protein as a strategy to reduce the immunogenicity of the kidney and support long-term graft survival after transplantation [32]. As LVs have a preferential capacity to transduce actively proliferating cells, they have gained particular attention in the treatment of renal cell carcinoma or genetic diseases such as polycystic kidney disease [33, 34]. Genetic engineering of the kidney may benefit from LVs due to their ability to integrate in the genome, therefore supporting a permanent expression of the transgenes in the transduced cells and their progeny.

2.3.4 Genetic Modification of the Kidney During Ex Vivo Machine Perfusion

In 2021, more than 10,000 patients were on the waiting list for a kidney transplant in the Eurotransplant region alone. Nevertheless, only about 4000 kidneys from deceased and living donors were transplanted [35]. This discrepancy between patients in need of a renal graft and number of kidneys available for transplant reflects not only the need for the development of strategies enabling the use of organs donated after circulatory death or from extended criteria donors but also the design of innovative strategies that prevent end-stage kidney disease or rejection after transplantation.

Ex vivo kidney perfusion (EVKP) emerged as a promising technology to support the expansion of the donor pool because it may circumvent the challenges posed by the increased immunogenicity of the graft due to ischemia reperfusion injury (IRI). EVKP allows for the assessment of organ quality and function during preservation. Recently, hypothermic machine perfusion became widely used in clinical practice because it showed to improve the outcome of kidney grafts after transplantation. Experimental and clinical studies have extended the application of EVKP not only to prevent IRI but also to promote healing and regeneration [5]. Furthermore, we have applied EVKP as a method to deliver lentiviral vectors for genetic engineering of the kidney toward reduction of the graft immunogenicity. Previously, we have showed the feasibility to genetically engineer porcine lungs by delivering lentiviral vectors encoding for shRNAs targeting β2-microglobulin and the class II transactivator to silence SLA class I and class II, respectively. Ex vivo machine perfusion of the lung under normothermic conditions has been demonstrated to be an efficient approach for the administration of lentiviral vectors. In this study, all regions of the lung were genetically engineered as shown by the expression of the reporter gene,

and the expression of SLA class I and II was efficiently downregulated [36]. Remarkably, the high efficacy of the use of ex vivo machine perfusion to genetically engineer complex tissue structures was re-confirmed in a hind limb perfusion rat model with lentiviral vectors at subnormothermic conditions. Different cell types were shown to be genetically modified including endothelial and epithelial cells as well as muscle cells. Furthermore, 87% of skin keratinocytes showed to express the reporter gene [37]. These data shows that the delivery of gene therapeutic agents such as lentiviral vectors during ex vivo organ perfusion is an efficient strategy to support the genetic engineering of organs and vascular composite grafts while supporting their preservation.

2.3.5 MHC Antigens as Targets in Transplant Rejection

Genetic discrepancies between donors and recipients of renal grafts pose a significant hurdle to a successful transplantation outcome. In particular, the high variability of the major histocompatibility complex (MHC) loci, in humans known as human leukocyte antigen (HLA), remains a relevant obstacle to long-term graft survival after transplantation. HLA-sensitized patients due to previous transplantations, blood transfusions, or pregnancies with panel reactive antibodies (PRA) higher than 80% have to wait longer for a suitable graft leading to increased morbidity and mortality [38]. Despite large progress in histocompatibility testing abrogated the risk for hyperacute rejection, acute and chronic antibody-mediated rejection persist as a key contributor to decrease renal graft survival. Donor specific antibodies (DSA) targeting mismatched HLA class I and II proteins are the key players during ABMR. Beside, DSA-mediated tissue injury caused by complement binding, a multitude of complement-independent antibody-mediated mechanisms may cause irreversible tissue damage. After binding their targets in the endothelium, anti-HLA class I antibodies trigger the activation of the endothelium and recruitment of immune cells. Also, DSA induces migration and proliferation of smooth muscle cells and stress fiber formation supporting tissue fibrosis. Furthermore, the recognition of the organ endothelium by anti-HLA class II antibodies have been associated with endothelial to mesenchymal transition in the absence of C4d deposition as well as supporting inflammation [39, 40].

Discrepancies at the HLA loci between donor and recipients may trigger T-cell-mediated rejection (TCMR). After recognition of the foreign organ, effector T cells cross the endothelium, infiltrate the graft, and initiate an interstitial inflammatory process upon interaction with host or donor antigen-presenting cells with recruitment of effect and memory T cells and macrophages. TCMR is characterized by a high production of IFN-y that induces the expression of different genes inducing the chemokines CCL5, CXCL9, CXCL10, and CXCL11. TCMR may cause irreversible nephron loss [41]. Hence, HLA is a major target to genetic engineering of the kidney toward preventing rejection after transplantation.

2.3.6 Genetic Engineering the Kidney to Downregulate MHC Expression

In previous studies, we have genetically engineered rat and porcine kidneys using lentiviral vectors encoding for short hairpin RNAs targeting β2-microglobulin (shβ2m) and class II transactivator (shCIITA) to silence MHC class I and II, respectively. As control, a lentiviral vector encoding for a nonspecific shRNA (shNS) was used. Furthermore, the vectors encoded for reporter gene sequences of a secreted form of the luciferase from *Oplophorus gracilirostris* (NanoLuc, NL) or Neongreen. Secreted NL offers the possibility to indirectly evaluate the vector activity by monitoring the levels of bioluminescence in easy to collect samples such as blood and plasma. The use of intracellular reporter genes such as Neongreen enables the precise quantification of the transduction efficiency. To evaluate the stability of the genetic modification, the kidneys were transplanted in MHC-mismatched allogeneic setting. In this model, kidneys of Lewis rats were retrieved and perfused with WME media supplemented with 5% BSA. The kidneys were perfused for 2 h with up to 0.7–2 × 10^{11} viral particles encoding for the shRNAs and NL as reported gene at 31–32 °C, flow rates of 9–12 ml/min, and a pressure of 80–95 mmHg. Afterwards, the free residual vector particles were removed by perfusion with a mixture of blood and WME. Then, the genetically engineered kidneys were cooled down, placed on ice, and transplanted orthotopically. Serum and urine (Fig. 2.1) samples were collected weekly to evaluate the expression of the reporter gene NL by measuring bioluminescence. Already 1 week after KTx, an increase in bioluminescence levels was observed. The bioluminescence levels detected in serum and plasma remained stable throughout the entire monitoring time of 6 weeks, indicating the stability of the genetic engineering.

Similarly, the MHC class I and class II silencing effect mediated by the specific shRNAs encoded by the lentiviral vector was maintained after KTx. β2-Microglobulin remained silenced by up to 71% in the renal tissues engineered for the expression of shβ2m in comparison to shNS-expressing kidneys. Also, CIITA transcript levels were downregulated by 70% in the renal grafts perfused with shCIITA-encoding vector particles in comparison to the control kidneys.

Size is a crucial parameter in genetic engineering of the organ, as it implies an adjustment of the vector dose used to perfuse the organ. Ex vivo organ perfusion for the delivery of LVs is an efficient strategy because it allows the recirculation of the LV through the organ minimizing wasting of the vector. However, the dose and the time of perfusion with the LV need to be adjusted to the size of the organ to achieve high transduction efficiencies even in large organs. Besides rat kidney, we also have shown the feasibility to transduce porcine kidneys. Land race pig kidneys were retrieved and connected to an ex vivo perfusion system (Kidney Assist, XVIVO, Groningen, The Netherlands) and perfused for 4 h with 1 × 10^{12} LVs encoding for Nanoluciferase as reporter gene. Endothelial cells were isolated from different regions of the kidney after perfusion and bioluminescence was measured to detect the expression of the reporter gene. Bioluminescence activity was detected in the

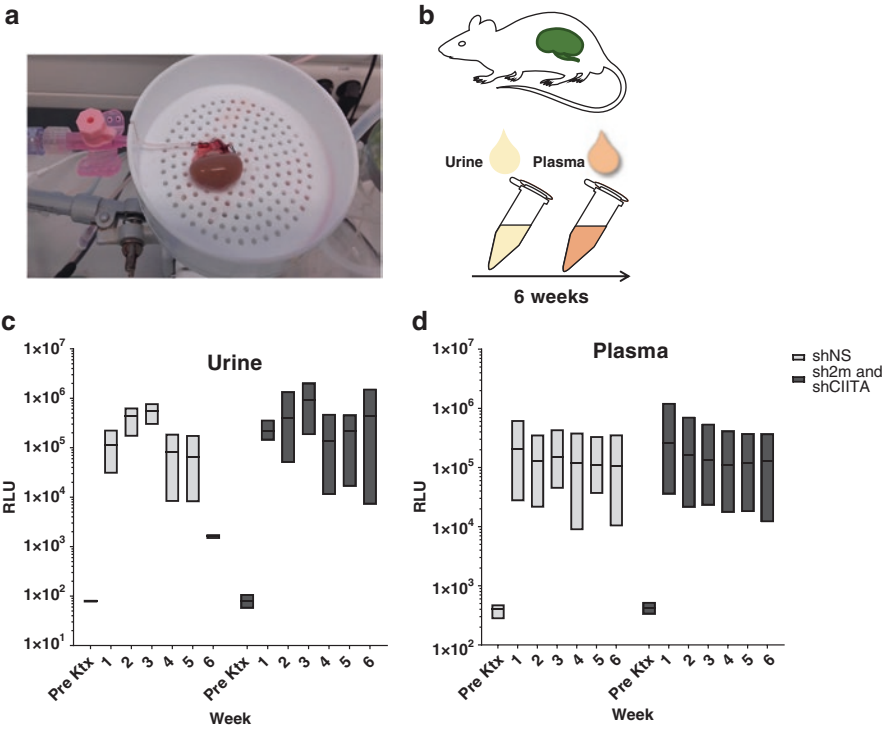

Fig. 2.1 Genetic engineering of rat kidneys. (**a**) Rat kidneys were connected to the perfusion system and perfused for 2 h with an LV encoding for shRNAs targeting β2-microglobulin and the class II transactivator (shβ2m/shCIITA) or a nonspecific shRNA (shNS) and nanoluciferase as reporter gene. (**b**) Genetically modified kidneys were transplanted. Urine and plasma samples were collected weekly during 6 weeks. Relative luminescence units (RLU) detected in (**c**) urine and (**d**) plasma

supernatant of endothelial cell culture isolated from the different kidney regions (Fig. 2.2).

Despite the feasibility to genetically engineer kidneys during ex vivo perfusion, it is important to elucidate the impact of the perfusion with the lentiviral vectors in the kidney tissue. Therefore, in previous studies, we have analyzed the levels of different injury markers and cytokines during EVKP. The detection of lactate dehydrogenase (LDH) is a widespread method to evaluate cell death in vitro because it is usually released from necrotic or apoptotic cells. Recently, LDH became a marker of tissue injury, and also plasma and urinary LDH levels could be correlated with the severity of kidney injury in AKI models [42–44]. Genetic modification of the kidney with lentiviral vectors did not increase the level of tissue damage compared with kidneys perfused without the vector. Already during hypothermic EVKP, the oxygenation of the perfusion solution showed to support the quality of the perfused kidney. During sub-normothermic perfusion or normothermic perfusion, there is an increased oxygen demand associated with higher metabolism. Oxygenation of the

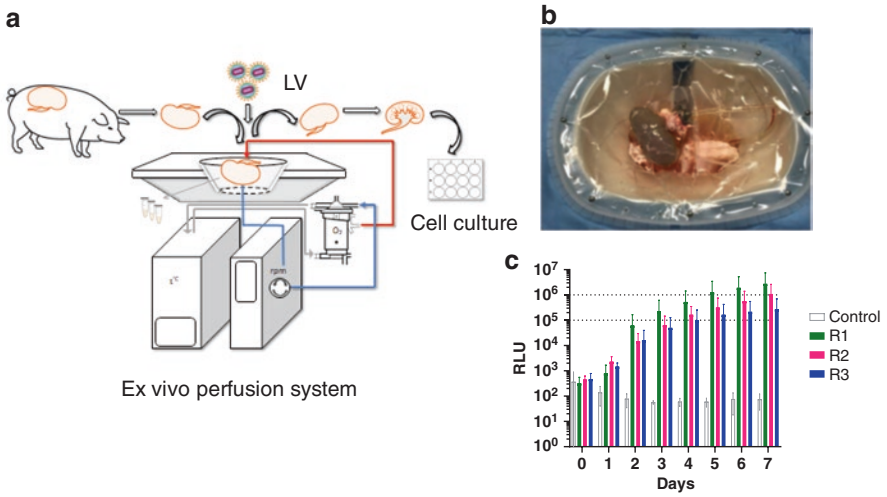

Fig. 2.2 Genetic engineering of porcine kidneys. Land race porcine kidneys were perfused 4h with lentiviral vectors (LV) encoding for nanoluciferase as reporter gene. (**a**) Schematic representation of the ex vivo perfusion of the kidney with LV. (**b**) Representative picture of a porcine kidney connected to the perfusion system. (**c**) Relative luminescence units (RLU) measured on the supernatant of endothelial cell cultures after kidney perfusion with LV. As control, endothelial cells of kidneys perfused without vector were used

perfusion solution may be beneficial to reduce oxidative stress and supporting the energy status and several groups are developing efficient oxygen carriers that might become an efficient alternative to red blood cells. Mainly hemoglobin-based oxygen carriers (HBOC) and perfluorocarbon-based oxygen carriers (PFCs) have gained plenty of attention. HBOC are capable to covalently bind HB in a similar manner to hemoglobin (Hb) in RBCs. M101 (Hemarina), a natural Hb biopolymer isolated from the lugworm "Arenicola marina," was the first HBOC tested in a clinical trial showing to support graft function after KTx and better kidney function [45]. Other studies showed that used HBOC-201 or Hemopure during subnormothermic EVKP exhibited similar renal flows as blood perfused kidneys and significantly less tissue injury than static cold stored kidneys [46, 47]. PFCs are hydrocarbons whose hydrogen groups were replaced by fluorine atoms and represent an alternative to HBOCs. Perfluorooctyl bromide and perfluorodecalin have been widely used PFCs. In particular, PFCs have been used during ex vivo heart perfusion [48]. The use of oxygen carriers as an additive to acellular perfusion solutions may in the future contribute to improve the genetic modification of organs by supporting the cell metabolism and preventing tissue injury.

Cytokines are essential regulators of immune responses and contribute for tissue homeostasis and host defense. After transplantation, cytokines are major drivers of inflammatory processes that might contribute to graft rejection. The process of ex vivo kidney perfusion per se has showed to alter the cytokine secretion profile of the kidney. In our studies we have evaluated whether perfusion of kidney with LVs

further modifies cytokine signatures in the renal tissue. In comparison to rat kidneys perfused without LVs, kidneys perfused with this gene therapeutic vector showed increased levels of MIP-1α, MIP-2, IP-10, IL-10, and EGF and decreased levels of IL-12p70, IL-17, MCP-1, and IFN-y [32]. Hence, perfusion with LVs affects the type of cytokines secreted by the kidney, and future studies will have to access the impact of this alteration in a transplantation setting.

Despite LVs represent an effective method to genetically engineer different biological graft types of different complexities, their use is associated with safety concerns such as insertional mutagenesis. Nevertheless, the application of third-generation self-inactivating LVs has recently been used in multiple clinical trials, and their mediated clinical success has opened the pathway to the first regulatory approval in 2017 [49]. In contrast to the systemic application of LVs that caused no or limited transduction of the target organ and unspecific transduction of the other tissues, we have showed that the delivery of the LV during EVKP enables the specific transduction of the kidney. After transplantation of the genetically modified kidneys, the vector remains localized to the graft. Therefore, the delivery of LVs via EVKP has the potential to decrease the risk for off-target effects and increase safety of the future use of genetically engineered kidneys.

2.4 Future Perspectives and Conclusion

Our studies have demonstrated the feasibility to silence the expression of kidney allografts as a strategy to decrease their immunogenicity toward increasing graft survival after transplantation. The delivery of LV during EVKP has been demonstrated to be an efficient method to genetically engineer the allograft during the preservation time. Hence, this allograft engineering approach represents a robust biotechnological platform strategy to permanently engineer kidneys. Beyond allografts, it could enable the development of innovative therapies through autograft engineering to treat kidney diseases based on genetic aberrations or preventing end-stage kidney disease by expressing protective genes.

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Chapter 3
Stem Cells: Use in Nephrology

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3.1 Introduction: Stem Cells and Kidney Diseases

Kidney diseases continuously raise serious concerns for human health and pose a challenging and costly public health problem at a global level. In recent years, a greater number of cases of kidney diseases worldwide have been reported, associated with aging and demographic transition processes, resulting from the increase in the population's life expectancy [1]. Hypertension, diabetes, and stress are multiplying factors, as well as socioeconomic, racial, and gender disparities, considered determinant factors for kidney diseases [2, 3].

The therapy for kidney diseases is intimately connected to its physiopathology [4]. The therapeutic maneuvers used to prevent the progression of kidney disease are not completely effective, whereas the treatment framework and therapeutic options, in addition to the limitation of certain drugs in acting on certain mechanisms, remain major obstacles. This scenario leads patients with kidney diseases to undergo replacement therapies such as dialysis, a temporary solution coexisting with an inferior quality of life for patients, or kidney transplantation [5]. Thus, there is a call to find new, more viable and effective strategies to prevent or stop progression, or even reverse kidney disease and, thus, to improve quality of life and patient survival.

Some steps have been taken in the past; however, the next necessary one seems to be toward stem cells and regenerative medicine. The use of stem cells and the ability to manufacture functional human tissue prove to be advantageous due to the possibility of their use in applications such as disease modeling and drug tracking

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and, ultimately, in tissue and organ repair and regeneration. However, human tissues are inherently complex and often organized into architectures composed of multiple cell types, extracellular matrix, and vasculature. These procedures increase with the complexity of structural organs [6]. Kidney is an intricate organ, made up of more than 26 different specialized cell types [7, 8], with a complex extracellular matrix of proteins and glycosaminoglycans, and organized into approximately 1 million microarchitectures, namely the nephrons. These structures are responsible for the regulation of volume, composition of body fluid compartments, maintenance of acid–base balance, excretion of metabolic waste, and production of hormones that control blood pressure and erythropoiesis [9, 10].

In recent decades, there have been numerous studies on the potential use of stem cells from different sources for the treatment of kidney diseases [11–13]. Stem cells can act by secreting bioactive paracrine factors and extracellular vesicles with immunomodulating and repairing properties of injured renal tissues [14, 15]. The evidence is complemented by the establishment of induced pluripotent stem cells (IPSC) and the targeted ability to differentiate into a renal lineage of these cells to self-organize and generate organoids for disease modeling, drug screening, and even for renal replacement [16].

This chapter summarizes an overview of the characteristics of different types of stem cells, organoids, mechanisms of action, clinical studies, and ethical issues on cell-based therapy for kidney diseases.

3.2 Sources of Stem Cells for Renal Therapy

There are a diversity of cells that can be employed as cellular therapy for kidney and can be helpful also for kidney physiopathological studies:

a. Embryonic stem cells (ESC) and induced pluripotent stem cells (IPSC)
b. Mesenchymal stem/stromal cells
c. Renal stem cells/progenitors cells

Some products from those cells elicit a better response than the proper cells. In this scenario, stem cell extracellular-derived vesicles (EV) are showing great results mainly on an experimental basis, and we will exploit it here.

3.2.1 Embryonic Stem Cells (ESC) and Induced Pluripotent Stem Cell (iPSC)

Embryonic stem cells (ESC) are obtained from the inner cell mass of blastocysts under certain culture conditions. From Thompson's pivotal experiment to nowadays, a lot of improvements have been made in the culture of these cells: use of defined medium, avoidance of animal-based products in culture, phenotypic

markers, genetic studies, and so on [17]. ESCs have two main characteristics: (1) self-renew and (2) huge potency to differentiate in other cells from body tissues, under specific factors and stimuli. This last characteristic defines them as pluripotent cells. Moreover, this high pluripotency is the appeal for regenerative medicine: you can generate the cell you need! [18].

Renal organogenesis is a highly complex process [19]. It results from the interaction of several cells and from different origins, stimulated by distinguished factors, elegantly reviewed by several authors [20–22]. More recently, with advances in global gene analyses, even single-cell transcriptomes, we now can understand the role of each cell and the multiple factors involved in this process [23]. From this knowledge, researchers have been working with ESC to recreate a kidney or, at least, to generate some cells [18, 24], although several multistep processes are still laboring and challenging. Time-dependent factors, dose-dependent stimulus, and cell-dependent interactions are necessary to generate one cell from the kidney [18]. And after generating this one, you should mix all cells together to create a kidney. In 2D culture conditions, it is quite impossible. Organoids or 3D cultured cells are simplifying those steps, and we are close to recreate a kidney in a petri dish. Organoids will be more detailed below. Then, it is possible to generate a precursor of a renal cell. Nephron progenitor cells (NPCs) and ureteric buds (UB) have been generated by differentiation-based protocols from ESC [25].

During renal organogenesis in mammals, Osr1+ cells give rise to the metanephric mesenchyme (MM), which condenses to form the cap mesenchyme (CM) [26]. MM gives rise to the nephrons and interstitium, while UB differentiates to elaborate the lower urinary tract from the collecting ducts to a part of the urinary bladder.

Protocols for differentiation of ESC to NPC or UB are different from each group. However some molecules and markers can be summarized (see Fig. 3.1) [27, 29–34]:

Due to ethical issues, tumorigenesis/teratomas formation, and all rejection problems, the use of ESC needs attention when translating to clinical therapeutics. Despite this, all knowledge that ESC can give us is invaluable [35].

In 2006, Takahashi and Yamanaka published an article that changed the research worldwide. They recreated a pluripotent cell from an adult cell [36]. Induced pluripotent stem cells (iPSC) are adult cells that go back to the past. Usually working with blood cells or skin fibroblast, some genes are integrated at the cell (OCT4, Nanog, SOX2 mainly) and then cells go back to its pluripotent stage, like an ESC stage. Due to its pluripotency and self-renew capability, iPSC once obtained work as an ESC cell and may generate as many cells as possible when stimulated. Groups have worked hard to obtain kidney cells from IPSC, using a lot of strategies (Table 3.1).

Cell replacement therapy (isolating cells from the same patient) and drug screening are the main attractive issues from IPSC research regarding kidney cell therapy. However, some issues still need investigation. There are few studies regarding epigenetics in kidney-derived IPSC. Since the cell is obtained from a human adult cell, a lot of genetic modifications have already happened [45, 46]. Moreover, genetic

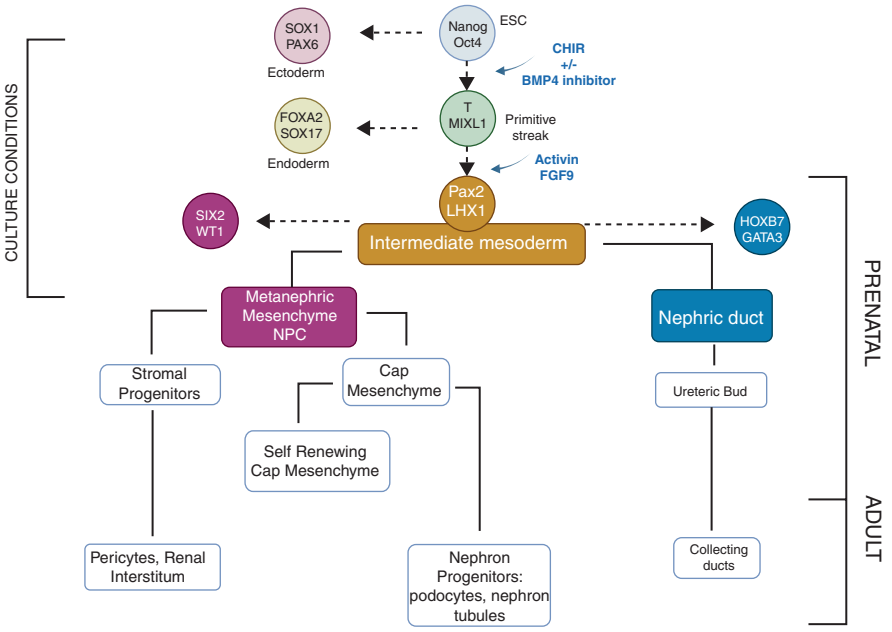

Fig. 3.1 Differentiation steps to generate nephron progenitor cells (NPC) and ureteric buds (UB) from embryonic stem cells (ESC) in culture conditions and following the lineage tree of renal cell types in human development. Stage proteins used as parameters for differentiation in culture conditions are identified in the circles. In blue, main molecules that must be present/absent at medium for differentiation in culture. Abbreviations: BMP, bone morphogenetic protein; FGF9, fibroblast growth factor 9; LHX1, LIM homeobox 1; LPM, lateral plate mesoderm; NPC, nephron progenitor cell; OCT4, POU class 5 homeobox 1; PAX2, paired box 2; ESC, embryonic pluripotent stem cell; SIX2, SIX homeobox 2; SOX2, SRY-box 2; T, brachyury; WT1, Wilms tumor 1. Image adapted from Takasato et al. [27] and Schumacher et al. [28]. Created with BioRender.com

abnormalities and tumorigenic concerns are still a problem since it is a culture cell and due to its pluripotency [47]. One positive aspect is that the ongoing studies have demonstrated that those kidney-derived cells are functional cells as seen in Table 3.1. Since organoids and those differentiated cells are not able to get vascularized, some researchers go further and repopulate decellularized kidneys with kidney derived-IPSC cells or organoids, and they have been showing better results than single-cell administration and a more feasible and closer clinical translation [48]. In the field of generation kidney-derived IPS, standardization of culture protocols urges attention. It is a difficult goal. There are a diversity of techniques to induce kidney cell generation and poor reproducibility that, nowadays, restrict scalability.

Despite clinical therapeutics for kidney-derived IPSC cells still needing to fight strong battles, IPSC easily creates an incredible tool: once IPSC is obtained from patients, we can exploit and better understand several genetic diseases [49–53].

Table 3.1 IPSC induction to generate kidney cells and its functional assays

Origin cell	Derived cells	Functional assay	References
IPSC	Podocytes	Cytoplasmic contractile response to angiotensin II, functional evidence of albumin uptake in the cytoplasm of iPS podocytes comparable to human podocytes, integration capacity of iPS-derived podocyte progenitors in an in vitro nephrogenesis reaggregation assay	Song et al. [37]
IPSC	Podocytes	transplantation method using spacers that release the tension of host kidney capsules, allowing the effective formation of glomeruli from human iPS cell–derived nephron progenitors	Sharmin et al. [38]
IPSC	Renal progenitors, i.e., nephrogenic intermediate mesoderm and metanephric mesenchyme	Intravenously infused iPSC-derived RPCs in a cisplatin mouse model	Imberti et al. [39]
IPSC	Kidney organoids	Dextran uptake by proximal tubules, upon implantation of the kidney organoids at renal capsule of immunocompromised mice they observed an increase in the number of vessels and glomeruli gradually acquired a much more mature architecture and the size-selective dextran handling	Low et al. [40]
IPSC	Ureteric epithelium and the metanephric mesenchyme in monolayer culture, following organoid culture conditions and fully kidney formation	Dextran uptake assay showing endocytic ability, cisplatin model at kidney organoid leading to cell apoptosis	Takasato et al. [41]
IPSC	Kidney organoids contain nephrons associated with a collecting duct network surrounded by renal interstitium and endothelial cells. Within these organoids, individual nephrons segment into distal and proximal tubules, early loops of Henle, and glomeruli containing podocytes elaborating foot processes and undergoing vascularization	Proximal tubules endocytose dextran and differentially apoptose in response to cisplatin	Takasato et al. [41]
IPSC	Metanephric mesenchyme	Glomeruli vascularized upon transplantation	Taguchi et al. [42]
IPSC	Kidney organoids containing podocytes, proximal and distal tubular cells, stromal cells and endothelial cells	Express renin	Shankar et al. [43]
IPSC	Nephron progenitor cells (NPC)	In vitro tubule-like structures in three dimensional culture systems	Kang and Han [44]

Chronic kidney disease experimental model (5/6 nephrotomy) was conducted to analyze the effects of IPS locally administered. Amelioration of CKD parameters was observed; however, tumor-like formations in 5 out of 8 were observed in the remnant kidneys [54].

We can summarize some of the potential studies using ESC and IPSC in kidney area in five approaches (see Figs. 3.2 and 3.3):

1. Generation of specific cells from kidney to understand it and to develop basic research.
2. Generation of cells to seed at scaffold or a decellularized kidney. Since vascularization is a problem at organoids, some researchers understand that replacement therapies must be throughout a real scaffold/decellularized kidney and repopulate these scaffolds with kidney cells derived from IPSC cells [55, 56].
3. Generation of organoids of the kidney to generate more realistic experimental models and drug tests. This subject will be explored below [57, 58].
4. Directly injected into humans. This strategy is being conducted for other diseases, such as Parkinson [59], macular degeneration [60], etc.
5. Modeling diseases and drug discovery: harvesting cells from genetic disease patients is possible to better understand several diseases [51].

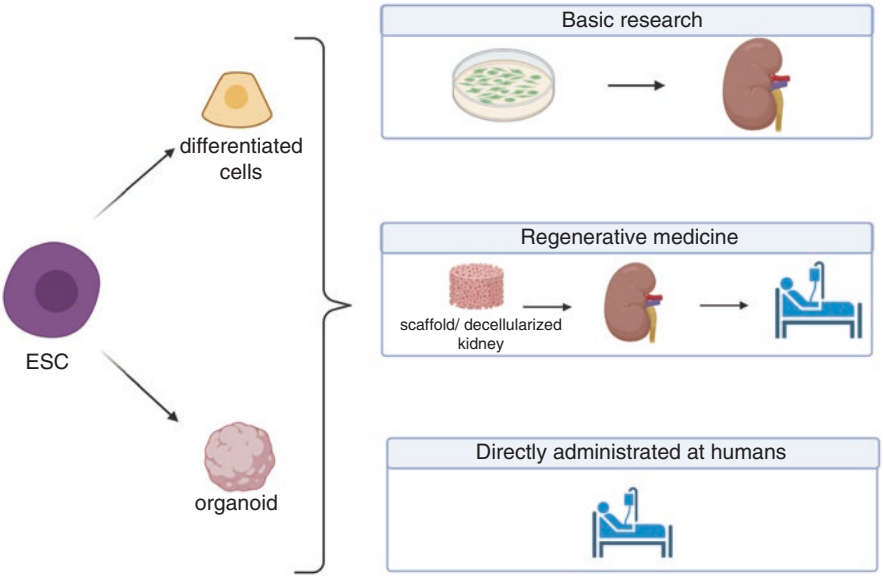

Fig. 3.2 Potential areas of ESC in kidney diseases. Embryonic stem cells (ESC) may be differentiated to the needed cell or to a complex of cells in 3D structure called organoid, under specific stimulus. At this point, they can be studied for basic research or injected in patients, throughout scaffolds or not. Direct administration of ESC at kidney disease in humans has not been tested. Created with BioRender

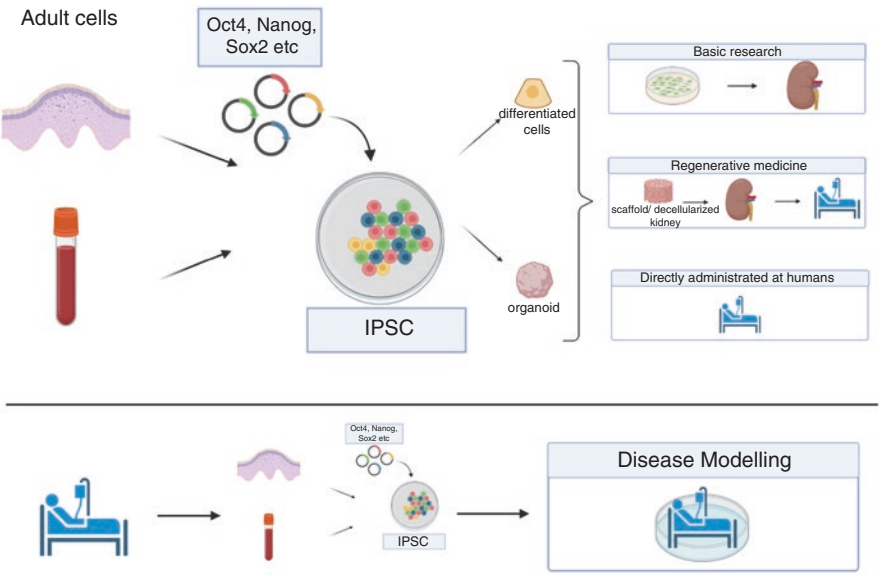

Fig. 3.3 Potential areas of IPS in kidney diseases. Since induced pluripotent stem cells (IPSC) have almost the same potential as ESC, the therapies and areas of studies of IPSC are quite the same as ESC. The advantage of using IPSC is that it is possible to take the cells from the patient, overcoming rejection in transplantation. Moreover, modeling disease is a huge tool for studying several genetics and metabolic diseases. Created with BioRender

3.2.2 Mesenchymal/Stromal Stem Cells

Mesenchymal stem cells, also known as multipotent mesenchymal stromal cells (MSCs), are the most studied cells in cellular therapy for kidney diseases—not only for kidney diseases but also for several illnesses.

These cells are collected from human body sites and then put at culture conditions to be expanded and to be administered into the patient (see Fig. 3.4). Mesenchymal stem cells are found in umbilical cord blood, adipose tissue, Wharton's jelly, bone marrow, dental pulp, and so on [61]. A recent consensus has standardized its abbreviation of MSCs from bone marrow as BM-MSCs, from adipose tissue as AD-MSCs and from umbilical cord as UC-MSCs [62]. Adult stem cells reside in areas where they are protected, namely niches [63, 64]. Some researchers point out that, since MSC can be found in virtually all sites of the body, perivascular areas may be the niche for part of MSC [65]. Other parts of MSC may have originated from nonperivascular sites [61, 66, 67].

How do you know if you collected the right cell and if they are the ones you need? Some consensus has been published and established some patterns. Dominici et al. [68], from International Society of Cell and Gene Therapy (ISCT), have described a minimum criteria to define MSC from bone marrow. Briefly, MSC should be plastic adherent, with positive extracellular proteins, mainly CD90,

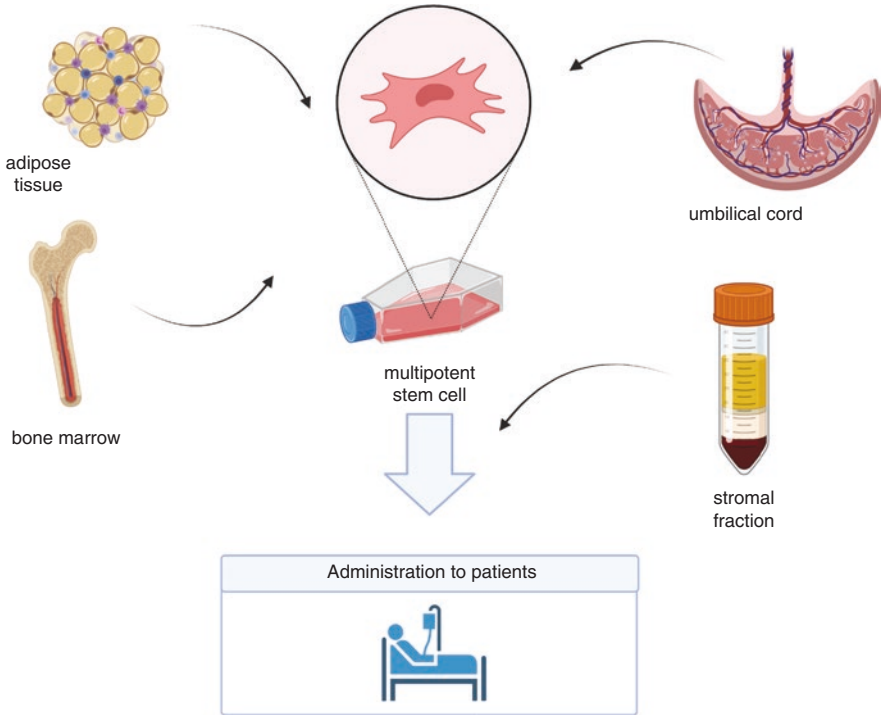

Fig. 3.4 Main sources of mesenchymal stem cell for clinical therapy. MSCs are cells expanded in vitro and then administered to patients. Stromal fraction of bone marrow or from adipose tissue may be used for clinical therapy with successful preclinical trials. Created with BioRender

CD73, and CD105, negative expression of CD11b, CD14, CD19, CD34, CD45, CD79a, and HLA-DR, and perform assays to characterize the multipotency properties (differentiation to adipocytes, chondroblast and osteoblast cells) [68]. In 2019, ISCT updated the consensus [69]; three new criteria have been added:

1. The origin of the MSC should be described: there are tissue-specific regenerative properties depending on the origin of the cell. For instance, CD34 expression is negative at BM-MSC and positive at AD-MSC [70].
2. Evidences of stemness in vitro or in vivo.
3. Functional assays.

However, this consensus does not define specific culture conditions, and it leads to a lot of differences and problems in quality, safety, and reproducibility to clinicals trials. In 2018, the marketing for MSC therapy has been authorized in Europe [71]. Nowadays, more than 250 trials are enrolled at Clinicals Trials.gov (studies found for: stem cell | kidney, also searched for Renal, Progenitor Cell, Process, and more, search date: October 6, 2021).

Since it has become a therapy, more attention should be paid to GMP manufacturing, quality control and safety, and efficiency tests. Regarding this issue, several articles have described some guidelines to achieve those goals [72–75].

The MSC's culture is heterogeneous. It is composed of fibroblasts, myofibroblasts, progenitors, among other possible cells. In this way, ISCT claims to analyze the functional properties of MSC that involves mainly its mechanism of action: analyze the secretion of trophic factors, the immunomodulation role, and its angiogenic pattern [76].

For kidney therapies, MSC treatment consists of administration of MSC expanded in vitro in patients. Since MSC is not fully recognized by the immune system, allogeneic transplantation is not a problem [77]. Administration of allogeneic MSC in patients does not elicit rejection, even with multiple infusions [77, 78]. On the other hand, the amount of cells, the MSC donor characteristics, the route of administration, periodicity or not of infusion, autologous or allogeneic MSC administration are not a well-standardized protocol and vary from study to study [79, 80]. How MSCs exert their therapeutic effect will be exploited below.

Among MSCs, adipose stem cells (AD-MSC) have several attractives to substitute BM-MSC: easy to collect and cultivate and quite the same properties of BM-MSC. Some differences have been observed, mainly in their response to injury. The source of MSC elicits differences in therapeutics responses [80]. Stromal fraction of bone marrow and from adipose tissue may be a noncultured option for cellular therapies. Indeed, these stromal fractions include a lot of other cells; they should be autologous administered, but they do not have the problems of a cultured cell: senescence and tumorigenesis [81, 82] (Fig. 3.4).

3.2.3 Renal Stem Cells

As mentioned above, virtually all tissues may harbor stem cells, some from a perivascular niche, other cells called progenitors from other niches that are unknown. The search for a tissue-specific adult stem cell is a huge task [63, 65, 83].

Is there a kidney renal progenitor? The fast regeneration of the kidney after an insult supports the idea of ready cells to repair. Highly turnover tissues harbor resident stem cells. Organs and tissue with low turnover may rely on progenitors cells [84]. At the kidney, progenitor cells may be the one closer to the lesion leading to this fast regeneration.

Several groups have been studying this population of cells. BrdU+-labeled retaining of cells [85], isolation of a highly proliferative cell from a nephron (named rKS56) [86], isolation of a CD133+ cell from kidney that expresses Pax2 (an embryonic marker) capable of generated tubules with epithelial markers [87], a Sca1+Lin- cell isolated from kidney that contribute to tubule repair [88] show that a kidney progenitor cell exists [89].

The parietal cells from Bowman's capsule, specially at the urinary pole, have been suggested as kidney progenitors. These cells were CD133+CD24+ (residual markers from embryonic kidney), and they differentiate in podocytes or tubule cells under culture conditions [90, 91].

Humphreys et al. elegantly described that the tubular epithelial regeneration after an injury is due to surviving epithelial cells, thus other cells/progenitors did not

contribute to its repair [92]. So, if the MSC or progenitor cells did not replace/differentiate into epithelial cells, the better outcomes obtained after their administration may be through its paracrine effects (see below at "Mechanism of Action").

Lindgren et al. described a possible tubular progenitor cell in humans. Sorting kidney cells ALDH^{high+} out, they found CD133+CD24+CD106- cells with regenerative properties. They can be located at the proximal tubule and at distal convoluted tubule [89, 93].

Renal papillae has also been described as a niche for kidney progenitor cells. Oliver et al. injected BrdU in mouse and rat pups and followed them for 2 months. After this period, they found BrdU retaining cells at the kidney papillae in numerous amounts [94].

Recently, a cell with stemness properties has been isolated from urine. Derived from renal papillae or from tubule, this cell is very attractive for cell therapy since it is obtained easily in a noninvasive way with similar properties of MSC [95].

The niche of kidney progenitor cells in adult kidney needs further studies [96]: proximal tubules, distal convoluted tubules, Bowman's parietal cells, papillae; should we consider multiple niches for kidney progenitors cells? (see Fig. 3.5).

In all those articles, the kidney cells show proliferative ratio, specific markers of embryonic cells, such as CD146, CD133, and CD24, form spheres at culture and when administered in animals models of kidney diseases, they lead to improvement of the disease and differentiation to tubules or podocytes. Epigenetics have a huge role in these cells. The final outcome/fate of a progenitor cell depends on its

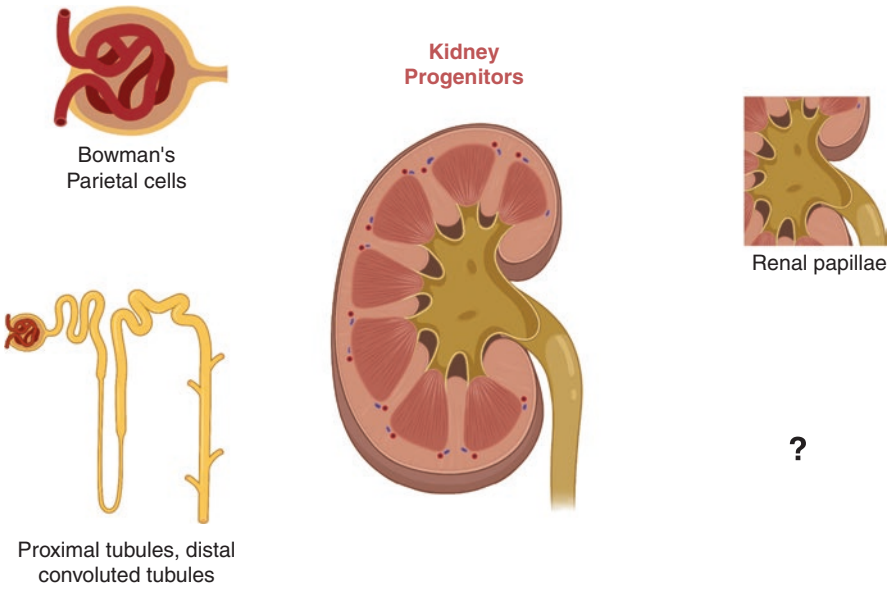

Fig. 3.5 Possible niches for kidney progenitors in adult kidneys. Those three sites may be potential areas for niches for kidney progenitors. Are there multiple niches? Created with BioRender.com

stimulus received. Injury is the main stimulus to a progenitor cell to differentiate: it is the basis of regeneration in cellular therapy [97, 98]. How injury leads to progenitor cell activation or its exhaustion is an area that still needs a lot of work.

3.2.4 Cell-Free Therapy: Extracellular Vesicles from IPS, MSC, and Kidney Progenitor Cells

Extracellular vesicles (EV) are an area of research that is increasing exponentially in the late years. EVs are vesicles composed of lipid bilayer with transmembrane contents and bear proteins, lipids, DNA, RNA, miRNA, etc., leading them to participate in several processes of cell-cell communication [99, 100]. Extracellular vesicles can be categorized by size and release from cell:

- Exosomes: 40–100 μm/budding from plasma membrane
- Microvesicles: 150–1000 μm/fusion of internal multivesicular compartments with plasma membrane
- Apoptotic body: 1000–5000 μm/cell fragmentation/blebbing

EVs are released by several cells (MSCs, cancer cells, immune cells, epithelial cells, etc.) and in several body fluids (blood, urine, milk, amniotic fluid) [100–103]. A detailed guideline summarizing the criteria to isolate and characterize EVs is defined by the International Society of EVs [104].

The main function of EVs is to regulate cell-cell communication [105]. In the first works of EVs, it was thought that EVs were only for cell clearance [102]. It is known that both resting cells and stressed cells release EVs. EVs released from cells in a disease environment may participate in the progression of the disease. In this sense, block EV release may be a therapeutic action to halt its progression. When EVs are released by donor cells, molecules from cytoplasm and/or membrane can come together. From this idea, the EVs can be used as disease biomarkers in therapies and studies of cell-cell communication.

Therapy with EV arises from the idea that those vesicles can carry "good" molecules if they are from "good" cells. The opposite is also right: "bad" cells generate vesicles with "bad" molecules, as described above. In this sense, those EVs can be used for disease biomarkers [101] and the understanding of disease progression. Nowadays, it is possible to engineer the donor cell to generate the EV you want. The EV donor cell can be upregulated or downregulated to specific molecules to modulate the recipient cell [101].

EVs cargo varies immensely, and it is related to donor cell microenvironment and to the stimulus of donor cell [102, 106]. EVs cargo are proteins, DNA, mitochondrial DNA (mtDNA), messenger RNA (mRNA), microRNA (miRNA), long noncoding RNA (lncRNA), circular RNA (circRNA), lipids, minerals, and also foreign molecules from infectious diseases [107, 108]. There are databases that summarize its cargo constantly: Vesiclepedia, ExoCarta, and EVpedia [109–111]. All these cargo can be absorbed by the recipient cell and can modify/reprogram its response [112].

Table 3.2 Extracellular vesicle therapies for kidney models, selected articles

Extracellular vesicles (EVs)	Kidney disease	Reference
Microvesicles from BM-MSC	Mouse AKI	Bruno et al. [114]
Microvesicles from BM-MSC	Human tubular cell/in vitro ATP depletion	Lindoso et al. [115]
Microvesicles from Wharton-Jelly MSC	Rat AKI	Zou et al. [116]
Extracellular vesicles from ESC	Mouse AKI and fibrosis after AKI insult	Yu et al. [117]
Extracellular vesicles from UC-MSC and UC-MSC overexpressing Oct4	Mouse AKI	Zhang et al. [118]
Exosomes from AD-MSC	Rat cisplatin AKI model	Lee et al. [119]
Exosomes from BM-MSC and from melatonin preconditioned BM-MSC	Rat AKI model	Alzahrani [120]
Extracellular vesicles from endothelial progenitor cells	Culture human glomerular endothelial cells and podocytes	Medica et al. [121]
Exosomes from UC-MSC	Rat unilateral ureteral obstruction (UUO)/fibrosis model	Liu et al. [11, 12]
Extracellular vesicles from BM-MSC	Diabetic model/streptozotocin	Grange et al. [122]
Exosomes from urine-derived stem cells	Diabetic model/streptozotocin	Jiang et al. [123]

EVs from several cells have been studied for kidney diseases from diagnosis to therapy [102, 113]. Table 3.2 summarizes some studies in EV therapy in the kidney models.

EV therapy is at its initial steps. Standardization of protocols, cargo definition, and routes of administrations may need to be analyzed cautiously.

3.3 Mechanism of Action of Stem Cells on Cellular Therapy

In literature, the use of stem cells in therapy in experimental models is always correlated with good outcomes, improvement of tissue, and amelioration of cell function. There is a lot of evidence that stem cell treatments are indeed a good option for several diseases/models. How does it work? How can a cell modulate the injury? We will review these questions below.

3.3.1 ESC and iPSC

Since ESC/IPSC therapy was established, the more attractive of this therapy is related to kidney replacement using scaffolds or a decellularized kidney filled with IPSC-derived renal cells, as better described below.

Injection of renal progenitors from IPS can also elicit a good response. Toyohara et al. have administered at the renal subcapsular the IPS-derived renal progenitors in

experimental models of AKI, showing improvement of renal disease. The mechanism of action is related to iPSC-derived renal progenitors secretion of growth factors such as HGF, VEGFa, and ANG-1 that are renoprotective and lead to tissue amelioration [124]. Another group injected IPS-derived renal progenitors intravenously in AKI model, leading to amelioration of renal impairment symptoms. They suggested that this could occur due to engraftment of IPS-derived renal progenitors to damaged tubule, proliferation, and acquisition of tubular epithelial phenotype [39].

3.3.2 MSC

In the field of MSC the mechanism of action is a more studied subject. Paracrine effects are the main mechanism of action of MSC that leads to modulation of inflammation and regenerative properties (anti apoptotic and angiogenic effects).

Nevertheless, it must be said that when we think about MSC therapy, it is the administration of MSC at a recipient patient, i.e., there is administration of exogenous MSC. We must bear in mind that the MSC mechanisms of action are what this cell should do in its niche/tissue. In a physiological state, MSC is quiescent. After an injury, the inflammatory response plays a role in regenerating the tissue, releasing a storm of cytokines and inflammatory factors. These molecules by its turn activate MSCs that come to downregulate the inflammation response and finish the repair process. If there is a balance between inflammatory response and MSC, tissue returns to its integrity and homeostasis. If this balance is not achieved, there is a disruption in the healing process, and diseases are established. Since endogenous MSC can not work properly due to pathologic state, exogenous MSC are administrated to the patient to heal and generated the homeostasis to the damaged area. And it mainly happens throught immunomodulation of inflammatory response [125–127].

Why do exogenous MSCs need to be administered to patients? Why are resident MSCs unable to exert their own function? Because resident MSCs are exhausted and/or depleted. Exhaustion of MSC occurs in metabolic alterations, such as obesity and aging, as well as in a pathogenic scenario. Obesity interferes with stem cell response. Mice fed with a high fat diet (HFD) have hair follicle stem cells depleted. HFD promotes a lipid-induced stress, which in turn activates IL-1R and inhibits SHH signaling [128]. AT-MSC from aging, diabetes, and obese donor patients leads to a different response in therapy, once these pathological states decrease the regenerative potential of the AT-MSC [129]. Neural stem cell also is impaired with aging [130].

Isolated BM-MSC from rat with CKD model showed signs of premature senescence: spontaneous adipogenesis, reduced proliferation capacity, active senescence-associated β-galactosidase, accumulation of actin, and a modulated secretion profile. So CKD inputs some modifications at resident MSC that impairs its functionality [131]. The same occurs with AT-MSC in a long-term exposure to uremic toxin leading to disruption of regenerative properties [132]. These data show that resident MSC can be modulated by metabolic and pathogenic stimulus and more importantly show the need to carefully assess the patient's suitability for autologous MSC transplantation.

Some authors suggested a way to stimulate resident MSC by modulating the niche and manipulating MSC in vivo [133]. This is an interesting area but still needs a lot of work. So if resident MSC is not working properly, exogenous MSC can help to cope with the injury. Once administered, the MSC biodistribution in the body is still controversial. There is evidence that MSC can be engrafted in the lung, liver, and kidney. MSC engraftment depends on the site of MSC administration. Most experimental models injected MSC intravenously; however, this decision depends on the mechanism of action needed [134, 135].

MSC can reach the injured site, in few numbers, despite the route of cell administration. Locally administration of MSC seems to be more effective than other routes. Exougenous MSC administered to patients may home to damaged areas throughout several chemokines and receptors, for instance CXCL12-CXCR4, CCL27-CCR10, and CCL21-CCR7 [136], and then exit the bloodstream. Ullah et al. greatly described strategies to improve this homing and migration of MSC for clinicals therapy [137].

Now, at damaged areas, MSC modulates inflammatory response in several ways. MSCs secrete IDO2,3, TGFb, PGE2, TSG6, and sHLA-G5 that act in immune cells, leading to an immunomodulation, mainly through increased population of regulatory T cells (Treg cells), suppressed proliferation, and activation of T cells, promoting regulatory DCs and M2 macrophages and then stimulating anti-inflammatory response [138–141]. More detailed mechanisms of MSC in adaptive and innate immune responses are recapitulated in well-written reviews [127, 138].

Some authors, on the other hand, suggested that the trapped MSC in the lung is the way MSC elicits its response. Monocytes and macrophages at lung phagocyte MSC and then change themselves to a regulatory pattern that are systematically distributed [142]. Since most clinical trials injected MSC intravenously, and these cells are trapped in the lung, it generated a controversy of how MSC exerts its function. Recent works suggest that the living MSC is not needed at the injury site. Some fragments of MSC cell or even MSC inactivated can elicit modulation of inflammation [143, 144]. It can change the previous works in the area regarding MSC mechanism of action and homing [145].

Besides modulation of inflammation, MSC has anti-apoptotic and angiogenic properties. Several works describe that MSC can secrete angiogenic factors, such as VEGF, FGF, HGF, placental growth factor (PGF), monocyte chemotactic protein 1 (MCP-1), stromal cell-derived factor 1 (SDF-1), and angiopoietin-1 (Ang-1). Regarding anti-apoptotic effects, MSC secretes survivin, VEGF, HGF, insulin-like growth factor-I (IGF-I), stanniocalcin-1 (STC1), TGF-β, FGF, and granulocyte–macrophage colony-stimulating factor (GM-CSF). MSC can also regulate reactive oxygen species (ROS) since it can produce HO-1 [139]. Modulation of ROS should also occur due to mitochondrial transfer via *tunneling nanotubes* (TNT) or via exosome transfer [146].

At kidney models, MSCs promote immunoregulation, anti-apoptotic effects, and angiogenic profile (Table 3.3).

Table 3.3 Exogenously MSC treatment at kidney models

	Model	Amount of cells and route of administration	Outcomes	Reference
Acute kidney disease (AKI)	40-min bilateral renal pedicle clamping	Intracarotid administration of MSC (approximately 10(6)/animal) either immediately or 24 h after renal ischemia	Improved renal function, higher proliferative, and lower apoptotic indexes, as well as lower renal injury and unchanged leukocyte infiltration scores	Tögel et al. [147]
	Sepsis-associated AKI/mouse cecal ligation and puncture operation	(1 × 10 cells intravenously) 3 h after surgery	Alleviate sepsis-associated AKI and improve survival, inhibition of IL-17 secretion and balance of the proinflammatory and anti-inflammatory states	Luo et al. [148]
	Polymicrobial sepsis induced by cecal ligation and puncture (CLP) in mice	5.0 × 105 BM-MSC cells from HO-1+/+ or HO-1−/− mice injected via the tail vein 2 h post-CL. Additional tail vein i.v. injections of 2.5×10^5 cells in 200 μl of PBS were given 24 and 48 h post-CLP. Lung-derived fibroblasts, at a dose of 5.0×10^5 cells as control were administered too	Amelioration of sepsis outcome, increased survival. After onset of CLP-induced sepsis, enhanced phagocytosis of bacteria by neutrophils and increased bacterial clearance	Hall et al. [149]
	Mouse cisplatin model	Human BM-MSC (5×10^5 cells) intravenously (i.v.) injected into tail vein	Decreased proximal tubular epithelial cell injury and ameliorated the deficit in renal function, resulting in reduced recipient mortality	Morigi et al. [150]
	Glycerol-induced mouse model	1×10^6 mice BM-MSC i.v. injected	Morphological and functional recovery	Herrera et al. [151]
	60 min bilaterally clamping of renal pedicles	Six hours after injury, MSC (2×10^5 cells) were administered intravenously	Morphological and functional recovery, reduced renal inflammation	Semedo et al. [152, 153]

(continued)

Table 3.3 (continued)

	Model	Amount of cells and route of administration	Outcomes	Reference
AKI > CKD	Folic acid model followed for 4 weeks	1×10^6 cells AT-MSC IP 24 h after folic acid	Reduced kidney fibrosis and chronic inflammation	Burgos-Silva et al. [154]
	Unilateral severe ischemia by clamping the left renal pedicle for 1h	6 h of reperfusion, 1×10^6 cells. Bone marrow mononuclear cells (BMMCs) were administered intraperitoneally	Reduced tissue inflammation, decreased fibrosis	Semedo et al. [155]
	Unilateral hypoxia followed for 6 weeks	2×10^5 AT-MSCs IP after 4h of injury or 2×10^5 IP AT-MSC at 6 weeks followed until 10th weeks	Reduced tissue inflammation, decreased fibrosis	Donizetti-Oliveira et al. [156]
Chronic kidney disease	5/6 nephrectomy	2×10^5 cell BM-MSC IV multiple doses	Reduced inflammation systemically and locally, reduce fibrosis progression	Semedo et al. [152, 153]
	UUO (unilateral ureteral obstruction)	10^6 human Wharton's Jelly-derived MSCs were injected into the aorta inferior to the renal artery after surgery in rats	Decreased fibrosis	Kheradmand et al. [157]
	UUO (unilateral ureteral obstruction)	Human MSCs (1×10^6/rat) immediately before operation	Exogenously administered MSCs significantly reduced these indicators of renal fibrosis, MSCs protect against obstruction-induced renal fibrosis, in part, by decreasing STAT3 activation and STAT3-dependent MMP-9 production	Matsui et al. [158]
	Chronic kidney disease in collagen4A3-deficient mice (Alport model)	At 6 weeks of age, COL4A3-deficient mice were divided into two groups that received tail vein injections of either 1×10^6 MSC in 200 µl isotonic saline or saline only at weekly until death	Renal parameters without changes, prevented the loss of peritubular capillaries, and reduced markers of *renal fibrosis*, that is, interstitial volume, numbers of smooth muscle actin-positive *interstitial cells*, and interstitial collagen deposits as compared to saline-injected COL4A3-deficient mice	Ninichuk et al. [159]

Table 3.3 (continued)

	Model	Amount of cells and route of administration	Outcomes	Reference
	Streptozotocin-induced diabetic nephropathy (STZ-DN)	2×10^6 Human UC-MSCs via the tail vein at week 6. Analyses after 2 weeks	Ameliorated functional parameters, such as 24-h urinary protein, creatinine clearance rate, serum creatinine, urea nitrogen, and renal hypertrophy index. In the kidney tissue, this improve of renal function were correlated with significant reductions in renal vacuole degeneration, lower inflammatory cell infiltration and less renal interstitial fibrosis	Xiang et al. [160]
	2K1C/Renal arterial stenosis	2×10^5 cells BM-MSC IV/weekly	Prevented the progressive increase of arterial pressure, reduced fibrosis, proteinuria and inflammatory cytokines, reduced fibrosis proteinuria and inflammatory cytokines and suppressed the intrarenal RAS	Oliveira-Sales et al. [161]

3.3.3 Extracellular Vesicles

Extracellular vesicles (EVs) have three main potential functions in kidney diseases: (1) diagnostic biomarkers, (2) progression of the disease, and (3) therapy. Jin et al. described the role of exosomes as diagnostic parameters for kidney diseases [101]. Karpman et al. have recently reviewed the role of EVs in pathophysiological [162, 163].

Several steps must be considered to generate exogenous EVs to therapy. Usually, a "good" cell is the one used. MSC is the most used cell to generate EVs. However, this EV area of study is calling attention and has been flourising. Nowdays it is possible to obtain therapeutic EVs from renal epithelia cell as well as from IPSC [163]. EVs release for donor cells can be increased during cellular activation and/or cell stress. After that, EVs are isolated and then ready for therapy [102].

Once intravenously administered, exogenous EVs can be found in the liver, spleen, and gastrointestinal tract [164]. As well as MSC, EV biodistribution depends on the route of administration.

How can EV cargo enter the cell? First, EVs must be near the recipient cell. Several receptors expressed at EVs may participate in this process, depending on the EV-cell origin. Moreover, the expression of these receptors can be modulated by bioengineering. To delivery EV's cargo, EVs must be internalized and the EV cargo delivery at the

recipient cell. EVs can be internalized by recipient cells by a variety of endocytic pathways, including clathrin-dependent endocytosis, and clathrin-independent pathways such as caveolin-mediated uptake, macropinocytosis, phagocytosis, and lipid raft–mediated internalization [165]. EV cargo enters the endosomal system in early endosomes (EEs); however, they are not degraded. This mechanism is still unknown [164].

EV cargo can act directly in the recipient cell. mRNA, microRNA, some proteins, and lipids can modulate the recipient cell [166]. Hade et al. have recently reviewed the molecules that are described to be EV-derived from MSC [167]. All the molecules, specially miRNA, as extensively described by several authors, can reprogramme the recipient cell, leading to amelioration of the kidney disease model [168, 169].

Several mechanisms of action of EVs are still unknown and need further studies: how do EVs reach the damaged cell? Is this amelioration obtained after administration of a transient process, since the amount of EVs cargo is limited?

3.4 Kidney Organoids

For decades, in vitro 2D culture and animal models have been widely used as an important research platform to address a range of scientific issues, from basic science to development, disease modeling, and evaluation of new drugs and therapeutics. Two-dimensional culture models are simpler systems, with relatively low cost and reproducible using primary or immortalized cells; however, they have limitations due to their simplicity. Primary renal cell cultures are defined as cells that were recently isolated from renal tissue. Recently renal epithelial cells were also obtained from human urine [170]. Their application is indicated because they mimic the physiological state of cells in vivo with more accuracy; however, they have limited growth capacity, due to the rapid process of dedifferentiation and with a predetermined number of cell divisions before entering senescence, with loss of their phenotype over time, which would make them unfeasible for long in vitro studies long term [171]. Despite these limitations, the use of primary renal cells still remains a reliable choice in studies of nephrotoxicity and basic renal cell functions [7].

Several immortalized cell lines of renal origin have been established due to their unlimited growth capacity and a more stable phenotype, which provides more reproducible results than primary cultures. Some immortalized human cell lines such as HK2, ciPTEC, RPTEC, caki-2, and other animal-derived cell lines as MDCK, LLC-PK1, NRK-52, and OK have been used because they maintain indefinite proliferation and sufficient phenotypic parameters for specific in vitro studies [171]. The RPTEC cells immortalized in two-dimensional cultures have been used for nephrotoxicity assays; however, they lack anion and cation transporters that are essential for drug excretion, making them unsuitable for predictive nephrotoxicity assays [172]. Disadvantages of these cells include the immortalization process is usually elicited by transfection and/or injection of Simian virus (SV40), papillomavirus (16E6/E7) genes, and human telomerase reverse transcriptase (hTERT) which can result in important changes in their characteristics and functions over time [173, 174].

In this context, two-dimensional cultures do not support growth in the vertical dimension resulting in abnormal polarity for specific renal cells [169]. Thus, the

lack of tissue-specific architecture and the absence of cell–cell interactions and cell–matrix interactions lead to loss of cell function and cannot accurately simulate the necessary microenvironmental factors and fail to modeling crucial elements of renal physiology that are highly influential in the study of the disease and the effectiveness of the drug for the treatment [175].

The use of experimental animal models are important tools for these studies, although they preserve the inherent complexity of interconnected tissues, they have shown little predictive and translational power for the human response due to discrepancy between species, which exhibit genetic and physiological differences in relation to basal metabolism, immune system function, and lifetime (de [176]). Most experimental studies use different species of animals such as mice, rats, hamsters, rabbits, zebrafish, guinea pig, xenopus toads, primates, dogs, and cats. In the field of therapeutics for kidney disease, most studies use rodents as a preclinical experimental model; however, these tested drugs fail in human clinical trials [177]. Each model has its own unique advantages and limitations [178, 179]. In vivo studies are time-consuming, of low yield and high cost as they require specific installations, adequate equipment and special training [180].

However, currently, many complex legal and ethical issues are raised about the pertinence of animal use leading to important restrictions on in vivo testing in the United States and Europe. The United States Environmental Protection Agency has prioritized the reduction of in vivo studies until the year 2035 and the Food and Drug Administration has established the use of the Principle 3 Rs (replacement, refinement, and reduction) in studies involving animal models [181]. This 3R strategy by Russell and Burch [182] suggests some ways to make animal experiments more humane, with minimal use of animals, that is, "reduction" in the total number of animals used in the experiment. This use must be carefully planned and "refined," and, if possible, higher animals must be "replaced" by alternative methodologies [178]. In 1995, the 4th R [183] was introduced which implies the addition of "responsibility" for the original three R's of Russell and Burch, based on the integrity and honesty of the results and the scientific correctness of proper and reasonable use of animal models necessary for research [184].

Thus, the existence of failures in replicating experiments in humans, the high costs spent, and the ethical and legal issues involved in the use of animals for disease models, tests of drugs, and therapy are under scrutiny. There is, therefore, a need for an intermediate path that can generate reliable forecasts and meet unresolved needs.

There is, therefore, a need for an intermediate path that can generate reliable predictions and meet needs not addressed by 2D models and animal models. With the improvement in 3D culture techniques, particularly the development of three-dimensional systems called organoids can help to overcome some of these limitations and concerns, making a "bridge" between studies based between in vitro and in vivo with great potential for applicability for disease modeling, personalized therapy, cancer research, and regenerative medicine.

Organoids constitute a complex three-dimensional multicellular collection, typically of human origin, derived from pluripotent stem cells, neonatal or progenitor cells, in which the cells spontaneously self-organize into differentiated functional cell types and present a structural and functional behavior similar to organ in vivo

[3, 185, 186]. Self-assembly and differentiation are essential characteristics of organoids resulting from the signaling pathways that regulate these processes provided by the extracellular matrix, growth factors in the medium and the constituent cell types [41, 187]. It is important to point out that the extracellular matrix is essential for the mechanical support of organoids in cell growth, migration, differentiation, and cell survival [188].

Organoids can be obtained from various sources such as pluripotent stem cells (PSCs), including embryonic stem cells (ESCs), and induced pluripotency stem cells (iPSCs) and tissue-specific adult stem cells [189, 190]. These distinct organoids have unique and complementary characteristics, as organoids derived from pluripotent stem cells mimic organogenesis during embryonic development and generally resemble fetal stage tissues, while organoids derived from adult stem cells recapitulate adult tissue [191]. WnT signaling has been identified as a key factor that allows the generation of organoids derived from adult stem cells [192]. PSC-derived organoids are generated through directed differentiation, mimicking specific combinations of growth factors that drive the induction of germ layers during development [177]. To date, several organoids derived from PSCs have been established that resemble various tissues, including functional organs such as brain, pancreas, intestine, liver, kidney [32], and heart, the last organ to be generated as an organoid [193] (Fig. 3.6).

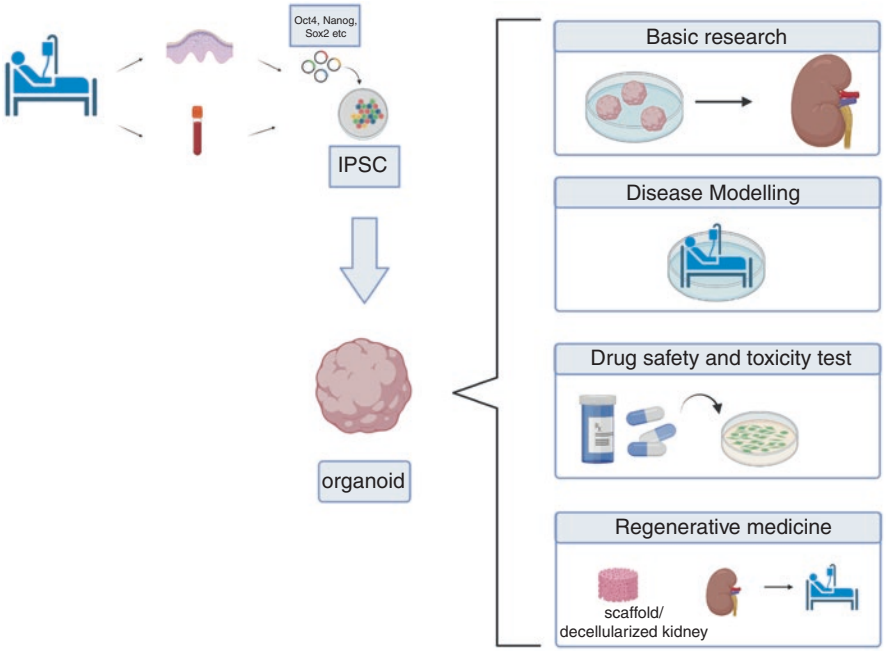

Fig. 3.6 Translational applicability of organoids. Organoids can be used for (1) studies of kidney development, aiming to understand human development and organogenesis processes; (2) disease modeling, to unveil the mechanisms that regulate and drive disease progression of various human pathologies; (3) drug efficacy and toxicity screening; (4) regenerative nephrology. Created with Biorender

3.5 Stem Cell/Renal Stem Cells Applied to Renal Tissue Engineering

Despite the great attractiveness of stem cell therapy, there are several obstacles to be overcome, such as the short duration of cell survival and function, the need for immunosuppression, in addition to the mandatory safety and fate studies of long-term implanted cells that limit its application [194]. Tissue engineering, as a therapeutic strategy, is based on the idea that bioactive substances facilitate the targeting, differentiation, and proliferation of stem cells seeded in three-dimensional scaffolds, leading to better cell engraftment [195]. Thus, this technology represents a new interdisciplinary field of knowledge, which aims to develop biological substitutes that mimic native tissue and can be used for the repair and regeneration of compromised tissues and organs [195, 196].

Complex organs such as kidney require an intact vascular network that can be reconnected to circulation after transplantation in the recipient in order to deliver nutrients and oxygen to the entire organ for clinical applications that must be processed in a three-dimensional (3D) fashion. Furthermore, given its highly organized multicellular structural complexity and the need for execution of vital essential functions, it would not be possible to reproduce these functions by traditional tissue engineering techniques. Only strategies such as decellularization and bioprinting are capable of generating functional and transplantable three-dimensional organs for future clinical application. Even in this context of difficulties in relation to renal complexity and its regeneration, tissue engineering proposes to help to simultaneously overcome the challenges inherent to dialysis, the need for organs for transplantation, and the prevention of patient exposure to immunosuppressive drugs.

Decellularization is a very attractive option to overcome these challenges, and it is anticipated that this new approach will be cost-effective in the long run, compared to the lifetime costs of dialysis or immunosuppressive drugs required for transplantation. The process of decellularization of xenogenic or allogeneic donor kidneys is the generation of whole organ scaffolds from which new kidneys are prepared, using the three-dimensional geometry, the vasculature, and components of the intact extracellular matrix, originating from the kidney tissue itself [197]. Through this strategy, vascularized acellular scaffolds are obtained intact, with total preservation of the vascular tree in order to facilitate the in vitro perfusion and reconnection to the bloodstream, which will provide nutrients and oxygen, in addition to the removal of metabolites after transplantation [198].

Furthermore, it has the essential advantage of providing structural integrity of the tissue, where synthetic and natural polymers used cannot replicate the precise spatial organization of cell architecture complex, as found in native renal tissue [199].

Several renal decellularization procedures have been described in the last years, in studies based on prolonged perfusion in the vasculature kidney through detergents or enzymes and successive washings [200]. These and other cell lysis solutions solubilize cell structures preserving intact the structural components necessary to maintain the mechanical and biological properties of the extracellular matrix (ECM) [201]. The vast majority of decellularization protocols depend on the use of

these detergents such as non-ionic Triton X-100 or the anionic dodecyl sulfate of sodium (SDS), but other existing techniques to assist this process include the use of alternating freeze-thaw cycles, shock osmotic and deoxyribonuclease to degrade nuclear material. In the face of exposure, an ideal protocol for decellularization will be one that efficiently removes all cellular material with less damage to the composition, to the biological activity and mechanical integrity of the extracellular matrix [202].

The recellularization of the resulting decellularized three-dimensional matrix of the kidney is the most complex and challenging phase due to the numerous differentiated cells that form the kidney [203]. Choosing cell sources to repopulate the resulting three-dimensional structure is important in obtaining a functional organ. The cells used should preferably be patient-derived to eliminate immunological rejection after implantation with ease of characterization and expansion, in addition to being functional in their new environment [197]. However, several cell types have been used for renal recellularization, including renal epithelial and endothelial cells, embryonic stem cells (ESCs), and IPSC.

Epithelial cells are an autologous source; however, they do not provide all cell types needed for kidney recellularization. Furthermore, cell expansion of these cells is not achieved due to the limited number of passages, making its clinical application unfeasible.

The use of ESCs in the recellularization process is limited by ethical issues due to the destruction of embryos, as well as because of in vivo teratoma formation [204]. Human IPSCs reprogrammed to be pluripotent cells avoid the ethical dilemma of embryonic cells, however IPSCs have already been shown to be tumorigenic, which limits their translation potential [56].

The recellularization methodologies used for decellularized renal matrices are critical to achieving success in repopulation whether it be cell distribution and differentiation [205]. In an attempt to circumvent these obstacles, different delivery routes were tested, when the cells were injected through the renal artery, they only reached the glomerular capillaries, while via the renal route they reached only the peritubular capillary [206].

Despite the promising results, the repopulation of decellularized renal matrices requires further optimization with the improvement of functional parameters, since the functionality of these organs is not yet accepted for transplants.

Bioprinting or organ printing**,** an extension of tissue engineering, recently defined as computer-aided additive biomanufacturing of cell tissue, applies additive manufacturing technology to structure living and nonliving materials with an organization bi- or three-dimensional pre-established, in order to directly produce biological tissue structures [207], substitutes that restore, maintain, or improve the function of a tissue or an entire organ. These emerging technologies are important tools to promote tissue regeneration with great potential for the bioengineering of living organs [208, 209]. Bioprinting systems use adaptations of additive manufacturing technologies and thus, based on their operating principles, can be classified as direct laser-induced engraving, inkjet printing (continuous or drop-by-drop), extrusion deposition, lithographic printing (stereolithography [SLA] and digital light

projection [DLP]), and electrostatic wiring [210]. Although bioprinting is a promising technology as an interface between engineering and tissues, each technique has its limitations.

Considering the prolonged time to print tissues and organs on a larger scale, a relevant disadvantage of encapsulating live cells in biomaterials is the need for the biomaterial cell suspension to be stored considerably in advance in the reservoir, which compromises cell viability and limits its bioactivity [207]. Mechanical resistance, structural integrity, and processability of biofabricated structures are also a common disadvantage among bioprinting techniques, as they use hydrogels with a high water content to favor biocompatibility [211].

Overall, cell encapsulation in biomaterials allows for cell patterning with potential for organ impression; however, the subsequent formation of extracellular matrix (ECM), digestion and degradation of the matrix of biomaterials, and the proliferation and colonization of encapsulated cells are not trivial. In order to overcome these problems, a new concept was introduced by Mironov et al. [212]. The proposal consists of tissue spheroids as building blocks that direct self-assembly for organ fabrication, demonstrating developmental morphogenetic principles such as cell organization and tissue fusion, based on the recognition that "nature is wise" [213, 214].

After bioprinting an organ, the produced structure needs to be transferred to a perfusion bioreactor, used to provide an ideal environment for the maturation process, by transporting nutrients, growth factors, and oxygen to the cells and extracting waste metabolic, so that the cells can grow and fuse, forming the organ [207].

Considering the path to transplantation in a human, this includes (1) modeling a three-dimensional model of an organ with its vascular architecture; (2) generation of a design for bioprinting, (3) isolation of stem cells, (4) differentiation of stem cells into specific target organ cells, (5) preparation and loading of organ-specific cells, vessel cells, blood samples, as well as the support medium, (6) bioprinting process followed by (7) organogenesis in a bioreactor and (8) transplantation. When building larger scale organs, the mechanical integrity of the bioprinted structures and their vascularization are the main challenges for the success of the approach in the search for the replacement or restitution of tissues and organs [215].

3.6 Clinical Translation of Stem Cells in Kidney Diseases

By searching for "Mesenchymal Stem Cell" at ClinicalTrials (www.clinicaltrials.gov), it retrieves 1270 studies between completed, recruiting, and not patients (other term ["Mesenchymal Stem Cell"], search date October 7, 2021). Adding the word "kidney" at the search, it results in 58 studies (conditions or diseases [kidney] + other term ["Mesenchymal Stem Cell"], search date October 7, 2021).

No trials with administration of exogenous exosomes/microvesicles or extracellular vesicles were found at ClinicalTrials for kidney disease. However, there is one work using patients and extracellular vesicles in kidney diseases. Forty stage III and

IV CKD patients were enrolled, and 20 patients received two doses of umbilical MSC-derived extracellular vesicles, showing amelioration of inflammatory response and improvement of kidney function [216, 217].

Hickson et al. summarize the progress of regenerative therapies into clinical translation in the four areas of nephrology: renovascular disease, sepsis-associated AKI, diabetic kidney disease, and kidney transplantation. The trials in diabetic kidney diseases for regenerative cellular therapies are evidently more prevalent since there is an exponential increase of diabetes in the world [218]. Completed trials using the exogenous MSC have been demonstrating amelioration of kidney diseases and no adverse effects in humans [217, 219]. However, it is difficult to compare all trials. These results can have interference of several parameters: lack of standardization on the production of the cells, lower quality and safety analysis, biases at donor MSC patients, route of administration and biodistribution of MSC in recipients, and mainly its mechanism of action in humans [220].

From the 58 clinicals trials, eight studies are completed (Table 3.4). All these studies showed safety and tolerability of MSC infusion in patients.

Table 3.4 Clinical trials with MSCs

w	Status	Study title	Conditions	Interventions	Locations	Publication
1	Completed	Mesenchymal stem cells transplantation in patients with chronic renal failure due to polycystic kidney disease	Chronic renal failure Polycystic kidney disease	Biological: intravenous injection autologous mesenchymal stem cells	Royan Institute Tehran, Iran, Islamic Republic of	Makhlough et al. [221]
2	Completed	Autologous bone marrow derived mesenchymal stromal cells (BM-MSCs) in patients with chronic kidney disease (CKD)	Chronic kidney disease	Biological: intravenous injection	Royan Institute Tehran, Iran, Islamic Republic of	
3	Completed	Induction therapy with autologous mesenchymal stem cells for kidney allografts	Renal transplant rejection	Procedure: kidney transplantation with MSCs infusion	Stem cell therapy center, Fuzhou General Hospital	
				Procedure: kidney transplantation without MSC infusion	Fuzhou, Fujian, China	

Table 3.4 (continued)

w	Status	Study title	Conditions	Interventions	Locations	Publication
4	Completed	MSC for occlusive disease of the kidney	Atherosclerotic renal artery stenosis	Drug: arterial infusion of autologous mesenchymal stem cells	Mayo Clinic in Rochester	Camilleri et al. [222]
			Ischemic nephropathy		Rochester, Minnesota, United States	
			Renovascular hypertension			
5	Completed	Hypoxia and inflammatory injury in human renovascular hypertension	Renal artery stenosis	Drug: mesenchymal stem cell	University of Alabama	Lerman [223]
			Ischemic nephropathy	Procedure: mesenchymal stem cell delivery with stent placement	Birmingham, Alabama, United States	
			Renovascular disease		Mayo Clinic	
			Chronic kidney disease		Rochester, Minnesota, United States	
					University of Mississippi	
					Jackson, Mississippi, United States	
6	Completed	Mesenchymal stem cells and subclinical rejection	Organ transplantation	Procedure: mesenchymal stem cell infusion	Leiden Universitary Medical Center	Reinders et al. [224]
					Leiden, Netherlands	
7	Completed	Allogeneic amniotic mesenchymal stem cell therapy for lupus nephritis	Lupus nephritis	Drug: human amniotic mesenchymal stem cell		
			Mesenchymal stem cells			
8	Completed	Evaluate the safety of CS20AT04 inj. in subjects with lupus nephritis	Lupus nephritis	Biological: allogeneic bone marrow derived mesenchymal stem cells	Hanyang university hospital	Jang et al. [225]
					Seoul, Korea, Republic of	

There are a lot of points to carefully look at and that need attention when translating stem cell therapy to humans. Moreover, transparency is needed [226]. The following questions reflect some concerns of procedures to culture the MSC that are quite open, and no standardization were determined.

1. Source of stem cell: from bone marrow, from adipose tissue, from IPS, etc., how these cells were isolated, etc.
2. MSC donor patient criteria: age, comorbidities related, pathogens exclusion, obesity, etc.
3. Cell culture conditions: use of defined mediums, the presence of fetal bovine serum, time of expansion, how passages were performed, how many passages, etc.
4. Preconditioning culture: how it was performed, etc.
5. Safety and quality control of cells before injection: viability test, karyotype tests, mycoplasma tests, pathogens tests, immunophenotype assays, secretion and function assays, etc.
6. Amount of cells injected at recipient patient and its periodicity
7. Route of administration: locally or systemically; whether systemic, arterial, or venous
8. Biodistribution: cells got trapped at lung, at liver, etc.?
9. Analysis of mechanism of action: systemic cytokine analysis before and after treatment, profile of immune cells before and after treatment, functional parameters of kidney before and after treatment, etc.
10. Recipient patient with inclusion and exclusion criteria well defined
11. Long-term follow-up after treatment: analysis of the recipient patients parameters after 1, 6, 12, 24, 72 months, for instance, to evaluate changes and tumorigenic potentiality, etc.

Regarding replacement therapies with organoids or scaffolds/decellularized kidneys with stem cells, they are in basic research yet due to kidney complexity [197].

Bringing EVs to clinical trials requires a lot of standardization protocols. Due to its infinitude of possible sources and internal cargo, a myriad of therapies can be generated. Drug delivery through EVs creates more parameters to increase variables for standardization. There are several EV isolation protocols, several characterization of EV protocols, EV half-life, storage conditions, biodistribution, dosage control, and route of administration that requires more studies before using them in humans [227, 228].

3.7 Ethics and Legal Regulation

With the large number of patients suffering from chronic and incurable diseases such as kidney diseases, interest in stem cells grows and generates great expectations in terms of possible benefits related to therapeutic applications. However, regardless of the potential and hope that stem cells will improve and save lives, there are many ethical, religious, legal, and security challenges and controversies to overcome.

hESCs are derived from the cell mass of embryos and have high levels of telomerase activity and normal karyotype. They are able to differentiate into cell types of the three germ layers under in vitro and in vivo conditions. However, human embryonic stem cell research presents a fundamental dilemma related to the moral status of the embryo from which embryonic stem cells are derived: is it morally acceptable to seek new therapies to cure disease at the expense of destroying a human embryo? Opponents of use argue that the embryo is capable of developing into a human being, and its destruction would be immoral and unethical. Proponents deny any moral status of the embryo whose potential benefits justify embryonic research [229]. This ethical dilemma is portrayed in different laws that regulate embryonic research around the world.

Safety issues related to hESC-based therapy are of primary concern for its clinical use. The pluripotency of hESCs allows these cells to differentiate into several different cell types, which would make it difficult to control after transplantation in vivo. When these cells are transplanted, tumors that contain the three germ layers can be formed and are called teratomas. Currently, it is believed that differentiating hESCs into the desired cell type before transplantation is the only way to prevent the formation of teratomas.

Thus, the use of stem cells contributes to disease modeling, drug discovery and testing, biobanks, organoids, and therapeutics, and it is important to always take ethical and legal considerations to each specific field of application.

3.8 Conclusion

Stem cell therapies and their subproducts, mainly EVs, are a promising therapy for kidney disease, with higher expectations as well as higher challenges. Basic research is still needed to understand the mechanisms and biological properties of stem cells. Moreover, standardization protocols, higher quality control parameters, therapeutic efficacy, and safety of using stem cells in humans are required for the flourishing of regenerative medicine using stem cells.

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Chapter 4
Proteomics and Biomarkers for Kidney Diseases Diagnosis

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Abbreviations

ACTN4	Actinin-4
AKI	Acute kidney injury
AQP2	Aquaporin-2
ARHGAP24	Rho GTPase-activating protein 24
BBMV	Brush-border membrane vesicles

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CD2AP	CD2-associated protein
CKD	Chronic kidney disease
DKD	Diabetic kidney disease
DNAJB9	DnaJ heat shock protein family (Hsp40) member B9
ESKD	End-stage kidney disease
EVs	Extracellular vesicles
FSGS	Focal segmental glomerulosclerosis
GBM	Glomerular basement membrane
IHC	Immunohistochemistry
ILVs	Intraluminal vesicles
LIMP-2	Lysosomal integral membrane protein type 2
MN	Membranous nephropathy
MS	Mass spectrometry
MVEs	Multivesicular endosomes
NAG	N-acetyl-β-D-glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
PAN	Puromycin aminonucleoside
PLA2R	M-type phospholipase A2 receptor
PTMs	Post-translational modifications
SCARB2	Scavenger Receptor Class B Member 2
SVs	Secretory vesicles
TRPC6	Transient receptor potential cation channel
YAP1	Yes-associated protein-1

4.1 Introduction

Kidney is an exquisitely complex organ that consists of multiple compartments with over 30 specialized cell types [1]. Kidney diseases of various pathologies could progress to a similar endpoint condition, referred to as end-stage kidney disease (ESKD), which requires kidney replacement therapy. The diagnosis of several kidney diseases needs kidney pathology through a renal biopsy, an invasive procedure that limits frequent evaluations. Currently, an emerging proteomic analysis in the field of nephrology for the identification of peptides, proteins, or molecules, as an understanding of entire cellular physiology instead of the concept of "one protein" or "one gene," [2] might lead to a more straightforward renal evaluation. Interestingly, a proteomic analysis could be beneficial in several aspects for nephrologists, including diagnosis, disease monitoring, treatment selection, and disease pathophysiology [3]. For the biomarker aspects, proteomic analysis on blood or urine samples is a powerful tool for discovering biomarker candidates or using proteomic pattern biomarkers. Additionally, the detection of abnormal responses of specific proteins, either quantitatively (protein abundance) or qualitatively (protein functions) through multidimensional analyses, could precisely identify phenotypic details of the patients with a more accurate diagnosis, the so-called deep phenotyping [4]. The application of omics-based technology could be a novel tool for achieving a final diagnosis with less invasiveness and more feasibility.

4.2 Concepts of Proteomics-Based Technology

Over millions of phenotype diversity created from the human genome, there is the manifestation of just over 20,000 protein-encoding genes [5]. Internal and external environments trigger multiple changes in the integrated system of protein synthesis and post-translational protein modifications to maintain homeostasis, generating functional multiplicity. Protein-coding sequences in the genome are transcripted into RNAs (transcriptome), which are translated into proteins (proteome) afterwards [6, 7]. Protein production via transcription-translation processes and protein degradation regulates the total number of proteins (protein abundance), affecting protein activities [8]. Each protein can be subsequently modified at the protein level to change the structure and property via post-translational modifications (PTMs), for instance, phosphorylation, ubiquitinylation, and acetylation [9]. The proteins are also bound to other biomolecules such as other proteins, DNA, RNA, or small molecules (protein intermolecular interactions), forming specific molecular complexes (interactome) (Fig. 4.1) [10]. Disruption of protein interactions leads to alteration of the functions of entire complexes more rapidly than the alteration in proteome abundance. Moreover, the translocation of such proteins into appropriate cellular compartments such as the nucleus, mitochondria, and endoplasmic reticulum (protein localization) [11] is needed to effectuate particular functions. The presence of defects in the preceding processes (protein abundance, PTMs, intermolecular interactions, and protein localization) could alter protein functions and cause inappropriate responses to stimuli [12].

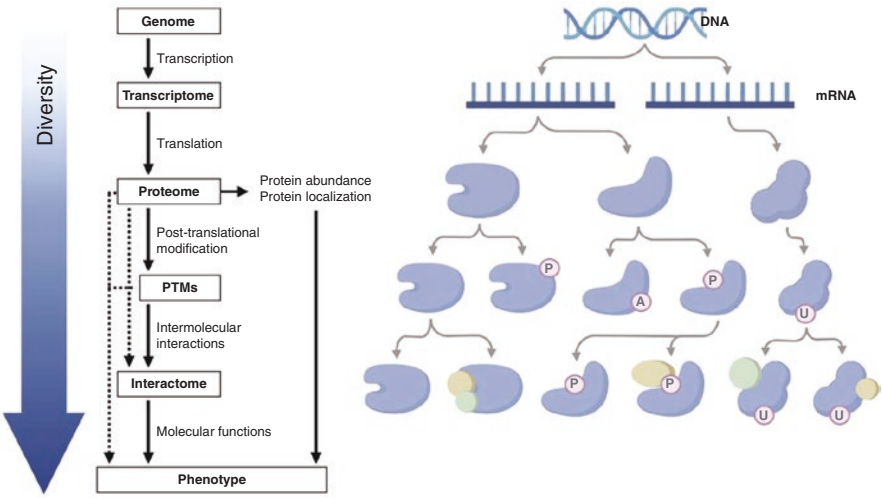

Fig. 4.1 The conceptual pathway from genome to phenotype expression. After DNA transcription and mRNA translation, the proteomes are modified to expand complexity of downstream molecules, leading to the extensive diversity of protein functions and phenotypes despite a distinct genome

To assess proteome expression, mass spectrometry (MS) is utilized to identify each protein by measurement of mass-to-charge ratio (*m/z*) through the two strategies, top-down and bottom-up approaches (Fig. 4.2). In the top-down approach, the mass spectrometer differentiates intact proteins. In contrast, in the bottom-up approach, the so-called shotgun approach, the complex proteins are digested into peptide fragments that carry charges in gas state [13]. Then, MS is used to identify each fragment. Matching the identity of peptide sequences and PTMs with those in the database, all proteins within a sample can be identified [14]. To enhance the performance of proteomic analysis, MS-based techniques such as sample preparation steps, ionization methods (matrix-assisted laser dissociation ionization and electrospray ionization) [15], fragmentation methods (liquid chromatography) [16], and multiple MS (tandem mass spectrometry) [17, 18] are recently improved.

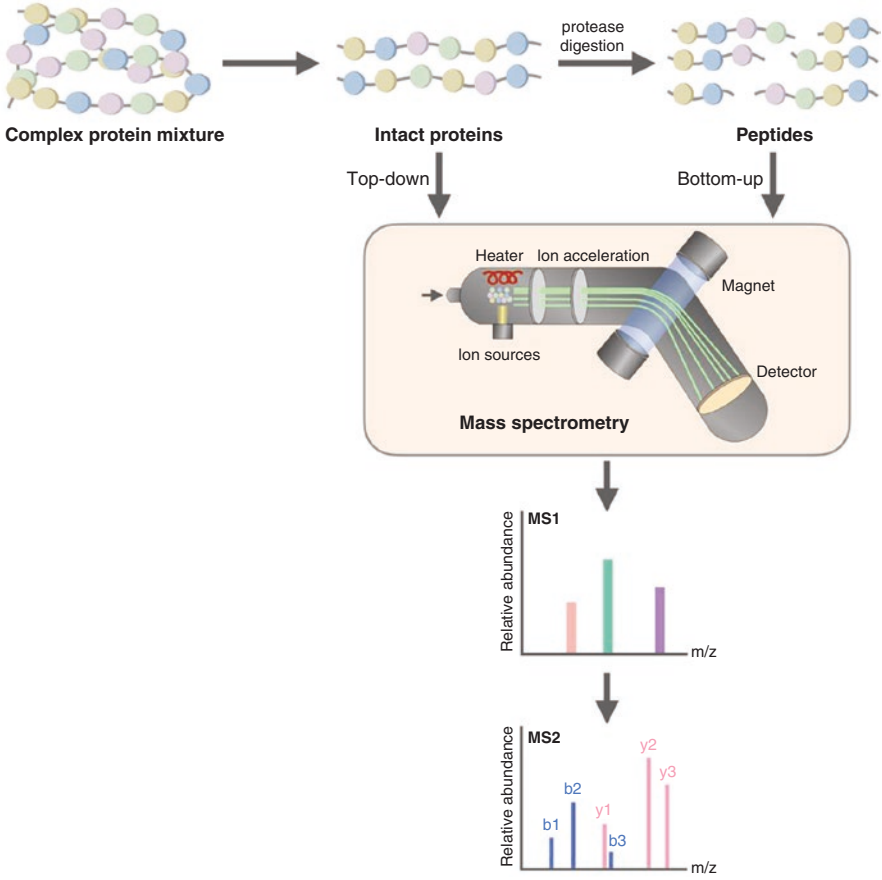

Fig. 4.2 Basic concepts of mass spectrometry-based proteomics analysis. Intact proteins and digested peptides are measured by mass spectrometer in the top-down and bottom-up approaches, respectively. The quantity of each fragment differentiated by mass-to-charge ratio (*m/z*) is shown as relative abundance. The pattern is matched to the dataset to identify the peptides and proteomes

Specific protein could also be labeled with stable isotope before the analysis to recognize sources of the samples and helps in quantification [19].

The proteomics-based analysis brings about extensive quantitative data of proteins or peptides. The essential step in biomarker study analysis is to filter pertinent and applicable proteins from a large number of the available data to translate into the list of biomarker candidates. Measurement of relative protein abundance or signal-to-noise ratio would identify a cutoff threshold for protein abundance [20, 21]. Comparison of the distribution between experiment and control samples, for example, using Bayesian method analysis would verify relevance to the pathophysiology of the diseases [22].

As aforementioned, proteomics is involved in complex biomolecular interconnections. It is lately integrated with other -omics methods to improve biomarker discovery and databases in multiple dimensions. The combination with the genomic approach, the so-called proteogenomics, helps determine the causal genomic variance that affects protein expression and functions, which focuses on genetic kidney diseases [23, 24]. The concurrent approach examining metabolites, which mediates protein modification chemically and enzymatically, helps verify the relevance of proteomic along with metabolomic changes, the so-called proteome–metabolome interactions [25, 26].

4.3 Sampling, Urine Normalization, Factors Affecting Proteomics

As kidneys play an essential role in urine production, changes in kidney physiology can induce corresponding changes in the urine [27, 28]. In contrast to blood sampling, urine sampling is noninvasive, easy to obtain over a length of time, and available in substantial volume. Urinary proteome has more stability at physiologic temperatures since proteolytic degradation activity has been completed when urine is retained in a urinary bladder [29, 30]. These make urine the better source for biomarker discovery [31]. However, there is a wide variation of urinary proteins and peptides abundance, depending on urinary concentration and volume, renal functions, as well as daily dietary intake, catabolic state, exercise, hormonal change, and circadian rhythm. Therefore, the rate of urinary protein and peptide excretion should be used for biomarker evaluation instead of the urinary concentration. Thus, the time normalization using the urine collection in a period of time, ideally 24 h, is the best way to measure the rate of biomarker excretion. Alternatively, the time normalization could be done by using certain "time-representative" markers such as levels of creatinine, cystatin-C, or N-acetyl-β-D-glucosaminidase (NAG) [32]. Nevertheless, studies on the correlation between these normalization factors and the interested biomarkers are required before proper use of these convenient normalizations. Additionally, another source of proteome data that contributes to the understanding of the pathogenesis of kidney diseases is the direct protein profiling and cellular imaging in the kidney biopsy sections, the so-called proteome-based

pathology [33]. However, this method requires adequate tissue sampling and proper tissue processes. Fresh frozen tissue collection is preferred over paraffin embedding protocol. The proteome-based pathology would promote the discovery of novel therapeutic targets beyond diagnostic tools [7].

4.4 Proteomic Analysis on the Specific Site of Kidney, an Initial Step of Biomarker Discovery

Each nephron consists of multiple compartments which perform particular functions. The glomerulus is the primary filtration barrier, which is a basic structure for urine production. In parallel, renal tubules play a role in the reabsorption and secretion of solutes, electrolytes, and water. Each specialized tubular segment holds distinct transporters to maintain the homeostasis of each solute along the tubules. Thus, a specific proteome represents the pathophysiology of particular compartments within the nephrons (Fig. 4.3) [34]. Because different kidney diseases initiate from the primary dysfunction in some parts of the kidney that might induce the secondary abnormalities (e.g., glomerular proteinuria induces tubular injury), the interpretation of proteomic analysis from a specific sample of kidneys needs careful interpretation. Hence, the precise sample collection without contamination by other parts of

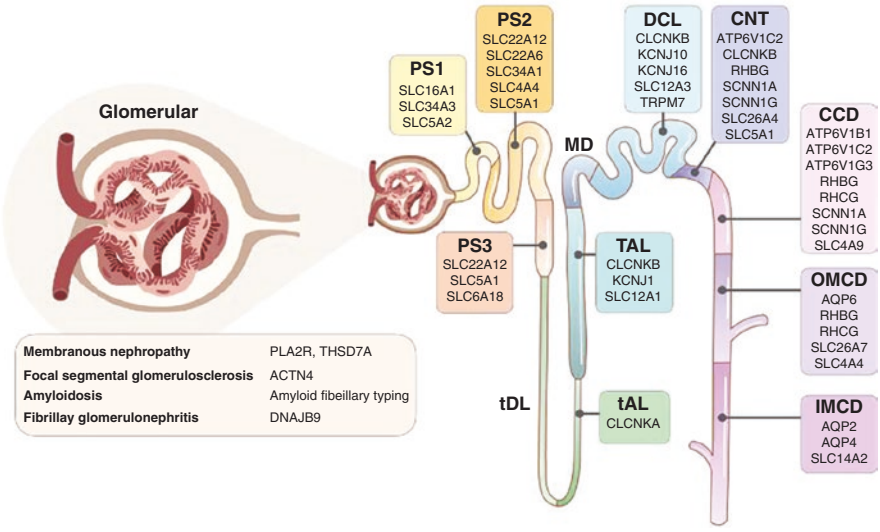

Fig. 4.3 Common protein expression of transporters and channels at glomerulus and specific segments along renal tubules. *CCD* cortical collecting duct, *CNT* connecting tubule, *DCL* distal convoluted tubule, *IMCD* inner medullary collecting duct, *OMCD* outer medullary collecting duct, *MD* macula densa, *PS* proximal tubular segment, *TAL* thick ascending limb of Henle's loop, *tAL* thin ascending limb of Henle's loop, *tDL* thin descending limb of Henle's loop

the kidney is helpful for the interpretation. For example, the tubular biomarkers derived from the analysis in glomerular specimens might be due to the tubular injury secondary to the glomerular defects, and the utilization of this tubular injury marker might not be appropriate for the diagnosis of glomerular diseases.

Nevertheless, an analysis of glomerular specimens is commonly used for the discovery of glomerular biomarkers. The glomerular filtration barrier is the three layers which consist of endothelial cells, glomerular basement membrane, and podocytes; proteomic analysis of glomeruli might have peptides from all of these cells. Practically, glomeruli are disrupted mechanically and enzymatically for single-cell suspension [35]. Podocytes are isolated from glomeruli in the form of fresh or immortal podocytes, which contain a number of gene expressions that regulate functions of slit-diaphragm such as podocin [36], nephrin [37], CD2-associated protein (CD2AP) [38], and transient receptor potential cation channel TRPC6, [39] and cytoskeleton structures such as actinin-4 (ACTN4) [40] and Rho GTPase-activating protein 24 (ARHGAP24) [41]. In parallel, an analysis of renal tubular specimens is commonly used for the discovery of renal tubular biomarkers.

Likewise, renal tubules consist of four dedicated segments: the proximal tubules, the loop of Henle, the distal convoluted tubule, and the collecting duct. Hence, the separation of these structural cells via specific methods, for example, Percoll density gradient centrifugation for proximal tubular cells [42], magnesium chloride precipitation for brush-border membrane vesicles (BBMV) from proximal tubules [43], and density gradient centrifugation for medullary thick ascending limb [44] and inner medullary collecting duct cells [45], is performed. The proteomic studies of each cell type include profiling of particular membrane transporters and their levels of abundance, and dynamic changes in such levels at different time points or different stimuli are published [46, 47]. To illustrate, Walmsley et al. [46] demonstrated proteomic profiling using BBMV isolated from proximal tubular cells of acidotic rats revealed an increase in sodium-dependent glucose transporter, lactate transporter, peptidases, and apical membrane protein that mediated endocytosis, but a loss in neutral amino acid transporters, glycolytic and gluconeogenic enzymes. These findings might promote glutamine deamidation within the tubular lumen. They also showed temporal changes in abundance levels of such membrane and associated proteins, proving the pathophysiology of adaptive changes in various phases of metabolic acidosis. Hence, studies from proper kidney parts might be helpful for the biomarker discovery in several renal diseases.

4.5 Proteomic Analysis on Urine Extracellular Vesicles

Extracellular vesicles (EVs) are a diverse group of membrane vesicles with diameters ranging from 50 to 1000 nm secreted by virtually all cell types [48]. As a subset of EVs, exosomes are EV at the size between 50 and 150 nm formed by the invagination of the cell membrane before forming as intraluminal vesicles (ILVs) in multivesicular endosomes (MVEs) by inward budding of the endosomal membrane before releasing

into the intercellular space [49]. Meanwhile, microvesicles and ectosomes are EVs at the size between 50 and 1000 nm, formed by outward budding of the plasma membrane that is associated with an alteration of membrane phospholipids polarity from several Ca^{2+}−dependent enzymes [48]. Hence, molecules on the EV surface are a fraction of the cell membrane. The evaluation of exosome surface molecules is similar to sampling a surface membrane, the so-called cell biopsy. Hence, MVB-derived exosomes and membrane-originated microvesicles exhibit overlapping vesicles in size, composition, and intra-vesicular contents, including the biogenesis factors (ALIX, TSG101, VPS4) and nucleic acid [48, 50]. On the other hand, secretory vesicles (SVs) are produced by the ER and Golgi apparatus, mostly with the specific vesicular contents (such as hormones and neurotransmitters) that fuse with the specialized cell membrane structures (porosomes) to excrete their contents [50]. Although urine exosomes are mostly produced by several urinary system cells (kidney, bladder, and prostate gland), small amounts of exosomes from blood circulation can be recovered in nephropathy with micro-bleeding. However, the large-scale unbiased analysis identifies the high abundance of proteins from the urinary system [51]. Interestingly, the structural protection of EVs protects the nucleic acid cargo from several enzymes allowing to use of the nucleic acid in EVs as biomarkers (Fig. 4.4) [52–54].

However, the variation in isolation method and sample normalization concerns urine exosome biomarkers and proteomic analysis [55]. Among all isolation techniques, the ultrafiltration method (low centrifugation before 200,000 × *g* high spin) and size exclusion chromatography relatively (an inexpensive and high throughput method) provide high-purity exosomes but with a relatively low exosome recovery are frequently used for the proteomic analysis of urine exosome [56, 57]. Meanwhile, density gradient centrifugation, filtration, affinity isolation (magnetic beads), and precipitation have some limitations for further proteomic analysis (including the low yield of urine EVs and a limited subpopulation of EVs and contaminants), which lead to the lesser proteomic studies with these isolations [48]. More importantly, urine collection and normalization issues are essential for the reliability of urine biomarkers. The time normalization, ideally all urine EVs from 24 h urine collection, is the most currently reliable method, despite a possibility of the day-to-day variation of EV excretion from the urinary systems. The limited knowledge of the normal physiology of urine EVs results in the inconclusiveness of using other methods of normalization (total EVs number, universal biomarker of EVs, mass spectrometry normalization, creatinine, osmolality, and renal functions) [50]. Despite these limitations, the high potential use of urine exosome biomarkers is demonstrated. For example, aquaporin-2 (AQP2) in urine exosome could be used to determine AQP2 balance in the collecting duct cells [58, 59] following a concept of "liquid biopsy" as urine EVs are samplings of molecules from the membrane of several cells in the urinary system [60, 61]. Moreover, several transcriptional factors and nucleic acids (mRNAs and miRNAs), which most are rapidly degraded in urine, could be detected in urine EVs of patients with glomerulopathy and tubulopathy [62–64]. Hence, the proper normalization with rapid urine EV isolation will lead to the clinical use of urine EVs, and proteomic analysis will be an essential tool for the biomarker discovery [65, 66].

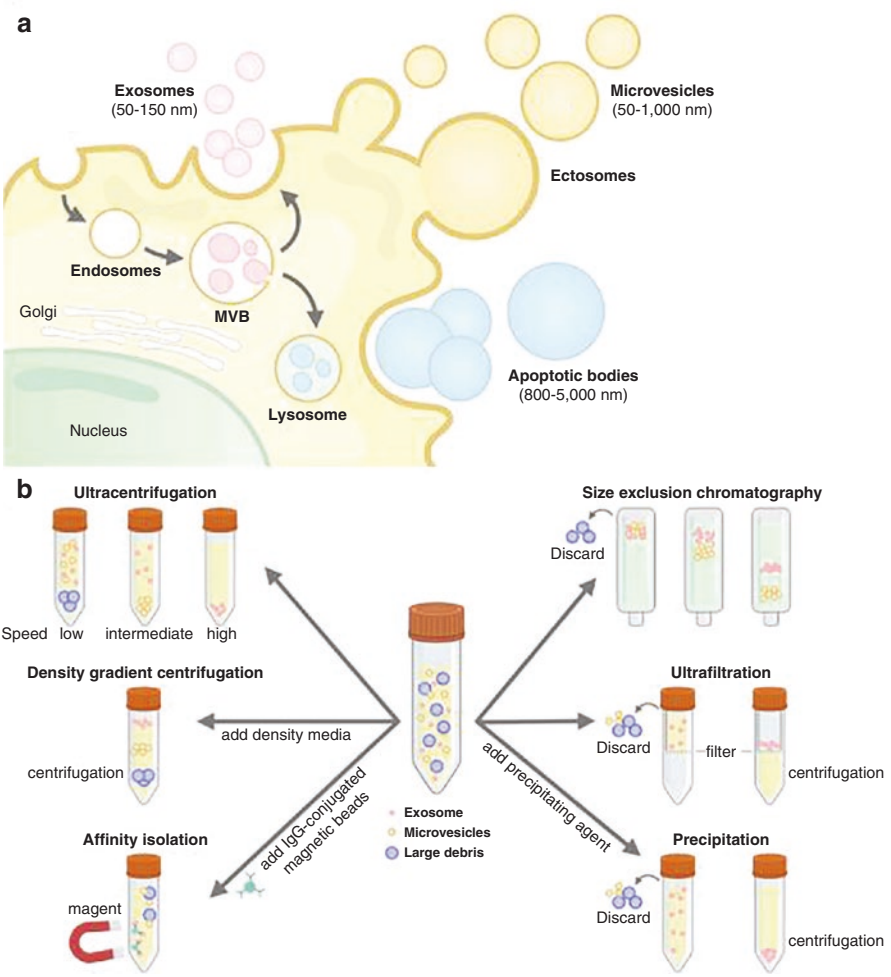

Fig. 4.4 The different sizes and biogenesis of exosomes, microvesicles, secretory vesicles, and apoptotic bodies (**a**) and the different methods of exosome isolation (**b**)

4.6 Current Biomarkers of Kidney Diseases Derived from Proteomic Analysis

4.6.1 Glomerular Diseases

At present, the pathogenesis of many glomerular diseases is not fully elucidated, and clinical manifestation is highly heterogeneous, even among patients with the same diagnosis. So, the proteomic approach is mainly employed to identify the more exact molecular mechanism of these diseases, which may further reveal novel

biomarkers that can be used to diagnose some diseases in a less invasive manner or to characterize a more precise phenotype for the patients with broader traditional glomerular disease diagnoses, the so-called deep phenotyping.

Identification of M-type phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7A and their autoantibodies as key pathogenetic factors in idiopathic membranous nephropathy (MN) is an early proteomic approach for elucidating glomerular disease pathogenesis by analyzing the interaction between serum from diseased subjects and glomerular extract from renal tissue of healthy people obtained from other purposes, identifying the causative proteins by mass spectrometry, and localizing the proteins in the nephron by immunohistochemistry (IHC) technique [67, 68]. However, some pathogenic molecules might not be circulating or identifiable in the serum sample. Broader and untargeted proteomic analysis in other biological specimens is needed. It reveals an increase in exostosin-1 and exostosin-2, which are later shown by IHC staining to present in the glomerular basement membrane (GBM) in PLA4R-negative MN renal biopsy. One-third of these patients have no detectable circulating autoantibodies to these proteins [69]. Analysis of non-traditional biosamples such as protein-rich urinary microvesicles also demonstrate increased lysosomal integral membrane protein type 2 (LIMP-2) or Scavenger Receptor Class B Member 2 (SCARB2), which co-localizes with IgG along GBM as confirmed by confocal laser microscopy, and there is no circulating autoantibody to LIMP-2 [70]. There have been emerging tissue proteomic findings in other glomerular diseases such as DnaJ heat shock protein family (Hsp40) member B9 (DNAJB9) for fibrillary glomerulonephritis and amyloid fibrillary typing in renal amyloidosis [33, 71].

In the disease with focal involvement, rather than diffused nature, such as idiopathic focal segmental glomerulosclerosis (FSGS), incorporating proteomic analysis with other -omic approaches and microdissected technology to compare protein abundance in involved and non-involved glomeruli could give rise to novel insight on disease mechanism, account for intra-patient or inter-patient variation in clinical manifestations, or even extrapolate to other proteinuric kidney diseases. This single-glomeruli proteomic approach needs special techniques to minimize loss from small tissue samples. Examples are many analyses of sclerotic glomeruli and renal tubular segments from focal glomerulosclerosis-induced mice and patients with idiopathic FSGS revealing increased lysosomal-associated membrane protein 1 (LAMP-1), SCARB2, and cathepsin B expression along with decreased alpha-actinin-1/4 and CD2 Associated Protein, which confirms the pathogenetic role of these molecules in proteinuria [72].

Apart from elucidating pathogenetic pathways and identifying novel biomarkers, the ultimate goal of the proteomic approach in glomerular disease is that the more apparent mechanism may lead to novel and more precise therapeutic targets, such as in the case of IgA nephropathy. The pathogenesis of IgA nephropathy has long been recognized as a multi-hit or multi-factorial process involving both environmental stimuli and immune dysregulation focusing on lectin pathway activation and susceptibility of the glomeruli for immune complex deposition and injury. However, recent proteomic analysis of microdissected glomeruli from renal biopsy specimens

of progressive IgA nephropathy patients, compared with those from non-progressed and non-diseased patients, reveals a high abundance of 28 classical and terminal complement system proteins which can classify progressors from non-progressors with higher precision than conventional clinical and histological characteristics, namely an area under the receiver operating characteristic (ROC) curve of 0.91 ($p = 0.001$) [73]. The finding, which is compatible with other IHC staining studies, renders multiple ongoing clinical trials investigating the efficacy of numerous alternative and terminal complement pathway inhibitors such as an oral factor B inhibitor iptacopan or LNP023 (APPLAUSE-IgAN, ClinicalTrials.gov identifier NCT04578834), a C3 cleavage inhibitor pegcetacoplan or APL-2 (ClinicalTrials.gov Identifier NCT03453619), a long-acting monoclonal C5 antibody (ClinicalTrials.gov identifier NCT04564339), and a small interfering RNA cemdisiran which reduces liver production of C5 (ClinicalTrials.gov identifier NCT03841448) [74].

Since most pathogenetic pathways of glomerular diseases relate to immune dysregulation, all therapies, even those guided by the precision approach, rely on immunosuppressive or immunomodulatory effects. The proteomic approach also sheds light on more direct therapeutic targets like precision medicine in the oncology field, though most are primarily confined to preclinical studies. An example is testing of phosphodiesterase-4 inhibitor rolipram in puromycin aminonucleoside (PAN) induced rats with podocyte foot process effacement since the combination of the bioinformatic analysis of proteomic profiles and modeling software suggest the role of protein kinase A in the reorganization of the actin cytoskeleton via a phosphodiesterase-dependent mechanism [75]. Another example is a study of Yes-associated protein-1 (YAP1) inhibitors for proteinuria reduction in rats with PAN-induced nephropathy. A proteomic analysis demonstrates an increase in YAP1, a transcriptional co-activator of fibrotic genes induced by mechanical stimuli and diseased state, even before the onset of proteinuria [76].

4.6.2 Diabetic Kidney Disease (DKD)

Since about 40% of diabetes patients eventually develop nephropathy and the rate of kidney function decline in patients with DKD is highly variable, with several patients reach ESKD, even with optimal standard therapy, a more personalized or precise approach has been recently introduced to this area with the help of systems biology, including the proteomic approach. Although exemplary proteomic implementation in DKD care should involve identifying pathogenetic pathways, prediction of DKD development [77], estimating the risk of developing unfavorable outcomes, helping in earlier or more precise diagnosis, and monitoring response after therapy, its utilization to date mainly focuses on evaluating progression rate and adverse renal or cardiovascular outcomes [78, 79]. Limitations for implementation include complex pathogenesis of DKD comprising endogenous, environmental, and dietary factors, lack of a gold standard for diagnosis without performing kidney biopsy, infrequent tissue sample obtaining, and lack of validation of many

proteomic profiles. This makes multiple proteomic studies in DKD have a small sample size and inadequate external validation.

However, there is a most studied and robust proteomic study in chronic kidney disease (CKD), including DKD, called CKD273 classifier, which comprises 273 urinary peptides related to intrarenal inflammation and extracellular matrix remodeling [80, 81]. CKD273 classifier predicts CKD progression and progressive albuminuria even in a normoalbuminuric state in addition to conventional clinical variables such as eGFR and albuminuria level in four studies. It even predicts unadjusted risk for ESKD and mortality in one study [82]. Moreover, the CKD273 classifier comprises many collagen-related peptides, along with some collagen fragment peptides associated with renal fibrosis from 42 kidney biopsy specimens. No such association is observed for conventional clinical parameters [83]. This classifier is the first to be tested on clinical efficacy from implementation to guide early initiation of aldosterone antagonists in DKD patients without albuminuria in the large-scale prospective Proteomic Prediction and Renin Angiotensin Aldosterone System Inhibition Prevention of Early Diabetic nephRopathy In TYpe 2 Diabetic Patients with Normoalbuminuria (PRIORITY) trial [84].

4.6.3 Acute Kidney Injury (AKI)

Due to the acute and critical nature of AKI, pathogenesis studies outside the preclinical field and therapeutic studies incorporating personalized medicine other than dialysis therapy are impractical. The implementation of a proteomic approach to human AKI essentially restricts identifying renal tubular biomarkers for early AKI diagnosis before the elevation of serum creatinine, such as neutrophil gelatinase-associated lipocalin (NGAL), alpha-1-microglobulin, beta-2-microglobulin, and alpha-1-antitrypsin. These candidate biomarkers derive originally from preclinical models and proteomic studies in subjects with different AKI phenotypes such as AKI staging, recovery status, or time to recovery, the so-called discovery phase, and finally be verified in external validation cohorts before introducing into clinical practice [85]. Of these biomarkers, NGAL has been tested by multiple studies to be predictive for severe AKI, dialysis requirement, and mortality after AKI in addition to early AKI diagnosis [86]. So, proteomic analysis in AKI can also give more insights into the mechanism of the disease, though indirectly, especially upon considering along with genomics, previous mechanistic studies, and the Human Protein Atlas for protein localization.

However, there are some limitations for implementation. Firstly, spot urinary collection in AKI may not be appropriate since the patients lose their steady-state, and calibration to urinary creatinine concentration or obtaining timed urine collection is a suggested solution. Secondly, candidate biomarkers may present low abundance in the biosamples, so the analyzing technique should be selected cautiously. Lastly, the appropriate cut point and external validation for any interesting outcome are important, especially for severe AKI, as the predominant population in most

cohorts are patients with mild to moderate AKI. This makes the area under ROC curves for AKI stage 3 lower than overall AKI for NGAL products [87]. This emphasizes the importance of the recruitment of more patients with various clinical phenotypes in the study cohorts.

4.7 Future Perspectives

Although proteomics has been promising for the discovery of biomarkers in kidney diseases, the ongoing advancements of proteomic technology, for example, the expansion of the high abundance peptide list and database, the improvement of labeling technologies, the refinement of sample fractionation, especially exosome isolation, and the reduction of sample contamination, can be expected to achieve even more remarkable discovery in the near future.

In addition, proteomics is an appropriate approach for the identification and the development of novel biomarkers from serum or urine, either of which can be easily translated to the use of non-invasive diagnosis and monitoring of kidney diseases in clinical practices. Though most urinary proteins could be derived from any part of the nephrons, the proteomic analysis could help identify and quantify segment-specific protein expression. Therefore, the enrichment strategy to identify renal tubular segment-specific biomarkers could be accomplished through proteomic techniques.

Apart from measurement of protein or peptide abundance, the analysis of proteins in the distal pathways such as PTMs and protein interactomes would lead to more extensive insights. Measurement of abundance and quantitative relationship, the so-called stoichiometric analysis, of such PTMs and interactomes would enhance discovering more specific biomarkers. Moreover, multi-omics analysis, the combination with other omics approaches such as genomic, epigenomic, transcriptomic, and metabolomic analyses, would generate comprehensive data to assess the biological processes of complex disease in each patient. The integration of multi-omics analysis in clinical practice would help in disease diagnosis, subtyping, and prognostication which would guide clinicians to precise decision-making.

Despite advances in proteomic methods for novel biomarker discovery, the applicable biomarkers in real clinical practice are still limited. After discovering the potential protein biomarkers, the validation study in a larger population is required to evaluate diagnostic accuracy of such biomarkers. Finally, approval of the local regulatory agencies and assessment of cost-effectiveness are mandatory before implementation in the clinical practice. Therefore, in the "discovery-validation-implementation" paradigm of the development of biomarkers, the latter two, which require more extensive clinical trials and perhaps the pharmaceutical industry's investments, are the crucial rate-limiting steps. The collaborations between researchers, clinicians, and the pharmaceutical industry are necessary to surpass such barriers and lead to a step closer to implementing the true "precision nephrology."

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Chapter 5
Single Cell Transcriptomics

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5.1 Introduction

Kidney is a highly complex organ with diverse cell types and structures. This cellular and molecular complexity hinders our ability to fully understand mechanisms underlying both homeostasis and disease. Conventional technologies for measuring the transcriptome have contributed substantially to our knowledge over the years. These technologies include microarray and RNA sequencing (RNA-seq) that quantitate messenger RNA (mRNA). RNA-seq has dramatically improved our understanding of molecular mechanisms in health and disease since its advent in mid-2000s. However, RNA-seq usually requires bulk tissue (thousands to millions of cells) for data generation. Such bulk transcriptomic profiling (bulk RNA-seq) is useful for cultured cells or relatively homogeneous tissue, but it is more limited when applied to a cellularly complex tissue like kidney, since signals from low abundance cell types are lost in the integrated sample [1].

The recent advent of single-cell RNA sequencing (scRNA-seq) technologies now allows transcriptomic profiling with single-cell resolution, resolving this

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limitation from bulk RNA-seq [1]. We and others have performed scRNA-seq on human and mouse kidneys [2–4], dissecting cellular heterogeneity in normal and diseased kidneys. These single-cell transcriptomic atlases have been utilized for dissecting molecular mechanisms of kidney diseases and identifying new therapeutic targets. Furthermore, scRNA-seq has the potential to serve as a diagnostic tool in the future. The scRNA-seq platform is commercially available, and the cost of sequencing is decreasing rapidly [5]. An increasing number of user-friendly bioinformatic tools now enable any researcher to utilize scRNA-seq without substantial expertise in computational skills. Thus, a basic understanding of scRNA-seq is becoming essential for both researchers and clinicians. In this section, we review the fundamentals of scRNA-seq from methodology to data analysis and its successful applications to dissect disease mechanisms in nephrology.

5.2 The Fundamentals of scRNA-seq

5.2.1 snRNA-seq vs. scRNA-seq

The first step in an scRNA-seq experiment is tissue dissociation. However, the kidney's cellular complexity and density complicates the task of generating a high-quality single-cell suspension representing all cell types. First, not all types of kidney cells are efficiently extracted from the tissue by cell dissociation procedures (with either enzymatic or mechanic methods). This is because kidney cell types have distinct physical and chemical properties and sizes, and therefore, some cells that are sensitive to external stimulus (e.g., glomerular cells) show poor viability, and some cells surrounded by connective matrix are more resistant to such dissociation such as stroma (especially for cells extracted from a fibrotic kidney). Second, kidney tissues, particularly for tissues obtained from human, are usually cryopreserved due to sample availability, but many cell dissociation protocols perform poorly with frozen tissues. Finally, the dissociation itself when performed at 37 °C causes artifactual cell transcriptional stress responses (e.g., immediate early gene induction), which confounds results. As a consequence, an increasing number of nephrology studies are adopting single-nucleus RNA-seq (snRNA-seq), in which cells are completely lysed to expose nuclei, and the single-nucleus suspension is used with other chemistries almost unchanged [2, 3].

An important difference between snRNA-seq and scRNA-seq approaches is that mRNAs captured in snRNA-seq are located within the nucleus instead and, therefore, are enriched for intragenic regions (introns) of pre-mRNAs that have not been spliced into mature mRNAs (due to higher polyadenine tracts within introns, allowing capture by polythymidine (poly-T) tracts on capture oligonucleotides). Due to the enrichment of these nascent transcripts, snRNA-seq may be more efficient in describing temporal effects of gene transcription events [6]. Importantly though, it is critical that snRNA-seq studies analyze reads from both exons and introns [2]. In a study comparing the efficacy of snRNA-seq vs. scRNA-seq on kidney tissues [2],

snRNA-seq showed equivalent throughput and gene detection sensitivity compared to scRNA-seq. This study also showed that snRNA-seq could improve identification of certain kidney cell types including podocytes, mesangial cells and endothelial cells, and significantly reduce dissociative artifacts. Although it is still under debate whether snRNA-seq might fully replace scRNA-seq, complementary roles exist for the two methods [7], and they are both actively in use. Overall, there is a rising use of snRNA-seq because it also offers more flexibility in current nephrology studies, for example, through biobanking of snap-frozen tissues for later analysis. For convenience, both methods will be referred to scRNA-seq going forward in this chapter.

5.2.2 scRNA-seq Methodology

Library generation in scRNA-seq includes RNA capture by poly-T oligos followed by the generation of complementary DNA (cDNA) via RNA reverse transcription (Fig. 5.1a). However, in contrast to bulk RNA-seq, these cDNA sequences must identify the cell from which the RNA originated. This is accomplished by inclusion of a cell-specific oligo with a sequence unique to each cell (i.e., a unique cell barcode). Methodologies for single-cell manipulation have rapidly evolved since being first described in 2009 [8]. The early methods, including limiting dilution, micromanipulation, and cell sorting, are based on manual isolation of individual cells [9] and as a consequence are low-throughput (low number of cells characterized) and will not be reviewed in this chapter.

Currently, the most widely used solution for single-cell omics is droplet microfluidics which enables automatic isolation of each cell by a partitioning system [10]. Briefly, the microfluidic device can generate an aqueous droplet sized to encapsulate a single cell by precisely controlling flow rates of water, oil, and the cell suspension (Fig. 5.1a). Then, a single cell and a microparticle (usually a bead or gel) conjugated with a distinct molecular barcode will be co-encapsulated into the droplet. The isolated cell is then lysed inside the droplet and mRNA binds to poly-T on the microparticle. cDNA is generated (either within the droplet or in bulk solution, depending on the technique) by reverse transcription. Representative technologies include Drop-Seq [11], InDrops [12], and the Chromium system developed by 10× Genomics [5]. One obvious advantage of droplet microfluidics is time efficiency compared with early methods: The flow rates of a microfluidics device and the concentration of a cell suspension are predefined, so cells can be processed by the automated device efficiently (currently completed within a 1-day workflow). Additionally, this technology exhibits high throughput (nearly 10,000 cells characterized in one lane for the 10× Genomics solution, for example), gene detection sensitivity (>3000 genes detected per cell), reliability, and reproducibility. Despite relatively high costs, droplet microfluidics has become a mature technology, and is increasingly used in nephrology research. Examples will be covered in the next section.

Besides droplet microfluidics, another approach to single-cell manipulation is the microwell (nanowell)-based assay such as CytoSeq [13] and Microwell-seq

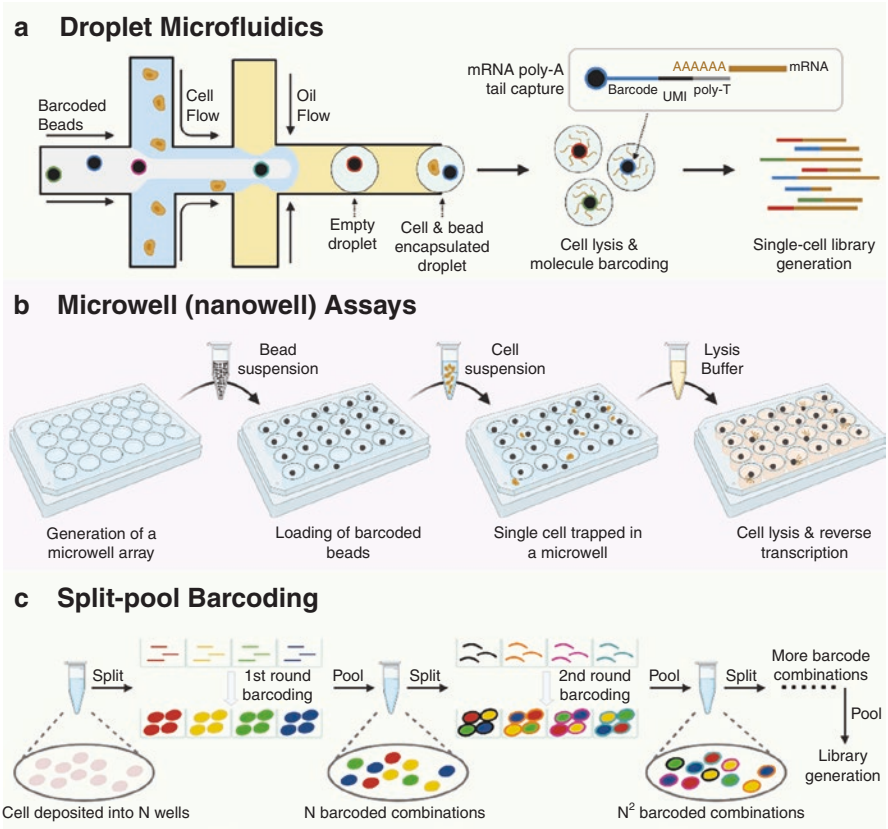

Fig. 5.1 Scheme of three single-cell manipulation methodologies. (**a**) In droplet microfluidics, single cells are isolated by a cell partitioning system which can co-encapsulate a cell and a barcoded bead in a droplet. Cells are lysed and mRNAs are captured by the poly-T oligos conjugated on the bead. (**b**) In microwell (nanowell)-based assays, a microwell array is generated and loaded with barcoded beads and cells. Each microwell is sized to trap one bead and one cell to enable single-cell capture. (**c**) In split-pool barcoding, in each well of a multi-well plate, a group of cells are deposited and indexed with well-specific barcodes. Then, cells are pooled and redistributed to another multi-well plate for the second round of molecular indexing. This step can be repeated and each cell will be indexed with a unique combination of several barcodes. *UMI* unique molecular identifier, *Poly-T* polythymidine, *Poly-A* polyadenine. (Created with Biorender.com)

[14]. In Microwell-seq, an agarose microarray containing 100,000 microwells is generated from a silicon micropillar chip, and the microwell can trap a single cell after a cell suspension is loaded (Fig. 5.1b). Compared to droplet microfluidics, this method enables removal of low-quality cells after cells are deposited into microwells, because cells can be inspected under a microscope to manually remove cells with poor morphology or doublets. Despite improved throughput and reduced costs, a major handicap of this assay is the generation of the micropillar chip which depends highly on specialized industrial fabrication capabilities.

One common feature of the two methodologies mentioned above is isolation of individual cells in a physical reaction chamber (i.e., droplet or microwell). A complementary and enabling technique has emerged recently. This single-cell manipulation method is termed split-pool barcoding (also called single-cell combinatorial indexing) and does not attempt to incorporate each cell with one unique cell barcode in a chamber, but instead marks a group of cells with sequential molecular indexes which ultimately differentiate individual cells by the unique combination of several barcodes (Fig. 5.1c). Featured technologies include sci-RNA-seq [15], SPLiT-seq [16], and SHARE-seq [6]. In sci-RNA-seq, for example, a group of cells (not individual cells) is placed in each well of a 384-well plate, and each well contains a unique cell barcode. Then, the cells from all 384 wells are pooled and then redistributed to wells of another 384-well plate with different barcodes in each well. The process is typically repeated a third time, resulting in a total of 384^3 (>50 million) barcode combinations. In this way, nearly all cells will be indexed with a unique combination of three different oligos. This method does not rely on specialized (and often expensive) equipment such as the microfluidics device, so gives access to single-cell technologies across a wide spectrum of laboratories. It also has advantages in its unprecedented throughput: million-cell datasets are easily within reach and costs are ~20× lower than current 10× Genomics solutions (~$0.01 per cell) [17]. The trade-offs include low gene detection rate and a very high requirement for labor—chiefly pipetting and exquisite molecular biology skills.

Although droplet microfluidics remains the most widely applied approach to scRNA-seq currently, other methodologies are emerging with enhanced throughput, gene detection sensitivity, and cost efficiency (Table 5.1). Researchers need to

Table 5.1 Comparison among modalities for scRNA-seq

	Microfluidics	Microwell-based assays	Split-pool barcoding
Throughput	1000–10,000 cells	~100,000 cells	Millions of cells
Gene detection sensitivity	High	Moderate	Low–moderate
Sample multiplexing	No	No	Yes
Required equipment	Microfluidics	Micropillar chips	Common 96-well plates
Library generation pipeline	Well established	Less optimized	Less optimized
Commercial availability	Yes	No	No
Bioinformatic resources	Rich	Limited	Limited
Representative platforms	10× Chromium; Drop-seq; InDrops	CytoSeq; Microwell-seq	sci-RNA-seq3; SPLiT-seq
Mouse kidney studies	[2, 4, 18–25]	[14]	[15]
Human kidney studies	[3, 26–31]	[32]	[33]
Multi-omics studies	[34, 35] (snATAC-seq)	NA	[6] (SHARE-seq)

determine the most appropriate scRNA-seq methodology depending on their study goals because each approach has unique advantages and limitations. For example, microwell (nanowell)-based assays are usually compatible with full-length transcript identification and split-pool barcoding methods can profile cells from multiple samples in one experiment (i.e., sample multiplexity), both of which are major limitations of droplet microfluidic single cell techniques [17]. Additionally, these methodologies can be leveraged to study not only single-cell transcriptome, but also other modalities (i.e., multi-omics) with modified chemistries.

5.2.3 Data Analysis

The amount of data generated by scRNA-seq is much larger than that generated by bulk RNA-seq. Furthermore, scRNA-seq suffers from sparsity—a large fraction of measurements recorded as zeroes, meaning that a given gene in a given cell had no reads mapped to it. This can either represent missing data (i.e., insufficient sensitivity) or alternatively a true absence of expression. How to handle the data sparsity of scRNA-seq is still controversial [36], since distinguishing between a true (biological) zero and a technical zero is challenging. scRNA-seq preprocessing and analytic methodologies have been developed to minimize these difficulties.

After library preparation and sequencing, the reads are preprocessed before analysis (Fig. 5.2a). First, the raw data are aligned on a reference genome to generate feature-barcode matrices with computational pipelines such as the CellRanger (10× Genomics) and zUMIs [37]. Both the tools internally utilize Spliced Transcripts Alignment to a Reference (STAR) [38] software to map each read to a reference assembly. CellRanger also offers basic analyses like clustering and visualization, lowering threshold to start scRNA-seq for the researchers who are not familiar with coding. After alignment, there are a number of publicly available computational packages for quality control, preprocessing, data visualization, and various secondary analyses. For example, Seurat [39] and Monocle [40] are most frequently used for these processes. Scanpy [41] is a scalable, Python-based package that can efficiently deal with a dataset of even more than a million of cells.

After mapping, low-quality cells are usually filtered out based on various indicators, such as number of detected genes, number of transcripts and mitochondrial gene percentage per cell. There are also other complementary packages to computationally remove technical artifacts. Ambient RNA exists in solution after tissue dissociation (especially with nuclear dissociation), providing false-positive reads in all cells. Computational packages like SoupX [42] and CellBender [43] correct ambient RNA in scRNA-seq data. Another example of technical artifact is doublet formation in a droplet–the co-encapsulation of two cells that will both share the same cell molecular barcode. There are several computational tools to deal with heterotypic doublets, including DoubletFinder [44] and Scrublet [45]. Development of these computational strategies against artifacts have improved the quality of downstream analysis. Next, the data are normalized, since sequencing depth alters

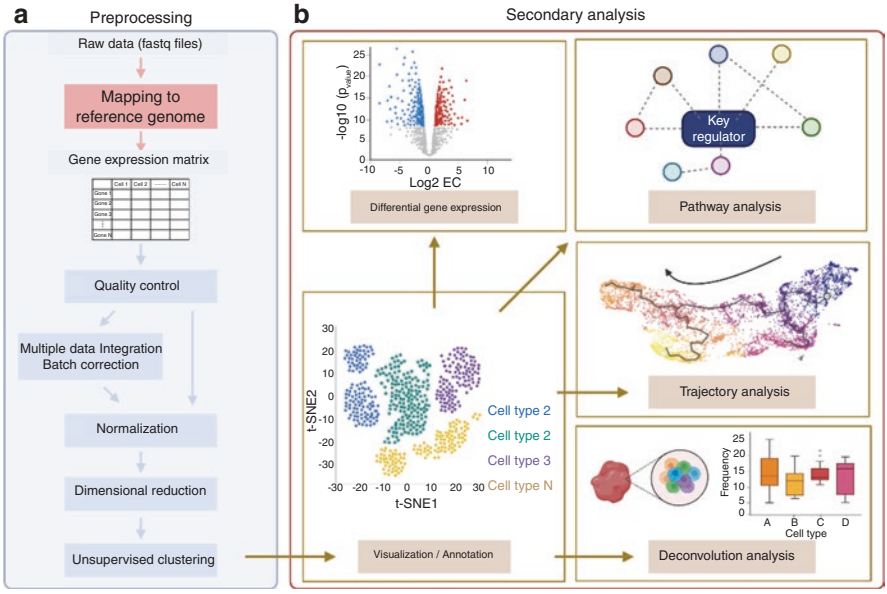

Fig. 5.2 Preprocessing and basic analyses for scRNA-seq data. (**a**) Preprocessing workflow for scRNA-seq includes mapping of raw files (fastq files) to reference genome to generate gene expression matrices, quality control, multiple dataset integration with batch effect correction, normalization, dimension reduction, and unsupervised clustering. (**b**) Subsequent secondary analyses including but not limited to differential gene expression analysis, pathway analysis, trajectory analysis, and deconvolution of bulk RNA-seq with scRNA-seq data. (Created with Biorender.com)

the raw count number of each gene. There are several normalization approaches in addition to simple log normalization adopted in Seurat [39]. For example, SCtransform [46] offers a probabilistic approach showed to perform better than conventional normalization approaches. When one needs to analyze several independent datasets, additional normalization may be needed to correct biological and technical batch differences. Seurat implements a set of methods to align similar cell populations across datasets by identification of "anchors" that are pairs of cells across the datasets with matched gene expression signature [39]. Another popular batch correction strategy is offered by Harmony that iteratively learns a cell-specific correction function [47]. The maturation of integration methodologies has achieved cross-species or even cross-modality integration to identify a shared cell type or cell states [39].

As mentioned above, scRNA-seq produces a tremendous amount of data compared to bulk transcriptomic profiling, and the resultant huge matrices are uninterpretable by the human brain. Hence, the single-cell data must be computationally reduced and clustered in an unsupervised fashion for visualization and downstream analyses. There have been numerous approaches for dimension reduction developed so far, although t-distributed stochastic neighbor embedding (t-SNE) and uniform manifold approximation and projection (UMAP) [48] are the most popular

approaches. Next, unsupervised clustering is performed on the dataset for classification and annotation of cell populations, where the Louvain and Leiden algorithms are the most widely used algorithms. An important caveat is that dimension reduction and unsupervised clustering are highly sensitive to user-defined parameters, so one needs to carefully determine based on indicators provided by the computational package (e.g., Jackstraw plot or Elbow plot on Seurat) and established marker gene expressions on resultant clusters. As reviewed above, there are numerous tools for normalization, data integration, and data visualization. Several published studies have compared and benchmarked various methodologies [49, 50].

After preprocessing, there are a variety of computational packages freely available for an array of analytic approaches (Fig. 5.2b). Differentially expressed genes (DEG) detected among the identified cell types or same cell states in different conditions provide numerous insights about cell states. The list of DEG is often applied to gene ontology analysis [51] or gene set enrichment analysis [52] to describe cell states. One might also want to compare DEG to GWAS-related genes to infer association of specific cell types or cell states with a certain genetic predisposition [53]. We could also infer gene regulatory networks and key transcription factors in the biological processes by a scRNA-seq pipeline named SCENIC [54]. In addition, machine learning has enabled various unsupervised analyses to reveal unpredicted findings from single cell datasets. For example, pseudotemporal ordering analysis is a computational approach on single cell data to infer the order of biological processes experienced by cells, such as differentiation or disease [55]. The resultant trajectory enables us to detect gene markers specific to an unrecognized transitional cell state or genes critical for cell fate decision. Another approach for inference of future cell state is RNA velocity that leverages the ratio of spliced and unspliced mRNA [56]. Furthermore, lineage tree reconstruction beyond these approaches has been developed by tracing somatic mutations on mitochondrial DNA captured in scRNA-seq [57]. Genome-based lineage tracing techniques utilizing scRNA-seq have been emerged with the evolution of CRISPR-Cas9-based gene editing [58]. Inference of ligand–receptor interactions is a common analytic method of scRNA-seq data. The development of bioinformatic tools like Connectome [59] or CellPhoneDB [60] offers landscape of intercellular communication in microenvironment. Several computational approaches such as CIBERSORT [61] and Bisuqe [62] have been published to infer cell type proportions in bulk RNA-seq data based on scRNA-seq data. Such deconvolution strategies based on scRNA-seq will broaden the usefulness of accumulated bulk transcriptomic data.

5.2.4 Complementary Technologies for scRNA-seq

Single-cell profiling approaches have been extended to various epigenetic analyses. For example, single nucleus assay for transposase-accessible chromatin using sequencing (snATAC-seq) adopts hyperactive Tn5 transposase to assess chromatin accessibility with single-cell resolution. Reconstruction of cis-regulatory DNA

networks or inference of transcription factor activity in snATAC-seq enables us to understand cell-specific regulatory mechanisms of transcriptome obtained in scRNA-seq [63]. We and others recently published multimodal single-cell atlases for human kidneys or mouse kidneys to understand comprehensive mechanisms of gene regulation in the kidney [34, 35]. Other single-cell approaches include single-cell profiling of histone modifications [64] or chromatin interactions [65]. Spatial transcriptomics is an emerging methodology to simultaneously obtain transcriptome and their locations [66]. Recently commercialized platform of spatial transcriptomics has broadened our understanding of spatial regulation of cell types and subpopulations in the organ. These emerging technologies have brought opportunities of integrative multimodal analysis combining scRNA-seq and complementary technologies. Integrative multimodal approaches are intensively reviewed by Stuart et al. [67].

5.3 Applications of scRNA-seq in Nephrology

5.3.1 scRNA-seq in Mouse Studies

Preclinical mouse models offer many advantages and have been widely adopted for scRNA-seq studies of kidney (Table 5.1). Advantages include high sample availability, low variation across individuals, and well-developed diseased models. In 2018, Park et al. profiled a healthy adult mouse kidney with droplet-based scRNA-seq and identified 16 major cell clusters with distinct transcriptome profiles [4]. They described a transitional cell state between two types of collecting duct cells, intercalated cells and principal cells, which highlighted the cellular plasticity of this population. The development of a healthy adult mouse kidney cell landscape was further accelerated by other studies, including a high-throughput profile with Microwell-seq [14] and construction of an atlas covering both sexes and different anatomical regions of the kidney [18]. Current works also focus on further improving the cellular resolution by sequencing a specific cell type purified from all populations and, therefore, can enhance identification of rare cell subtypes in kidney. In addition, scRNA-seq is actively used to study molecular events in mouse nephrogenesis that regulate central lineage commitment in organ development. For example, Combes et al. [19] characterized developing mouse kidney cells on E14.5 and E18.5, which identified huge heterogeneity of nephron progenitor cells and described the developmental trajectory of ureteric epithelial cells at the single-cell resolution.

Based in part on success in characterizing healthy mouse kidneys, scRNA-seq has also been employed to interrogate mechanisms of disease pathogenesis in a variety of mouse kidney disease models, including diabetic kidney disease [20], kidney transplant [21], unilateral ureteral obstruction (UUO), and renal ischemia reperfusion injury (IRI). In UUO, for example, scRNA-seq has been successfully leveraged to study profibrotic and proinflammatory phenotypes of proximal tubular cell (PTC) [2], injury responses of myeloid lineage cells [22] and contribution of monocytes to the myofibroblast pools in fibrosis [23]. In addition, scRNA-seq has

promoted our understanding of molecular mechanisms that trigger transition from acute to chronic kidney injury in the IRI model. Kirita et al. [24] sequenced samples over the time course of IRI and provided a comprehensive kidney injury atlas, and discovered a novel diseased cell state of PT that is derived from failed tissue regeneration and named as failed-repair proximal tubular cell (FR-PTC), which is supported by other following studies [25]. Therefore, successful use of scRNA-seq in mouse studies can help illuminate cellular drivers of kidney disease pathogenesis and help identify new therapeutic strategies.

5.3.2 scRNA-seq in Human Kidneys

We and others have published single-cell transcriptomic atlases of human kidneys that have enormous value for dissection of molecular mechanisms in human kidney diseases [3, 26–30, 33] (Table 5.1). For example, Young et al. [26] performed scRNA-seq on healthy and cancerous kidneys and described the similarity of gene expression signature between renal cell carcinoma and a subtype of PTC that expresses VCAM1. We identified NF-κB activation and loss of HNF4A activity in VCAM1-expressing PTC subpopulation based on multimodal single-cell analysis on human kidneys [34]. Interestingly, the molecular signature of this subpopulation showed close similarity to FR-PTC in injured mouse kidneys described above [24, 25]. These scRNA-seq studies successfully described PTC subpopulations related to human kidney diseases [34, 24, 25]. scRNA-seq on diseased kidneys have also provided insights for disease mechanisms and pathophysiology. For example, scRNA-seq on human kidneys with early diabetic kidney disease revealed alteration in gene expressions including disturbed electrolyte absorption and reabsorption in renal tubules [27]. Zheng et al. generated scRNA-seq data on IgA nephropathy kidneys and described a proinflammatory molecular signature of mesangial cells with aberrant IgA deposition. Their findings provided lines of evidence suggesting tubulointerstitial inflammation and fibrosis through crosstalk between mesangial cells and immune or tubular cells [28]. Dissection of immune cell heterogeneity is another important application of scRNA-seq. Arazi et al. [29] described the immune cell landscape on lupus nephritis kidneys, identifying proinflammatory and inflammation-resolving responses of various immune cells. scRNA-seq on the biopsy specimen of transplanted kidney described two monocyte subtypes with or without *CD16* expression and endothelial cells with resting or activated states [30]. One activated endothelial subpopulation expressed genes consistent with the pathologic diagnosis of antibody-mediated rejection [30]. These findings underscore the heterogeneity and complexity of immune processes in pathogenesis of kidney diseases. scRNA-seq has also been applied to understand developmental processes. Cao et al. [33] generated comprehensive single-cell transcriptomic atlases on four million single cells from 15 fetal organs including the kidney. They identified renal progenitor populations, describing transcription factors regulating lineage specifications in developmental kidneys [33].

5.3.3 scRNA-seq in Kidney Organoids

Human kidney organoids have been studied for dissecting processes of organogenesis or disease mechanisms, drug screening, and development of regenerative therapy. Recently, application of scRNA-seq to kidney organoid was found to be useful for studying kidney organoid. We performed scRNA-seq on kidney organoids to describe comprehensive picture of organoid cell states in time course. Based on scRNA-seq data, we established a new strategy to reduce off-target cells by inhibiting brain-derived neurotrophic factor pathway [68]. Subramanian et al. performed scRNA-seq to validate that kidney organoids transplanted under the mouse kidney capsule decreased off-target cells [69]. Furthermore, a novel kidney organoid protocol combining metanephric mesenchyme and ureteric bud-like cells was established to improve PTC maturation and reduce off-target cells, confirmed by scRNA-seq [70]. These works suggest that scRNA-seq is useful to analyze the quality of organoids and to establish better protocols for kidney organoid differentiation.

5.3.4 Publicly Available Data Source

As there are an increasing number of published single-cell studies, usually the raw sequencing data is available for download and reanalysis. These raw datasets are usually available on Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) via an accession number from the publication. There are also several online data visualization tools offered by individual research groups. For example, we established KIT (http://humphreyslab.com/SingleCell/): a webtool for visualization of any gene of interest on our mouse and human kidney single-cell atlases. The Susztak lab also offers a similar visualization tool for their single-cell data (https://susztaklab.com/). Azimuth (https://azimuth.hubmapconsortium.org/) is a webtool that offers annotated reference dataset for various organs, including kidney [39]. One can upload any dataset that will be automatedly processed, analyzed, and annotated with cell types. Azimuth is also useful for visualization of target genes on the reference healthy kidney dataset which consists of beyond ~60,000 kidney cells [31].

5.4 Future Perspectives

scRNA-seq is a powerful tool to dissect cellular heterogeneity and disease mechanisms in the kidney. The capacity of scRNA-seq to describe disease-specific cell states also suggests its potential for diagnosis and precision medicine by describing cell-specific transcriptional profiles in disease. Indeed, we and others have successfully performed scRNA-seq on kidney biopsy specimens [28, 30]. It has already revealed new candidate therapeutic targets, for example, the NFkB pathway in proximal tubule cells that fail to repair after AKI [24]. In antibody mediated rejected of

kidney transplants, we have used scRNA-seq to describe activated endothelial cells that are most likely the driver of this insidious disease [30]. In addition, the Kidney Precision Medicine Project is utilizing scRNA-seq to categorize and sub-phenotype both AKI and CKD [31]. At present, scRNA-seq applications are limited to research but one can envision a time when it might be applied for diagnostic purposes. Hurdles that would need to be overcome to realize this goal include the high cost and relatively slow turnaround of the technique. Recently, scRNA-seq on urinary cells of subjects with diabetic kidney disease and healthy subjects was performed, and almost all kidney cell types along with bladder cell types were identified [71]. The cell type composition in urine was specific to the subject and relatively stable among different collection methods, suggesting the potential of urine sediment as a diagnostic tool. Accumulating single-cell datasets on urine and further decreased costs of scRNA-seq will accelerate the use of scRNA-seq in accurate diagnosis of kidney diseases and precision medicine.

The first wave of single-cell studies has been largely dependent on the commercially available droplet-based scRNA-seq platforms [11]. Currently, many studies are profiling multiple samples from diverse background and disease stages, given the genetic and environmental heterogeneity of human subjects. This demands improved throughput, and the emerging single-cell methods such as split-pool barcoding [17] offer one solution by providing million-cell datasets in one experiment with reduced costs. Another potential bottleneck is the requirement of advanced computational power and user bioinformatic skills.

5.5 Conclusion

We have reviewed current scRNA-seq techniques, emerging complementary technologies and their application in nephrology. scRNA-seq has dissected cellular heterogeneity with distinct gene expression signatures in human and mouse kidneys, providing numerous insights for disease mechanisms and potential therapeutic targets. It has already been successfully applied to better understand kidney development, AKI, CKD, diabetic kidney disease, and renal cell carcinoma. Ongoing evolution of single-cell technologies and maturation of bioinformatics will continue to offer substantial opportunities for both researchers and clinicians in nephrology.

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Chapter 6
Gut Microbiota and Chronic Kidney Disease

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6.1 Epidemiological Scenario

Chronic kidney disease (CKD) is a syndrome characterized by the progressive decrease in the function or structure of the kidneys, which lose their functional capacity to filter blood and maintain the body's homeostasis. It is associated with high morbidity and mortality rates, posing a major challenge to public health as it has a major socioeconomic impact [1]. Adult patients are identified with CKD when they present, for a period equal to or longer than 3 months, a glomerular filtration rate (GFR) lower than 60 mL/min/1.73 m^2, or GFR higher than 60 mL/min/1.73 m^2, but with evidence of kidney structure damage. Some indicators of kidney damage are albuminuria, renal imaging changes, hematuria, leukocyturia, persistent hydroelectrolyte disturbances, histological changes on renal biopsy, and previous kidney transplantation. Albuminuria is defined by the presence of more than 30 mg of albumin in the 24-h urine or more than 30 mg/g of albumin in an isolated urine sample adjusted by urinary creatinine. Major causes of CKD include diabetes, hypertension, chronic glomerulonephritis, chronic pyelonephritis, chronic use of anti-inflammatory drugs, autoimmune diseases, polycystic kidney disease, Alport syndrome, congenital malformations, and long-term acute kidney disease [2]. The prevalence and incidence of CKD in many countries are unknown, but they stand out as being highly prevalent and are associated with an increased risk of cardiovascular disease, severity, and death. The United States estimates 14.8% prevalence of CKD in the adult population from 2011 to 2014 and 703,243 cases, with 124,114

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new cases in 2015, showing an incidence rate of 378 patients per 1,000,000 people (pmp), with 87.3% of these on renal replacement therapy. In Latin America, the incidence was 167.8 pmp in 2005 and, in Brazil, 431 pmp in 2004 [3]. Global data from 2013 showed that reduced GFR was associated with 4% of deaths worldwide, i.e., 2.2 million deaths. More than half of these deaths were caused by cardiovascular disease, while 0.96 million were related to end-stage renal disease [2].

Epidemiological surveys addressing the disease are still scarce in regard to CKD risk factors before renal replacement therapies. Among these, the CKD Study, the National Health and Nutrition Examination Survey (NHANES), and the United States Renal Data System (USRDS) Report pointed out the increased prevalence of CKD at ages over 70 years and the association of the disease with hypertension in the United States and with diabetes in Mexico [3].

Chronic kidney disease is estimated to have affected about seven million individuals in 2017. As CKD is related to a large socioeconomic burden and a high mortality risk, identifying causal factors for CKD is important to elucidate biological pathways that are potential therapeutic targets [2].

6.2 Gut Microbiota and Chronic Kidney Disease

The human microbiome, a term derived from the Greek *mikros* (small) and Latin, *bio* (life) and *oma* (group or mass), is characterized by a set of microorganisms, their genomes, and the environmental conditions present in tissues and different parts of the human body [4]. There is extensive search to understand the human microbiome, its metabolites, its action on the host, and the importance of the complexity of these relationships in health and disease. Thus, advances in high-throughput sequencing methods have paved the way for decoding bacterial genomes from different parts of the human body, a fundamental basis for microbiome analysis. Besides, the use of new technologies, especially those related to "omics" technologies—genomics, proteomics, metabolomics, and others [5].

There is evidence of the likelihood that the development of the human microbiome begins formation in the womb, continues at birth, and beyond [6, 7]. However, the origin and means of transmission of early childhood microorganisms are poorly understood. Ferreti et al. (2018) obtained interesting findings: it was possible to identify a very high microbial diversity and strain heterogeneity in the pioneer infant intestinal microbiome; another important factor is that microbial strains present in infants, for which there is strong evidence of transmission from their mothers, are more likely to adapt and persist in the infant intestines; however, they gradually decline over time shifting to acquire strains from the environment and different places [8].

The set of these microorganisms that make up the microbiome is called microbiota, which is composed mainly of bacteria, viruses, fungi, protozoa, and archaea; they colonize the human body in the cutaneous, oral, respiratory, gastrointestinal, and genitourinary tracts [9]. The largest amount is present in the gastrointestinal

tract, which hosts more than 100 trillion microorganisms, including at least 1000 different species of bacteria [10]. It is also in the gastrointestinal tract (GI tract) that we find most of the immune system, thus associating it as an indicator of health [11]. The microbiota has an influence on the modulation of the immune system in both health and disease, for this and other factors are associated in the development or cause of chronic noncommunicable diseases (NCDs) [12]. This perspective of the microbiome universe is relevant in the current panorama of global public health, since NCDs have been the leading cause of death in the world population [13].

There is a connection between intestines and kidneys that can be classified into metabolic and immunological pathways. In the metabolic pathway, mediated by metabolites produced by the gut microbiota, an inadequate diet is the possible inducer, resulting in a high production and accumulation of toxic substances, called uremic toxins (UTs) in the intestinal environment, such as indoxyl sulfate (IS) and para-cresyl sulfate (PCS) (Fig. 6.1). They are excreted by the kidneys, and the accumulation of these elements in the intestines causes damage to the mucosal barrier leading to increased intestinal permeability (leaky gut). This leads to the influx of endotoxins and UTs into the kidneys via the circulation, which contributes to renal inflammation. In the immune pathway, the gut microbiota activates intestinal immune cells as well as modulates the profile of immunoprogenitor cells in the bone marrow. In this scenario, activated immune cells and pro-inflammatory cytokines

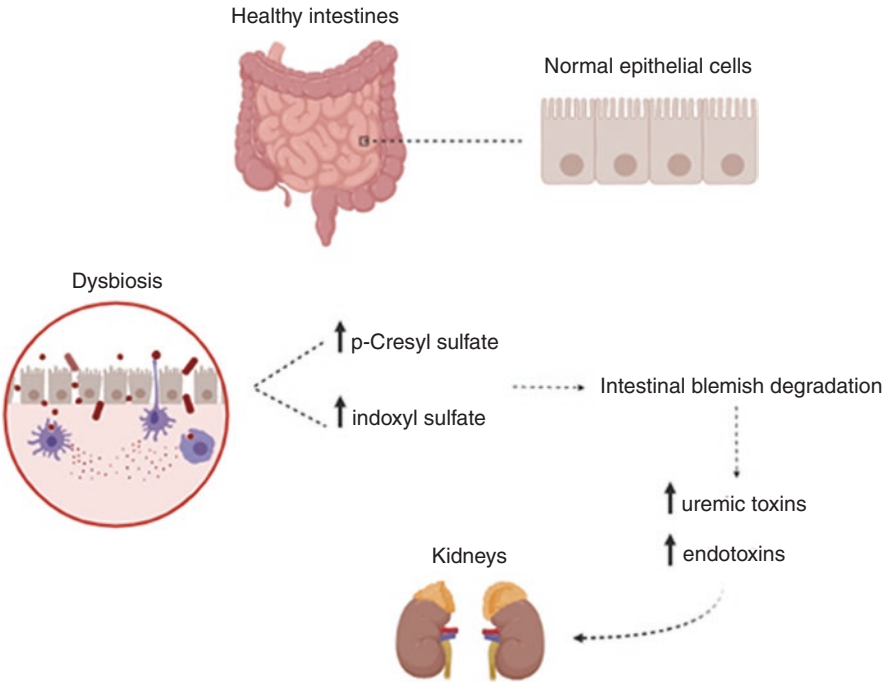

Fig. 6.1 Connection between gut microbiota and chronic kidney disease

contribute to renal inflammation via the circulation. In addition, there is an increase in plasma levels of the soluble plasminogen activator urokinase receptor (suPAR), caused in the early phase and progression of CKD. The concentration of bacteria and their products in the circulation considerably contributes to chronic low-grade inflammation, which plays a critical role in the maintenance of many chronic non-communicable diseases (NCDs), being hypertension and CKD among them [14]. The results of IS and IPS accumulation include reactive oxygen species production, cardiac fibrosis, inflammation, inflammatory gene expression, activation of the renin-angiotensin-aldosterone system, renal tubular damage, and others [15].

UTs from IS, PCS, and TMAO (trimethylamine *N*-oxide) induce in CKD risk factors such as inflammation, oxidative stress, and fibrosis. Cardiovascular injury is the leading cause of morbidity and mortality in CKD patients, and studies point to dysbiosis as a factor that plays a significant role in this context [1].

6.2.1 Microbiome: An Evolutionary Perspective

In the course of evolution, humans have adapted not only to climatic and environmental conditions but to the food and resources available. Humans have undergone anatomical, physiological, and metabolic changes, mainly due to the availability of food from different regions and climate. Some anthropologists claim that the eating patterns of Paleolithic human ancestors significantly influenced neural expansion, increasing the size of the brain and decreasing the size of the gastrointestinal tract [16–18]. These dietary patterns of pre-agricultural hominin diets are useful for understanding how the current Western diet may predispose modern populations to chronic diseases.

The Paleolithic diet was based on plants, vegetables, animals and fish they hunted, and insects with their by-products, such as honey, quite different from today's diet, based on processed and ultra-processed foods, high in salt and sugars [19]. This Paleolithic nutritional transition to the Western diet, associated with the lack of corresponding genetic adaptations, causes significant deleterious changes in metabolism [20]. With this new Western lifestyle and diet almost everywhere in the world, overweight and chronic diseases are also increasing rapidly in developing countries. Some observed features of this dietary adaptation process include: an increased production of reactive oxygen species and oxidative stress, development of hyperinsulinemia and insulin resistance, low-grade inflammation, and an abnormal activation of the sympathetic nervous system and renin-angiotensin, which play key roles in the development of chronic diseases. This explains the close relationship between obesity and a wide range of comorbidities, such as type 2 diabetes mellitus, cardiovascular disease, and others. Lifestyle changes according to genetic makeup, including diet and physical activity, can help prevent or limit the development of these diseases [21].

Diet as a major modulator of the microbiome has also shaped microbiota profiles throughout human evolution [22]. The microbiota has evolved to achieve homeostasis in the host in response to profound changes in lifestyle, especially in the last

10,000 years [23]. Studies and knowledge of how the microbiome changed during evolution are rare. In an attempt to understand some aspects, Schnorr et al. (2014) explored the Hadza gut microbiota from Tanzania, a modern hunter-gatherer population that lived as Paleolithic humans. This study showed the first map of the composition of the Hadza microbiota that reflects functional adaptation to a foraging lifestyle, with high bacterial diversity and enrichment in fibrolytic microorganisms (such as xylan degrading Prevotella and Treponema), representing adaptations to provide SCFA from their plant-rich diet. However, despite the absence of Bifidobacterium and an enrichment of potential opportunists such as Proteobacteria and Spirochaetes in this population, they have relatively low rates of infectious diseases, metabolic diseases, and nutritional deficiencies compared to other groups established in northern Tanzania [24, 25].

Based on these aspects, the modern paleolithic diet was developed, characterized by the consumption of vegetables, fruits, roots, nuts, seeds, eggs, fish and lean meat, excluding grains, dairy products, oils, cereals, legumes, salt, and refined sugar, and has gained attention for its multiple health benefits [26]. Based on these characteristics, Barone et al. (2019) sought to identify in a small group the effects of the modern paleolithic diet (MPD) on microbiota structure and diversity in Western urban populations. Despite limitations, the findings suggest that MPD may be a means of counteracting the risk of losing the bacterial memory that accompanied our ancestors throughout human evolutionary history. Another important finding was that Paleolithic dietary traits may indeed act synergistically in maintaining the homeostasis of microbial diversity.

Furthermore, the high intake of monounsaturated fatty acids found in MPD suggests that these may play a role in supporting high microbiota diversity, which can be explored in further studies. However, some factors still need to be elucidated, such as how long these benefits will remain, genetic and/or lifestyle factors [27].

Although some studies suggest potential benefits of the modern paleolithic diet in obese patients and those with type 2 diabetes in the medium and long term, by improving insulin sensitivity, glycemic control, and leptin levels and reducing total fat mass and triglyceride levels [28–30], special attention is needed when following a paleolithic diet for a long time because the percentages of macronutrients are far from the nutritional recommendations, until some factors are elucidated in further longitudinal studies and randomized clinical trials, fully evaluating the impact on host health over time. In addition, the presence of some warning signs, such as overrepresentation of bile- and fat-loving microorganisms, requires attention to potential long-term health effects [27].

6.3 Microbiota and Its Implications for Pathologies

The composition of the gut microbiota is influenced by several factors, such as diet, medication use, the intestinal mucosa, psychological and physiological stress, age, the immune system, and the microbiota itself [31]. This change in the quantitative

and qualitative composition of the microbiota resulting in microbial imbalance is called dysbiosis, and this process explains the microbiota–illness relationship [32].

As a consequence, it is possible to observe an increase in intestinal permeability, with the breakdown of the intestinal barrier and the epithelial occlusion zone, causing an increase in the translocation of microorganisms, their metabolites, endotoxins, lipopolysaccharides (LPS) that cause the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), and translocation of other substances into the circulation, providing a low-grade inflammation and deregulation of the immune system [33]. This chronic low-grade inflammation leads to increased systemic levels of bacterial products, insulin resistance, and a concomitant effect on plasma lipids [34].

The increased intestinal permeability and penetration of antigens alter the physiological functions, leading to activation of innate immunity generating inflammation at chronic levels and may be related to the clinical progression of neuropsychiatric diseases, through the gut–brain axis [35]. The gut–brain axis is the ensemble of the enteric nervous system (ENS) and the central nervous system (CNS). This bidirectional connection is maintained by several modulators and can be influenced by external and internal factors that affect the gastrointestinal tract (GI tract) [36]. The gut microbiome has a strong influence on the motor function of the GI tract, as well as on the modulation of the immune system and neuroendocrine intercellular signaling [37].

Other ways that the microbiome interacts with the host is through various metabolites, including short-chain fatty acids, the bile acid pathway, and via the trimethylamine (TMA)/trimethylamine-N-oxide (TMAO) [32]. The microbiome is in intense metabolic activity producing numerous biologically active compounds, which can be transported in the circulation, distributed to various sites and influencing essential biological processes. Furthermore, some endogenous gut endotoxins, such as lipopolysaccharides (LPS), indoxyl sulfate, and p-cresyl sulfate, which are related to important metabolic functions related to atherosclerosis and potentially contribute to the pathogenesis of cardiovascular disease (CVD) as well as chronic kidney disease [38–40].

In the early stages of CKD, the microbiome is dysbiotic. Due to reduced renal function, the high concentration of urea generates a high influx of urea into the gastrointestinal tract, where microbial ureases catalyze the hydrolysis of urea and generate large amounts of ammonia. A byproduct of ammonia, ammonium hydroxide, increases the intestinal pH, causing mucosal irritation and interference with the growth of commensal bacteria, favoring the establishment of intestinal dysbiosis [41]. Dysbiosis favors the growth of microorganisms that possess enzymes capable of generating uremic toxins, such as indoxyl sulfate (IS), *p*-cresyl sulfate (*p*-CS), indole-3-acetic acid (IAA), and trimethylamine *N*-oxide (TMAO), stimulating inflammation and oxidative stress contributing to the progression of kidney damage, as well as increased intestinal permeability and consequent dysregulation of the immune system [42, 43].

GCFAs play an important role in regulating metabolism and inflammation and are involved in health and disease through signaling molecules, including

modulation of autonomic systems and systemic blood pressure, as well as inflammatory responses and other cellular functions. Some of physiological functions include inhibition of histone deacetylases (HDACs), activation of G-protein-coupled receptors (GPCRs), chemotaxis and phagocytosis modulation, induction of reactive oxygen species (ROS), altering cell proliferation and function, altering intestinal barrier integrity, having anti-inflammatory, antitumorigenic, and antimicrobial effects, and altering intestinal integrity. Some GPCRs, particularly GPR43, GPR41, and GPR109A, have been identified as receptors for GCFA [32, 38].

In addition to these mechanisms, there is a relationship between microbiota and TMAO and SCFA, in which some of these metabolites interact with other endocrine hormones, such as ghrelin, leptin, glucagon-like peptide-1 (GLP-1), and YY-PYY peptide4 [44, 45]. SCFAs provide necessary energy for the intestinal epithelium, regulating lipid metabolism and stimulating incretin production. The microbiota transforms choline, L-carnitine, and phosphatidylcholine from the diet into trimethylamine (TMA). In this way, hepatic flavin monooxygenase (mainly FMO3 and FMO1) converts TMA to TMAO [45].

6.4 Microbiota and Its Influence on Therapy

Exploring the diversity of the microbiome requires an evolutionary perspective to fully understand it [46]. Some recent bioinformatic methods for functional metagenomics are used to understand microbial composition and next-generation sequencing that focuses on taxonomic assignments through DNA sequences, allowing previously uncultured bacteria to be identified in order to gain understanding of the "new." Metagenomics refers to a collective genome of microorganisms from an environmental sample that informs the microbial ecosystem. Direct metagenomic sequencing uses a "shotgun" approach to directly compare with reference genomes or gene catalogs, improving taxonomic resolution and allowing the observation of single-nucleotide polymorphisms and other variant sequences with functional capabilities determined by comparing the sequences with functional databases. These microbial nucleic sequences are then used as a proxy to estimate organism identity and the relative abundance of complex microbial communities [32].

With the knowledge of the result of these new technologies undertaken, new therapeutic tools have been developed, such as probiotics, prebiotics, and synbiotics, with important applicability in clinical practice [47]. The World Gastroenterology Organization (WGO) defines probiotics as live microorganisms that, when administered in adequate amounts, have a beneficial effect on the health of the host. Prebiotics are ingredients that are selectively fermented by the gastrointestinal microbiota, causing specific changes in the composition and/or activity of the gastrointestinal microbiota, their growth, activity, or both, providing health benefits to the host. A combination of products containing prebiotics and probiotics is called synbiotics [48].

There is a relationship between the supplementation of probiotics, mainly *Lactobacillus* and *Bifidobacteria*, and significant improvements in mood, anxiety, and state of depression and reductions in cortisol and pro-inflammatory cytokines [49]. Some other beneficial functions have been attributed to *Lactobacillus plantarum* supplementation in intestinal disorders, such as inflammatory bowel diseases, metabolic syndromes, dyslipidemia, hypercholesteremia, obesity and diabetes, and aspects of brain health involving psychological disorders [40].

Next-generation sequencing (NGS) technology is part of a future prospect of integrated sequencing systems for genome and transcriptome exploration that aims to overcome unmet research and clinical challenges, such as in oncology. NGS has enabled the development of new molecular subclassifications of putative clinically meaningful tumor, particularly through gene expression analysis with RNAseq [50]. This has the ability to delineate the phase of variants in the HLA antigen recognition site with non-coding regulatory polymorphisms. These relationships are critical for understanding the qualitative and quantitative implications of HLA gene diversity and provide a rich resource for linking HLA regulatory polymorphisms to their HLA antigen recognition site to understand the specific expression of allotypes and haplotypes and their consequences for disease susceptibility [51].

6.5 Conclusion

Science is breaking new ground with the exploration of the genome and transcriptome in time and space to develop new specific "drugs" and predictive biomarkers for individualized drug-sensitive therapy. This future perspective is essential not only in the production of drugs but also to understand the processes and mechanisms that are involved in the course of pathology.Conflicts of InterestNone.

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Chapter 7
Artificial Food and the Future of Nutrition for Kidney Health

Fabiana Baggio Nerbass and Denise Mafra

7.1 Introduction

With estimates of the global population reaching a plateau at nine billion in 2050, a significant increase in food production over the next three decades is imperative. Concurrently, enormous quantities of food must be produced to protect the environment and create resistance to climate change.

In addition, dietary habits are strongly linked to health status. High intake of ultra-processed food (UPF) and processed meat with food termed as "ready-to-go-food" or "fast food" causes damage to both human and planetary health. People are at high risk of developing non-communicable diseases (NCDs), such as type II diabetes mellitus, obesity, hypertension, and kidney diseases, when the intake of unhealthy food is high. An unhealthy diet is closely related to kidney health, and the concept of food as medicine should be used to prevent or mitigate poor health [1]. Thus, the future of food production should also favor people's access to dietary patterns that improve the overall health and quality of life.

In this chapter, we have discussed some aspects related to dietary patterns, nephrotoxins from the diet, and agricultural production in terms of kidney diseases. We have also discussed the current practices and future trends of food production, especially artificial food, mainly the most studied cultured meat. Finally, we have examined how the future of nutrition and food production can better promote both human and planetary health.

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7.2 The Present

7.2.1 Dietary Patterns and Kidney Diseases

In nutritional epidemiology, dietary pattern analysis has emerged as an approach to investigate the relationship between the effects of the overall diet and synergistic effects of nutrients resulting in cumulative effects on health and disease instead of evaluating individual nutrients or foods [2].

From 1960 to 2015, agricultural production was more than tripled, with significant expansion in the use of water, land, and other natural resources. In the same period, there was remarkable industrialization of food, and the consumption of processed, packaged, and prepared food has increased worldwide [3]. Although these technologies have advantages such as increasing shelf life, microbiological safety, and increased access due to lower cost, they negatively impact diet quality.

The Global Burden of Disease Study 2017 reported that unhealthy diets might cause most of the burden of chronic, NCDs, such as hypertension, cardiovascular disease, cancer, diabetes mellitus, and chronic kidney disease (CKD) [4].

Approximately 10% of the world's population lives with CKD, and its main risk factors are hypertension and diabetes mellitus [5], which are related to unhealthy Western diets [6], characterized by high consumption of UPF, salt, and sugar. This dietary pattern is well known for its pro-inflammatory properties. Several researchers have shown an association between this diet and a decline in renal function [7, 8]. In contrast, healthy dietary patterns, characterized by higher intake of fruit and vegetables, fish, legumes, cereals, whole grains, and fiber and lower intake of red meat, salt, and refined sugars, are associated with nephroprotective outcomes [9, 10]. A meta-analysis including 630,108 adults who were followed up for 10.4 ± 7.4 years has shown that healthy dietary patterns are associated with a lower incidence of CKD [9]. The DASH diet (low intake of red meat, processed food, sodium, and fat and high intake of nuts, fruits, legumes, vegetables, and whole grains) is associated with a low incidence or prevalence of CKD. This association has been shown in prospective studies such as the Atherosclerosis Risk in Communities study [11], Tehran Lipid and Glucose Study [12], and Korean National Health and Nutrition Examination Survey [13]. In addition, a healthy dietary pattern is associated with a lower mortality risk in patients with CKD [14].

Nephrolithiasis or kidney stones is another very prevalent kidney disease. A review of epidemiological data from seven countries found a prevalence rate of 1.7%–14.8%, which seems to be rising [15]. The etiology of kidney stones is multifactorial and involves the interaction of environmental and genetic factors. The current dietary guidelines recommend increasing fluid intake, maintaining a balanced calcium intake, and similar to CKD, reducing dietary intake of sodium and animal proteins and increasing intake of fruits and fiber [16, 17].

Reducing healthy diet intake (food rich in dietary fiber, bioactive compounds, minerals, and vitamins) leads to loss of antioxidants and anti-inflammatory properties of food [1]. In addition, as diet is the primary modulator of the gut microbiota,

unhealthy diets promote gut dysbiosis, which has been linked to inflammation and cardiovascular disease in patients with CKD [18, 19].

Taken together, changes in food system production provoked an impaired quality pattern of diets, leading to high consumption of processed food and UPF, including high saturated fats, sugar, salt, additives, and pesticides. In addition to the influence of dietary patterns on kidney health, an unhealthy diet can affect kidney disease.

Food additives are widely used in the food industry to improve safety, shelf-life, taste, texture, and appearance. Here, we have focused on two widely consumed elements present in many processed foods—sodium and phosphate. Excessive sodium and phosphate intake is associated with a high risk of hypertension and kidney diseases. It is also directly and indirectly associated with cardiovascular events [20].

Despite existing dietary guidelines, sodium intake is usually higher than the recommended value (1.5–2.3 g/day) in populations worldwide [21, 22]. In 2010, the average world consumption was 3.95 g Na/day, equivalent to 10 g of salt daily. The world's largest sodium consumers are in Asia, Eastern Europe, and tropical Latin America, where the daily average exceeds 4 g Na/day. This is exemplified in countries such as China (4.8 g), Thailand (5.3 g), Turkey (4.1 g), and Brazil (4.1 g) [23].

High sodium intake has also been observed in CKD populations [24, 25]. However, there is good evidence supporting the positive effects of decreasing sodium intake on surrogate markers of cardiovascular events (blood pressure) and progression of kidney disease (albuminuria) [26, 27]. Positive effects are also found for kidney stone prevention using dietary approaches that include low sodium intake. This results in a reduced incidence of kidney stones [28] as sodium intake is directly associated with urinary calcium excretion.

In low- and middle-income countries, most of the sodium consumed comes from salt added during cooking or from sauces. In most high-income countries, however, sodium intake can be reduced by a gradual and sustained reduction in the amount of sodium added in processed food since it is the main sodium source of their diet [29]. Therefore, high-income countries that invested in public campaigns to decrease added salt and implemented a gradual and sustained reduction in the amount of salt added to food by the food industry have shown positive results in reducing sodium consumption [29, 30].

Phosphate additives are also present in various food products. However, due to the high consumption of processed food in the last century, there are concerns regarding whether chronic high consumption of phosphate may be toxic and impact kidney health [31].

The recommended dietary allowance (RDA) of phosphate for adults is 700 mg/day [32], which is easily achieved by a varied diet. However, phosphate consumption can exceed this amount by two or more times in individuals and populations who consume a significant quantity of industrialized food items. Estimation of phosphate consumption by dietary recalls is challenging and largely underestimated, especially in population consuming high amounts of processed food. Such estimations are difficult since phosphate content is not available in industrialized food item labels. In addition to increasing the total intake, the bioavailability of inorganic phosphate from additives is higher than that of organic phosphate present in natural sources [17].

High phosphate intake (from red meat and processed food) appears to be associated with kidney damage, although the exact biochemical mechanism has not been fully elucidated [33]. However, it is well documented that high phosphate plasma levels are associated with a higher risk of overall mortality in patients with CKD [34, 35].

7.2.2 Nephrotoxins from Diet and Agricultural Production

Nephrotoxins present in consumed water, food, and the environment may also play a role in kidney diseases. This concern was raised due to the current epidemic of CKD of non-traditional cause (CKDnt) leading to kidney failure mainly in young male agricultural workers without traditional risk factors, mainly in Central America and Asian countries such as Sri Lanka [36, 37].

Although the etiology has not been fully elucidated in any region [38], the research has focused on consumption of contaminated water as the primary risk factor in Asian hotspots. The possible contaminants include heavy metals, glyphosate, and other agrochemicals [39, 40]. However, contamination of food has been poorly investigated. Some studies have found hazardous amounts of lead and cadmium in a typical diet of a region; however, these contaminants were not found in other studies [41–43].

In Latin America, heat stress and dehydration are the most frequently studied causative risk factors of kidney disease [44–46]. However, a recent longitudinal study conducted in Mexico that assessed kidney functioning of migrant and seasonal farmworkers pre- and post-harvest found a significant decrease in kidney function that was more pronounced in those who worked in the conventional field than in those who worked in the organic field. Thus, the authors suggest that pesticide exposure should be considered in combination with heat stress and dehydration [47].

Cases of acute kidney injury, mainly in Brazil and China, have been linked to Haff disease, a type of human rhabdomyolysis characterized by the sudden onset of unexplained muscular rigidity and an elevated serum creatine kinase level within 24 h after consuming cooked aquatic products [48].

Toxins from tropical plants, such as Djenkol beans [49], star fruit (Averrhoa carambola) [50], poisonous mushrooms (Amanita phalloides) [51], and cotton seed oil (gossypol), can also cause acute kidney injury [52].

7.3 The Future

7.3.1 Food Production and Introduction of Artificial Food

Food systems can nurture human health and support environmental sustainability; however, they are currently causing adverse effects. It has been estimated that more than 820 million people have insufficient food and many more consume low-quality diets that cause micronutrient deficiencies and contribute substantially to

diet-related NCDs [53]. The currently expanding food production and economic growth have resulted in a huge economic burden on the natural environment. "Almost one-half of the forests that once covered the Earth are now gone. Groundwater sources are being depleted rapidly. Biodiversity has been deeply eroded. Every year, the burning of fossil fuels emits billion of tons of greenhouse gases into the atmosphere, which are responsible for global warming and climate change" [3].

The world's growing population and increasing human welfare will necessitate a 30%–70% increase in food production over the next three decades. Concurrently, large quantities of food must be produced to protect the environment and create resistance to climate change. However, it is unclear whether we can sustainably feed a global population of 11 billion in 2100. This question has been extensively explored in FAO's report—The future of food and agriculture: Trends and challenges, 2017. The consensus view is that current systems are likely capable of producing enough food, but to do so in an inclusive and sustainable manner will require major transformations involving international agencies, local governments, scientists, agricultural and food industries, consumers, and others [3].

The food industry successfully introduced different artificial foods and ingredients in the last century to substitute natural sources, including sweeteners, colorings, and flavorings. Foods from non-meat sources (soybeans or wheat), such as seitans and tofu, have existed for many centuries, whereas cellular agriculture has been introduced in the last decade. Artificial meat production may be a more sustainable alternative for producing high-protein sources using technologies such as genetic modification and cloning. Although in vitro meat culturing techniques have been explored, they are presently under discussion.

Approximately 70% of all agricultural land is used for livestock production, and artificial meat products may help reduce greenhouse gas emissions compared to conventional meat production [54]. The other benefits include those regarding animal welfare issues, food safety, public health, and the need to face the increasing worldwide population and associated protein demand [53]. In 2020, this novel product was approved for sale in Singapore, and only the future will tell whether it will meet complex consumer demands [55].

The National Aeronautics and Space Administration designed artificial meat from myoblasts in suspension culture in 2002. In 2014, Dr. Mark Post developed cultured meat [56], and Maastricht University produced the first cultured beef burger. Cellular agriculture is also linked to the production of starch and cellulose, such as amylose and amylopectin, hyaluronic acid, chitosan, soy protein, and bread-derived scaffolds [57].

Studies have shown how to construct steak-like meat using several sources of bovine cells such as adipose-derived stem cells, pluripotent stem cells, and satellite and muscle stem cells. With cell fiber synthesis from tendon-gel-integrated bioprinting, a bioreactor is used to increase cell number [58, 59]. The popularization of this meat produced in vitro, also called cultured meat or clean meat, is growing rapidly; however, the cost of production is still high. Another problem is the low acceptance of cultured meat. A systematic review (including 91 articles on consumer acceptance of different sources of proteins) showed that in addition to uptake

of insects, the acceptance of cultured meat is also low [60]. Negative acceptance is related to neophobia [61].

According to Djisalov et al. (2021), cultured meat is also beneficial because there is no treatment with hormones or antibiotics. In addition, it reduces food-borne illnesses and diseases such as avian and swine influenza [62]. In addition, the composition and flavor can be modulated by controlling fat, mineral, and vitamin content and can be customized according to different nutritional requirements [57, 63]. Another interesting point is that "designer meat" can be produced using nanotechnology-based methods to improve the nutritional value, flavor, and bioavailability of nutrients [64].

The organoleptic properties and regulations regarding production, labeling, and marketing deserve more discussion. Furthermore, more studies on the risks regarding food safety, ethical perspectives, and health are needed. The nutritional equivalence between traditional and cultured meat is debatable. However, the next step in food production involves artificial food production, and studies showing the pros and cons of their use are needed urgently.

It is important to note that many biochemical metabolisms, such as conversion of glycogen to lactate in the post-mortem of the anima and muscle contraction by actin and myosin using calcium, do not exist in cultured meat. More research is needed to determine whether these differences interfere with the nutritional composition of cultured meat [64].

Regarding nutritional composition, protein is the most important nutrient in red meat and cultured meat is made with the best protein source, such as muscle cells or cytoskeletal proteins [63].

Concerning the dietary protein recommendation for patients with CKD, it is important to recommend a low protein diet to patients with CKD on pre-dialysis treatment [17]. Cultured meat is a good source of protein, and therefore, studies evaluating the effects of cultured meat on these patients need to be conducted.

7.3.2 Future of Nutrition for Kidney Health

Healthy dietary patterns, usually characterized by higher intake of vegetables, fruits, legumes, nuts, whole grains, fish, and low-fat dairy and lower intake of red and processed meats, sodium, and sugar, have been associated with CKD prevention, progression, and mortality [9, 14, 65]. Healthy dietary patterns reduce the risk of albuminuria and CKD [9]. Thus, based on current knowledge, nutrition experts recommend a plant-dominant low-protein diet for conservative management of CKD [66]. The best scenario seems to be the one that promotes and supports universal access to a healthy dietary pattern based on organic foods, more fruits and vegetables, and less red meat and processed foods. In addition, personalized nutrition is a promising approach at an individual level, which manages and integrates heterogeneous and patient-specific molecular, clinical, and anamnestic data to achieve individual well-being [67].

High red meat intake is linked to many cardiovascular diseases, cancer, and kidney diseases, in addition to high greenhouse gas emissions. Therefore, reducing its consumption is urgently required. Cultured meat intake could reduce the risk of these chronic diseases and reduce food-borne illness and nutrition deficiency, thus making it a promising candidate for a sustainable diet [68]. Artificial food can reduce significantly greenhouse gas emissions, water use, and energy consumption [69]. However, controlling nutritional composition is very important and is unclear, mainly regarding micronutrients such as vitamin B12 and iron. Another discussion is regarding cell multiplications as some dysregulation can occur and lead to adverse effects on human health [70, 71].

Overall, this chapter reinforced the findings of previous studies that an unhealthy diet is harmful to human and planetary health and included the negative consequences for patients with kidney diseases. Therefore, these changes should be urgently proposed and implemented. This chapter discussed the introduction of artificial food, cellular agriculture, and cultured food as an alternative strategy to replace the unhealthy diet (Fig. 7.1).

In terms of technical issues, methods to increase the production of cultured food are being optimized, but industrial-scale production is not yet possible. However, this research is in the infancy stage in the health field, and many gaps in knowledge exist. These gaps cannot be resolved if there are adverse effects on human health, and no study has been performed in patients with kidney diseases. In addition, it is

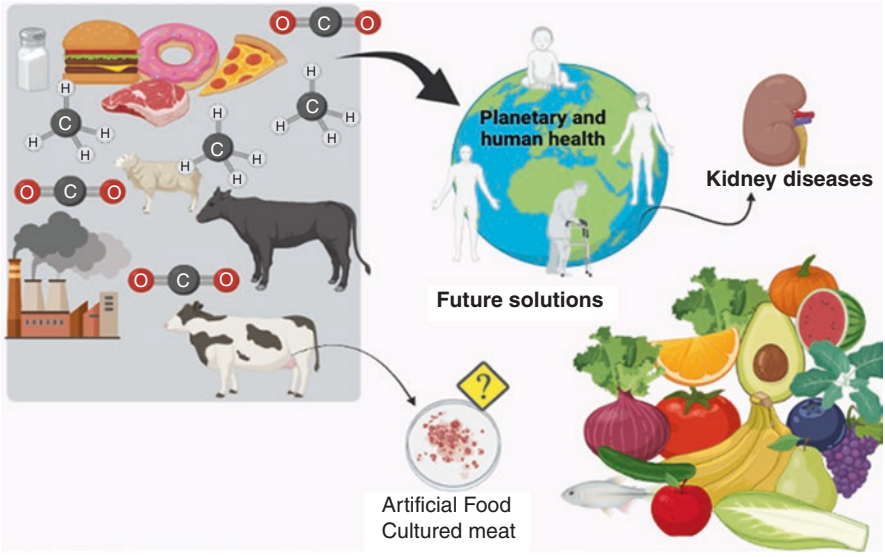

Fig. 7.1 Future of nutrition for kidney health. The food production system and high red meat and ultra-processed food intake lead to alteration in the environment equilibrium and increased risk of non-communicable diseases, including kidney diseases. Therefore, discussions and actions for the future of nutrition are urgently necessary. (Image created with Biorender)

unclear whether cultured meat will be a food option in the future, and whether this food is good for human health.

For many people, cultured meat is still science fiction, and it can also be seen as an unnatural food. Finally, considering that unhealthy food consumption needs to change urgently in patients with kidney diseases, we need more educational programs to encourage healthy and sustainable diet consumption.

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Chapter 8
Novel Drugs for Kidney Diseases Treatment

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8.1 Introduction

Kidney diseases characterized by acute kidney injury (AKI) and chronic kidney disease (CKD) development remain important causes of increased morbidity, mortality, and health costs in short and long term in different clinical contexts [1]. Kidney diseases have been treated with different drugs, including immunosuppressants for glomerular diseases and kidney transplant, anti-hypertensive drugs and hypoglycemics for hypertensive and diabetic kidney diseases (DKD) [2]. Despite that, there is no effective treatment for AKI, and therapeutic interventions targeting CKD progression are largely limited to control of hypertension and hyperglycemia, multidisciplinary treatments (blood glucose control, blood pressure, and lipid control), appropriate weight management, guidance for diet and smoking cessation, RAS inhibition drugs (angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, and mineralocorticoid receptor antagonist by suppressing the action of aldosterone).

Diverse kinds of insults caused by diabetic chronic affection contribute for renal tissue damage, increasing the risk of developing irreversible processes such as CKD [3, 4]. The main molecular mechanisms have been implicated in glucose-mediated vascular damage. Among them, mechanisms of hyperglycemia-induced damage, including increased polyol pathway flux and intracellular formation of advanced glycation end-product (AGEs), activation of protein kinase C and increased flux through the hexosamine pathway [5]. However, the pathophysiological mechanisms

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involved in DKD still not all elucidated, and the treatment strategy for DKD remains the same with that of conventional diabetic nephropathy due to in part to the lack of therapeutic targets.

Other relevant context is associated with renovascular disease (RVD), which is an important cause of secondary hypertension and CKD, increasing morbidity and mortality rates [6]. RVD is caused by endothelial dysfunction with atherosclerotic lesions in the renal vessels, causing reduction in renal blood flow with stenosis and hemodynamic abnormalities, and also a pro-inflammatory stimuli and pro-fibrotic pathways [6]. The care of RVD patients was based on the assumption that restoring renal artery patency would improve renal function [7]. New studies have been developed to define the mechanisms of irreversible renal injury and develop novel therapies to improve renal outcomes in these patients. Anti-inflammatory, pro-angiogenic, and mitoprotective interventions have emerged as powerful strategies and showed promising results [8].

Hence, novel strategies that minimize AKI and halt the progression of CKD are urgently needed [3, 9, 10]. Novel promising renoprotective drugs have been investigated, aiming to assess new possible therapies for different kidney diseases, such as DKD and non-diabetic kidney disease, CKD-related anemia, polycystic kidney disease (PKD), after kidney transplantation, and renovascular hypertension [2, 7]. Among them, this chapter will highlight the SGLT2 inhibitors, NF-E2-related factor 2 activator, hypoxia-inducible factor prolyl hydroxylase inhibitor, incretin-based drugs, AGE inhibitor, epigenetic regulator, mitochondria-targeted agents, CKD anemia treatment drugs (HIFs), and PKD-specific drugs (V2 receptor antagonists).

8.2 SGLT2 Inhibitor Drugs

SGLT2 is a sodium and glucose cotransporter mediated by Na-K ATPase in the proximal tubular basement membrane. Drugs that inhibit this cotransporter difficult the proximal tubular reabsorption of glucose, promoting urinary glucose excretion aims to decrease glycemia [4]. Hence, the inhibition of SGLT2 became an important therapeutic target meanly for diabetic patients from the first half of the twentieth century [11]. Between 2012 and 2015, the European Medicine Agency (EMA) and the US Food and Drug Administration (FDA) approved three SGLT2 inhibitors: dapagliflozin, canagliflozin, and empagliflozin, for reducing plasma glucose in persons with type 2 diabetes mellitus (T2DM) [11]. Despite signals of possible cardiovascular risk due to SGLT2 inhibitors and that could promote AKI through volume depletion and other hemodynamic effects, a large cohort studies showed reduction in cardiovascular events, including cardiovascular death and prolong life in patients with T2DM [11, 12].

Recently a large number of research studies demonstrated that SGLT2 inhibitors exhibited substantial significant renoprotection, improving estimated glomerular filtration rate, decreasing death due to kidney dysfunction and others effects. The benefits of SGLT2 inhibitors is independent of their blood glucose–lowering effects

and may be mediated by natriuresis and glucose-induced osmotic diuresis, reducing intraglomerular pressure and then albuminuria, inflammatory mediators, and others indirect effects [13].

8.2.1 Dapagliflozin

Dapagliflozin is a highly potent and selective inhibitor of SGLT2, being one of the first drugs of this group. This drug reduces glucose levels in both fasting and postprandial periods, from the first dose and continuing for up to 24 h between doses [14]. In some clinical trials, it was found that with 10 mg of dapagliflozin daily there was a reduction in baseline HbA1c between 0.82% and 0.97%, which corresponds to a 21.7–29.6 mg/dL reduction. Another clinical trial carried out in patients who had never received any treatment for diabetes, it was verified that this drug in association with metformin, another antidiabetic drug, has its action more visible when compared to the same ones in monotherapy both in the reduction of basal HBA1c, as well as in weight reduction [15].

An important factor in these clinical studies involving this drug is that both the drug and its metabolites do not influence the action of cytochrome P450, thus minimizing the interaction with other drugs. However, it is not recommended to use it with antihypertensive drugs such as diuretics, as there is a risk of volume depletion [16]. In further studies related to pharmacokinetics, it was found that there is no need to adjust the dose by race, sex, age, and mild to moderate liver or kidney disease. However, as its mechanism of action is related to renal function, this drug may not have the same efficiency and effectiveness in patients with mild to moderate renal failure. Among some of the most relevant adverse effects, urinary tract infections stand out, usually associated at the beginning of treatment, which can range from mild to moderate [17].

In the Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease (DAPA-CKD) trial, the long-term efficacy (median of 2.4 years) and safety of the dapagliflozin was evident in patients with CKD, independently of the presence of type 2 diabetes [18]. In comparison with placebo, dapagliflozin group had better prognosis for kidney outcomes and kidney function. For the primary outcome of even occurrence, the hazard ratio (HR) for dapagliflozin use was 0.61; 95% confidence interval [CI], 0.51–0.72; $P < 0.001$. Moreover, a protector profile was observed due to dapagliflozin use with composite of a sustained decline in the estimated GFR of at least 50%, end-stage kidney disease, or death from renal causes and for the composite of death from cardiovascular causes or hospitalization for heart failure. Only for death, dapagliflozin group presented better survival in comparison to placebo. Interestingly, these effects of dapagliflozin were similar in participants with type 2 diabetes and in those without type 2 diabetes and were considered safety was confirmed [18].

Other clinical studies also confirmed the important efficiency and safety of the dapagliflozin use. In a Japanese population, dapagliflozin exerted beneficial effects

similar to sitagliptin and metformin on glycemic parameters, reducing body weight and improved serum adiponectin levels [19]. In a series of renal transplant cases with both pre-existing diabetes and post-transplant diabetes mellitus, the use of the dapagliflozin in the standard dose of 10 mg helped to achieve satisfactory control with favorable effects on blood pressure and weight with no adverse effects on renal function [20]. All patients had optimal levels of immunosuppressant medications with no interference observed in the pharmacokinetics of these medications and no episode of graft rejection.

8.2.2 Canagliflozin

Advanced studies with canagliflozin have detected a significant reduction in HbA1c from approximately 0.81% with 100 mg to 1.03% with 300 mg/day of the drug. Moreover, patients who received canagliflozin showed a significant weight loss [21]. However, there were higher rates of urinary tract infections by both bacteria and fungi due to canagliflozin use. The FDA demands post-marketing trials of the drug, mainly to investigate cardiovascular disease, as well as pharmacovigilance studies for suspected adverse reactions [22].

The CREDENCE (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation) trial was performed to assess the effects of the SGLT2 inhibitor canagliflozin on renal outcomes in 4200 patients with type 2 diabetes and albuminuric chronic kidney disease [23]. The trial had a median of 2.62 years of follow-up. The use of canagliflozin in this trial had effect for glycated hemoglobin, with mean level at 13 weeks being lower in the canagliflozin group than in the placebo group by 0.31% points (95% CI, 0.26–0.37). On average, levels were lower in the canagliflozin group for systolic blood pressure, diastolic blood pressure, and body weight. The geometric mean of the urinary albumin-to-creatinine ratio was lower by 31% (95% CI, 26–35) on average during follow-up in the canagliflozin group [23].

Canagliflozin showed efficacy to reduce outcomes related to doubling of serum creatinine, end-stage kidney disease, estimated GFR <15 mL/min/1.73 m^2, and renal and cardiovascular death (such as myocardial infarction, or stroke). There was less change in the estimated GFR slope in the canagliflozin group than in the placebo group (−3.19 ± 0.15 vs. –4.71 ± 0.15 mL/min/1.73 m^2 per year). During the first 3 weeks, there was a greater reduction in the estimated GFR in the canagliflozin group than in the placebo group (−3.72 ± 0.25 vs. –0.55 ± 0.25 mL/min/1.73 m^2); however, subsequently, the decline in the estimated GFR was slower in the canagliflozin group [23].

Some characteristics of safety include any adverse or serious adverse events and decreased hyperkalemia due to canagliflozin use [15]. However, other adverse events were similar with placebo, such as the risk of lower-limb amputation, with rates of 12.3 versus 11.2 per 1000 patient-years in the canagliflozin group and the placebo group, respectively (hazard ratio, 1.11;95% CI, 0.79–1.56), the rates of fracture (hazard ratio, 0.98; 95% CI, 0.70–1.37), and the rates of diabetic

ketoacidosis were lower but higher in the canagliflozin group than in the placebo group (2.2 vs. 0.2 per 1000 patient-years) [23].

A Canagliflozin Cardiovascular Assessment Study (CANVAS) trial with type 2 diabetes patients, the composite outcome of sustained doubling of serum creatinine, end-stage kidney disease, and death from renal events occurred less frequently in the canagliflozin group compared with the placebo group (1.5 per 1000 patient-years in the canagliflozin group vs. 2.8 per 1000 patient-years in the placebo group; hazard ratio 0.53, 95% CI 0.33–0.84), with consistent findings across prespecified patient subgroups. Annual eGFR decline was slower (slope difference between groups 1.2 mL/min/1.73 m^2 per year, 95% CI 1.0–1.4) and mean of albuminuria was 18% lower (95% CI 16–20) in participants treated with canagliflozin than in those treated with placebo. Total serious renal-related adverse events were similar between the canagliflozin and placebo groups [24].

Other studies showed the improvement in plasma kidney biomarkers due to canagliflozin use. Canagliflozin decreased KIM-1 compared with placebo in individuals with type 2 diabetes in CANVAS trial [25]. Moreover, early decreases in TNFR-1 and TNFR-2 during canagliflozin treatment were independently associated with a lower risk of kidney disease progression, suggesting that TNFR-1 and TNFR-2 have the potential to be pharmacodynamic markers of response to canagliflozin [25].

8.2.3 Empagliflozin

The Empagliflozin Cardiovascular Outcome Event Trial-Removing Excess Glucose (EMPA-REG OUTCOME) is the major trial evaluating effects of empagliflozin on cardiovascular diseases and renal outcomes of type 2 diabetic patients [26]. This study was not powered to evaluate renal outcomes. However, important renal characteristics were evaluated. The cardiovascular benefits in patients treated with empagliflozin contributed for a significant reduction in important renal endpoints compared with placebo [27].

In a study with EMPA-REG OUTCOME trial, was developed using randomly assigned patients with type 2 diabetes and an estimated glomerular filtration rate of at least 30 mL/min/1.73 m^2 of body-surface area to receive either empagliflozin (at a dose of 10 mg or 25 mg) or placebo once daily [28].

Kidney abnormalities occurred in 525 of 4124 patients (12.7%) in the empagliflozin group and in 388 of 2061 patients (18.8%) in the placebo group, and a significant relative risk reduction of 39% and benefits occurred in across the two doses of empagliflozin. Moreover, progression to macroalbuminuria and doubling of the serum creatinine level occurred less in the empagliflozin group than placebo (11.2% vs. 16.2% and 1.5% vs. 2.6%, respectively), with a significant relative risk reduction in both 38% and 44%, respectively) [28]. The initiation of renal-replacement therapy occurred in 0.3% in the empagliflozin group and in 0.6% in the placebo group, a significant relative risk reduction of 55%. However, the use of empagliflozin was not associated with a significant reduction in the rate of albuminuria [28].

Other study evaluating the effects of empagliflozin on cardiovascular morbidity and mortality in patients with type 2 diabetes, the urosepsis was reported in 0.4% in the empagliflozin group and 0.1% in the placebo group, but there was no imbalance in overall rates of urinary tract infection, complicated urinary tract infection, or pyelonephritis [29]. In addition, there were no relevant changes in electrolytes, and the proportions of patients with confirmed acute renal failure were similar in the two study groups.

From the EMPA-REG OUTCOME trial, patients with prevalent kidney disease at baseline, empagliflozin reduced the risk of cardiovascular death by 29% compared with placebo (hazard ratio [HR], 0.71; 95% confidence interval [CI], 0.52–0.98), the risk of all-cause mortality by 24% (HR, 0.76; 95% CI, 0.59–0.99), the risk of hospitalization for heart failure by 39% (HR, 0.61; 95% CI, 0.42–0.87), and the risk of all-cause hospitalization by 19% (HR, 0.81; 95% CI, 0.72–0.92) [30]. These effects remain consistent across categories of eGFR and urine albumin–creatinine ratio at baseline and across the two doses studied (empagliflozin 10 mg and 25 mg) [30]. In other EMPA-REG OUTCOME trial design, the patients with type 2 diabetes and established cardiovascular disease were randomized 1:1:1 to receive placebo, empagliflozin 10 mg or empagliflozin 25 mg, once daily. The results showed that empagliflozin caused reduction in cardiovascular and all-cause mortality, hospitalization for heart failure, and incident or worsening nephropathy [31].

8.3 NF-E2-Related Factor 2 Activator

Although current treatments, such as RAS inhibitor and SGLT2 inhibitor, can only slow down the decline of GFR, NF-E2-related factor 2 (Nrf2) activator is a novel drug with the potential to improve the GFR of DKD patients [32]. Nrf2 is a transcription factor which regulates the wider range of downstream pathways, aiming to maintenance of cellular homeostasis under stress and internal regulatory mechanisms. Nrf2 is a transcription factor that acts in regulation of defensive responses to oxidative stress. Hence, chronic diseases such as CKD, which have oxidative stress and inflammation as an important cause, may be expected to benefit from Nrf2 activators with clinical application. Several Nrf2 activators are at various stages of clinical development using clinical trials for CKD and DKD, autosomal dominant polycystic kidney disease, and focal segmental glomerulosclerosis [32].

All diseases that have oxidative stress and inflammation could be the treatment targets for Nrf2 activators [33]. Clinical trials of Nrf2 activators for several kidney diseases are now ongoing. Bardoxolone methyl (BARD) is a cyanoenone triterpenoid based on natural product oleanolic acid and one of the most potent Nrf2 activator known to date. An improvement of estimated glomerular filtration rate (eGFR) due to BARD use was incidentally observed and its renoprotective effect has been highlighted since the trial [34, 35].

Studies have indicated that Nrf2 activators have protective effects, both in vitro and in vivo, partially mediated via inhibition of transforming growth factor-β1 (TGF-β1) and reduction of the extracellular matrix [36]. Furthermore, a synthetic

inducer of Nrf2, bardoxolone methyl, increased renal function in diabetic patients [37]. It was shown that Nrf2 activators sulforaphane and cinnamic aldehyde improved albuminuria, minimizing kidney damage [38]. Finally, these studies conclude that Nrf2 activation represents a strong therapeutic target that is important in diabetic patients, where oxidative stress is an important mediator of progression of DKD [39].

Regarding BARD, an important phase 2 study with patients presenting CKD and type 2 diabetes (TSUBAKI) study aimed to determine if patients without risk factors can avoid fluid overload and whether changes in eGFR with bardoxolone methyl reflect true increases in GFR. BARD treatment demonstrated an increase in eGFR with no serious adverse events in patients with stage 3–4 CKD [40]. The increase in eGFR was maintained 4 weeks after the cessation of BARD, which was administered for 52 weeks. Subsequently, the Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes (BEACON study) was conducted as a phase III clinical trial [35]. Although BARD continued to demonstrate a robust eGFR-reverting effect, the BEACON study was prematurely terminated after a median period of 9 months since there was increased hospitalization or death from heart failure (relative risk, 1.83; 95% CI, 1.32–2.55; $P < 0.001$). Moreover, increased albuminuria is other controversial findings against safety of renoprotective effect of BARD [32].

8.4 Hypoxia-Inducible Factor Prolyl Hydroxylase (HIF-PH) Inhibitors

Hypoxia-inducible factor (HIF) is a key transcription factor in the response to hypoxia that produces a physiologic response to reduced tissue oxygen levels by activating the expression of certain genes. The purpose of this adaptive homeostatic response is to restore oxygen balance and protect against cellular damage while oxygen levels are being restored [41].

HIF activation may have a renoprotective effect by ameliorating a vicious cycle of chronic hypoxia, which has been previously tested in numerous experimental animal models [4, 42]. The long-term observation of ischemia-reperfusion injury (AKI-to-CKD transition model) was reported to reduce renal fibrosis by the administration of HIF-PH inhibitor [43].

HIF-PH inhibitors are likely to become an important tool for anemia management in patients with CKD. Most studies suggest important effects for anemia in CKD and several molecules that inhibit HIF-PH enzymes are under development for treating anemia in patients with CKD [41, 44]. HIF-PH inhibition leads to endogenous erythropoietin production and enhances the availability of iron to the erythron. Published clinical trials show increased Hb levels with physiologic blood levels of endogenous erythropoietin. The most promising molecules include Roxadustat® (FibroGen, Astellas, and AstraZeneca), Vadadustat® (Akebia), Daprodustat® (GlaxoSmithKline), and Molidustat® (Bayer), presenting investigation status from phase 2 until phase 3 [45–49].

Recently, the Asian Pacific Society of Nephrology HIF-PHI Recommendation Committee, formed by a panel of 11 nephrologists who have clinical experience or been investigators in HIF-PHI studies, recommended that physicians can consider HIF-PHI as alternative for correcting and maintaining hemoglobin level for renal anemia both in dialysis-dependent and non-dialysis-dependent CKD. Iron status should be evaluated before HIF-PHI use and correcting iron deficiency before initiation of HIF-PHI (ferritin > 100 ng/mL and TSAT > 20%) for all CKD patients [50]. In addition, anemia in CKD should be controlled using erythropoietin stimulating agents (ESA) or HIF-PHI after sufficient iron supplementation. In some cases, they suggested the preferable use of HIF-PHI rather than ESA, if the cause of ESA hyporesponsiveness is unknown or difficult to manage due to defective iron utilization and or due to difficulty in ESA drug adherence [50].

However, the long-term use of these agents may contribute to existing tumor survival and grow due to HIF activation in hypoxic. It is not possible to state whether HIF-PH inhibitors offer an advantage regarding cardiovascular end points at comparable target Hb levels. Results of ongoing trials will elucidate the short- and long-term benefit and risks profile of these agents to better define their role as an alternative to erythropoiesis-stimulating agents and iron supplementation in patients with CKD with anemia [41].

8.5 Incretin-Based Drugs

Incretin, which is secreted from the gastrointestinal tract after food intake, promotes insulin secretion from pancreatic β-cells as blood glucose rises, creating a hypoglycemic effect. Incretin-based therapies are a major innovation that stimulates insulin secretion and reduces glucagon secretion, resulting in a decrease in hepatic glucose production [51]. There are two classes of drugs based on this system: GLP-1 receptor agonists, such as exenatide and liraglutide, and DPP-4 inhibitors, which slow down the endogenous degradation of GLP-1 by inhibiting DPP-4 [52].

AMPLITUDE-O study was a randomized placebo-controlled trial conducted at 344 sites in 28 countries and sought to evaluate the drug efpeglenatide in participants with type 2 diabetes and some history of cardiovascular or kidney disease. The randomization process was stratified according to the use of sodium-glucose cotransporter-2 inhibitors. This study was carried out with a total of 4076 participants, of which 2717 received efpeglenatide and 1359 received placebo. Some signs and symptoms presented by the participants who received the drug were diarrhea, constipation, nausea, vomiting, abdominal distension, among others. Such symptoms were felt more often by participants who received the drug than by those who received the placebo. This study found that participants with type 2 diabetes who had a history of cardiovascular disease or current kidney disease, the risk of cardiovascular events would be lower among those who received weekly subcutaneous injections of efpeglenatide at a dose of 4 or 6 mg than those participants who received only the placebo, allowing to conclude the effectiveness of the drug for type 2 diabetes [53].

Studies have evidenced a combined renoprotective effects due to GLP-1 receptor agonist. Patients in treatment of Liraglutide presented a protective profile in relation to composite renal outcome, such as persistent macroalbuminuria, persistent doubling of the serum creatinine level and an estimated glomerular filtration rate of 45 mL or less per minute per minute per 1.73 m^2 of body-surface area, the need for continuous renal-replacement therapy (end-stage renal disease), or death due to renal disease [54].

Important cohort studies, including Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial and Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes (SUSTAIN-6), and Exenatide Study of Cardiovascular Event Lowering (EXSCEL) study and the Evaluation of Lixisenatide in Acute Coronary Syndrome (ELIXA), the main effect was by reduction in the development of overt albuminuria, with no differences in other parameters [4, 55, 56]. In dulaglutide versus insulin glargine in patients with type 2 diabetes and moderate-to-severe chronic kidney disease (AWARD-7) trial, which compared dulaglutide and insulin glargine, the decreased albuminuria and eGFR reduction in the dulaglutide group was more evident [56].

8.6 Mitochondria-Targeted Agents

Diverse evidences for mitochondrial dysfunction in AKI and CKD have led to a search for mitochondria-protective drugs. Defects in mitochondria function are key players in AKI and CKD. Mitochondria have an increased expression in proximal tubular epithelial cells, where the nephron performed incredible and decisive kidney function [9]. Inhibition of electron transfer in electron transport chain leads to electron leakage in complex 1 and complex 3 and the formation of reactive oxygen species (ROS) and cardiolipin peroxidation, causing further establishing a direct feeding cycle of mitochondrial oxidative stress and progressive energy deficiency [9].

In AKI, ischemic insults inhibit mitochondrial respiration, switching to glycolytic metabolism with dramatic reduce of ATP production. Moreover, during this process, Na + -K + -ATPase activity is inhibited and intracellular Na + increases and mediates cell swelling [57–59]. The lack of ATP also causes other consequences as cytoskeleton breakdown and detachment of endothelial cells, loss of brush border and cell detachment of proximal tubular cells and podocyte effacement [9, 60]. Hence, recovery of ATP is essential for cell survival, which depend on the recovery of mitochondrial function. During ischemic damage, the mitochondrial damage difficult the ATP production during a decisive moment for the cells, causing different kinds of cell death [61].

The mitoprotective drug elamipretide (ELAM) exerts important renoprotective effects in several models of kidney injury. A phase 2a trial with ELAM during renal angioplasty with stenting showed greater increase in total GFR and improved cortical perfusion, suggesting a role for targeted mitochondrial protection to minimize procedure-associated ischemic injury and to improve outcomes of revascularization

for human ARAS [62]. Moreover, these changes were associated with reductions in systolic blood pressure and improvement in some kidney biomarkers profile. ELAM treatment had favorable IGFBP7 and TIMP-2 (more promising predictors of AKI [63]) rise at 24 h in response to a protective role in the face of contrast and procedural hazards. This same study suggested that ELAM reduced acute changes in tissue during hypoxia and had no evidence of tissue injury using NGAL and creatinine ARAS [62].

Mitoquinone mesylate, also known as MitoQ, is a mitochondria-targeted agent that restores the antioxidant efficacy of the mitochondrial respiratory complex [64]. MitoQ is a protective agent against oxidative damage in metabolic diseases and was studied in renal disorders. In preclinical studies with MitoQ, these mitochondria-targeted approaches seem to protect renal structure and function without affecting systemic metabolic parameters, suggesting that it may be possible to combine these novel targeted agents with standard therapies that target blood pressure, glucose, or lipids [65]. A phase 4 trial with MitoQ as a dietary supplement for CKD is not yet open for recruitment.

Table 8.1 presents a summary of everything that was discussed during this chapter, showing the pharmacological classes, drugs, mechanisms of action, therapeutic

Table 8.1 Promising drugs, pharmacological class, mechanisms, and therapeutic effects

Pharmacological class	Drugs	Mechanism of action	Therapeutic effects
SGLT2 inhibitory drugs	Dapagliflozin Canagliflozin Empagliflozin	Sodium glucose cotransporter receptor inhibitor (SGLT2)	Glucose reabsorption in the renal proximal tubule Glucosuria
NF-E2-related factor 2 activator	Methyl bardoxolone	Regulation of inflammation and oxidative stress	Protective effects Reduction of extracellular matrix Increased kidney function Improved albuminuria
Hypoxia-inducible prolyl hydroxylase factor (HIF-PH) inhibitors	Roxadustat Vadadustat Daprodustat Molidustat	Oxygen balance restoration Protection against cell damage	Reduction of renal fibrosis Erythropoietin production Increased Fe availability
Incretin-based medications	Liraglutide Exenatide Lixisenatide	GLP-1 increase Increased insulin synthesis and secretion	Glucagon reduction Delay in gastric emptying Satiety
Agents targeting mitochondria	Mitoquinone mesylate	Increased tubular epithelial cells Restoration of antioxidant efficacy of mitochondrial complete respiratory	Increased total glomerular filtration rate Protective agent against oxidative damage

Source: The authors themselves (2021)

effects, and whether or not these drugs have FDA and/or EMA approval, correlating with the importance of drug to be approved in agencies so that it is made available to the general population, so that it can make use of them.

8.7 Final Tasks

Despite the economic and health consequences of kidney diseases, there have been very few innovations in the prevention and management of them over the last decade [1]. Renal replacement therapy remains the most used way for the treatment of kidney disease in final stages. Novel technologies have contributed to favor access to healthcare and following to improve outcomes. Machine learning, which is a technique that uses mathematical algorithms that improve learning through artificial intelligence, has been applied, with great success, to provide diagnosis with high accuracy and to determine better quality of care [2]. However, despite dialysis and transplant have show significant improvement in the last years, it remains associated with decreased life expectancy and increased mortality and economic costs. Challenges related to more early and precise diagnosis of kidney disease are needed to improve the knowledge about crucial targets to avoid kidney disease progression. Finally, new renoprotective renal drugs need to be implemented in more countries, aiming to evaluate it impacts along clinical kidney disease management of different populations.

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Chapter 9
3D Printing in Nephrology

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9.1 Bioprinting

9.1.1 History of Bioprinting

The number of patients with end-stage kidney disease continues to increase worldwide. According to a survey, approximately 3.8 million patients received this treatment as of 2017 [1]. Currently, the only treatment options for these patients are dialysis and organ transplantation. Dialysis supplements the kidneys' filtration function, but the kidneys have a complex structure and perform various functions other than filtration, such as maintaining homeostasis and producing hormones; therefore, organ transplantation is necessary to obtain these functions.

However, the number of donor kidneys for organ transplantation is limited, and new technologies to regenerate kidney functions are being pursued to compensate for this shortage. Among these technologies, organ regeneration through regenerative medicine, mainly using induced pluripotent stem cells (iPSCs), is expected to be the most promising. Bioengineering technology, which has made significant progress in recent years, offers new possibilities for creating kidneys. If the organ could be replaced entirely, it would free patients from the burden of current renal replacement therapy and improve their quality of life.

9.1.2 Tissue Engineering

While there are many diseases for which organ transplantation is the only treatment option, attempts to create artificial organs by combining medical and engineering knowledge have been vigorously promoted in recent years, and clinical applications have been developed for some organs such as artificial joints [2], blood vessels [3], and hearts [4]. At the same time, it became possible to increase the number of cells by isolating and culturing those from tissues and organs removed from the body, and research was conducted to regenerate organs using these cultured cells [5].

In particular, the concept of "tissue engineering" was defined by Langer and Vacanti in 1993, and it was proposed that the three elements of "cells," "growth factors," and "scaffolds" are essential for the biofabrication of three-dimensional (3D) organs and tissues [6].

Initially, various organs were regenerated by seeding cells on a scaffold for cell culture in two dimensions and feeding them with growth factors. However, the original complex structure and organ microenvironment could not be reproduced in two dimensions, and it was necessary to support cell growth in three dimensions. Therefore, 3D scaffolds based on tissue engineering have been used, but they do not fully mimic tissue structures or meet all the requirements for tissue regeneration. Conversely, bioprinting is a new approach to solve the current problems associated with tissue engineering, and bioprinting technology has been rapidly advancing [7].

Moreover, stem cell research centering on embryonic stem cells (ESC) and, later, iPSCs have led to the expectation of organ and tissue regeneration using cells, and researchers around the world are now entering the field of regenerative medicine.

9.1.3 Bioprinting Methods

Additive manufacturing, also known as 3D printing, has brought about innovative advances in industry, medicine, and art [3]. This technology has been applied to tissue engineering to create biological tissues by 3D printing cells, biocompatible materials, and others [8].

The technology, also known as bioprinting or biofabrication, can be broadly classified into microextrusion, inkjet bioprinting, laser-assisted printing, and other technologies. These technologies usually use bioink, which contains cells and scaffolds in liquid ink, is solidified by temperature change or photo-crosslinking, and finally forms a 3D structure containing cells [7, 9, 10] (Fig. 9.1). Other technologies include scaffold-free biofabrication, which prints cells without a scaffold [11].

Microextrusion is a method of mechanically or pneumatically printing material onto a substrate using a computer-controlled needle [7, 13–15]. Living cells are affected by shear forces, but shear thinning and thermally cross-linkable bioink can improve cell viability and enable 3D printing with cellular components [11]. It is possible to create tissues with high cell density and even print vascular tissues, but they still require improvements in the printing speed and resolution [10].

The inkjet bioprinting method uses a drop-on-demand printer device to reproduce high resolution by printing small droplets of bioink at appropriate locations using digital information [7, 10, 16]. It uses piezoelectric and thermal forces to eject the bioink [17, 18]. When acoustic force is used, it can be combined with ultrasound to properly control the speed and size of the droplets and adjust the cell concentration [7]. The lack of pressure and heat effects lead to improved cell viability, but there are problems associated with using a 15–25 kHz frequency, which can cause damage to the cell membrane [18]. Thermal inkjet printers are less expensive but have disadvantages such as clogging and size variations [10, 18].

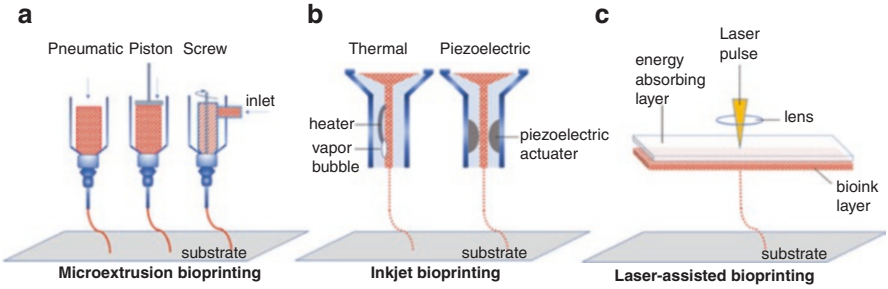

Fig. 9.1 Bioprinting methods. (**a**) Microextrusion bioprinting. (**b**) Inkjet bioprinting. (**c**) Laser-assisted bioprinting. (Adapted from [7, 10, 12])

The laser-assisted bioprinting method consists of a pulsed laser beam, lens, absorbing layer, and bioink layer [7, 10]. Because it can achieve a resolution of one cell per drop on the micrometer scale [15], it is expected to mimic detailed structures in organ regeneration and is rapidly becoming popular but incurs high costs [19, 20].

Each method has different characteristics, and it is necessary to consider the most important factors in bio-3D printing: surface resolution, cell viability, and the use of biomaterials for printing [7]. Although these innovative techniques allow the placement of cells in any desired location in three dimensions, as discussed previously, there is a risk of exposing cells to heat, shear forces, and many problems are encountered, such as cell viability, uniform distribution of cells in the bioink, cytotoxicity of the bioink itself, and cell damage caused by laser irradiation.

A liquid scaffold was used in one study, with the cells as the bioink. Bioink scaffolds can be broadly divided into natural and synthetic polymers. Natural polymers include alginate, collagen, hyaluronic acid, and silk fibroin, which are highly hydratable and compatible with biological tissues [21]. Synthetic polymers include polylactide-co-glycolide, polyethylene glycol, poly-L-lactic acid, and poly-ε-caprolactone. Although the degradation products of synthetic polymers are harmless, they are not physiological and may cause inflammatory reactions due to biodegradation [22]. However, these bioprinting methods assume the use of a scaffold.

In contrast, we have developed a completely new bioprinting method, the Kenzan method, which utilizes the characteristics of spheroids, which are aggregates of cells, to fuse and laminate spheroids onto fine needles using a controlled robotic system [23–25]. The details of the Kenzan method are described in the following sections.

In addition to the aforementioned printing methods, an integrated tissue organ printer has been developed to provide good cell viability and vasculature [26]. In recent years, in vivo bioprinting technology has been developed, in which cells and materials are directly injected into the body and printed, and bone bioprinting has been reported in bone defects [27, 28]. If this technology advances, it may be possible to regenerate the injured area immediately after trauma or tumor removal by bio-3D printing. Although bioprinting is still in development, it is a beneficial method for regenerative medicine.

9.2 Kidney Bioprinting

9.2.1 Kidney Function

The kidney filters blood, removes waste, and maintains homeostasis. Additionally, it has various functions such as the production of renin and erythropoietin and activation of vitamin D. Histologically, there are about one million nephrons in each of the left and right kidneys, and the nephrons are composed of renal corpuscles and tubules. The corpuscles are composed of glomeruli and Bowman's capsules, and the tubules are composed of proximal tubules, the loops of Henle, and distal tubules [29].

Renal blood flow in humans can be as high as 1200 mL/min from the renal artery, which branches directly from the aorta, and the outflow occurs via the renal vein to the inferior vena cava. In addition, there is a very fine network of blood vessels, including imported small arteries, renal corpuscles (glomeruli), exported small arteries, peritubular capillaries, and peritubular veins. A tiny portion of renal blood flow is used for gas, nutrient, and waste exchange in the kidney itself, but it is mainly used for filtration in the glomerulus [29].

The kidney was also the first organ to be substituted with artificial materials and the first organ to be successfully transplanted [30]. However, the kidneys are one of the most challenging organs to fabricate artificially because of their nonuniform and complex structure and because they require a great deal of vascularization.

9.2.2 Phantom

It is essential to understand the kidneys' anatomy to perform the appropriate surgical procedures.

The importance of understanding the anatomy of organs has increased with the advancement of surgical techniques, such as laparoscopic surgery, in recent years. However, there is a limit to the amount of training that can be provided using a traditional cadaver, and there are few opportunities for surgeons to have direct contact with the organs and deepen their anatomical understanding.

Imaging techniques such as X-rays are useful in the preoperative structural assessment of organs. Although computed tomography and magnetic resonance imaging data can be used to create 3D images and 3D-printed models, a faithfully reproduced 3D-printed model has been shown to be superior to a 3D image because it provides visual and tactile information about the organ's anatomy. 3D-printed models can be used for simulated surgeries to determine the extent of tumor resection, trauma, and congenital malformations before surgery [31] and implants based on 3D printing technology are also being actively developed [32].

In the field of urology, 3D-printed kidney phantoms are used to simulate kidney stones, kidney cancer, kidney transplantation, and other surgeries to provide medical professionals with useful information. They are also used for educational purposes to help patients understand their own diseases [33]. However, the kidney phantom is made of non-biological materials and cannot replace the actual organ.

9.2.3 Kidney Bioprinting

As mentioned above, renal replacement therapy for patients with end-stage kidney disease remains a challenge. The development of functional kidney structures using biomaterials is being actively pursued as an alternative technology, and bio-3D printing technology is attracting attention as one of the methods.

Atala, a surgeon at Wake Forest University, USA, is a leading expert in regenerative medicine and has reported numerous studies on kidney regeneration. In 2011, he gave a TED Talk presentation on tissue engineering, in which he surprised the world by showing a 3D-printed human-scale kidney structure [34]. However, although kidney regeneration research has been conducted globally since then, a bio-3D printed kidney with complete structure and function has yet to be completed [35].

The macrostructural complexity of tissues and organs can be broadly classified into four levels: flat tissues, tubular structures, hollow structures, and solid organs, with fabrication difficulty increasing in that order [35]. The kidney, a solid organ, is one of the most challenging organs to regenerate, and many challenges are associated with kidney regeneration. These challenges include the technical difficulty of reproducing the complex structure of the kidney, choice of cell type, and the selection of appropriate biomaterials to maintain the kidney's structure and function [12, 36–38]. As mentioned above, the kidney has a multifunctional and extremely complex structure, and it is also composed of more than 20 different types of highly specialized tissues, making kidney bioengineering difficult.

At present, bio-3D printing technology is used to fabricate parts of the nephron, as it is difficult to regenerate the entire kidney. An interstitial interface has been fabricated by seeding multiple fibroblasts, human umbilical venous endothelial cells (HUVECs), and proximal tubular epithelial cells on 3D-printed biomaterials to mimic living tissues and drug responsiveness has been confirmed [39]. A proximal tubular structure was fabricated, in one study, by bioprinting a 3D model that reproduced the size and curvature, and the proximal tubular epithelial cells seeded on it were shown to have morphological and functional characteristics closer to those of the proximal tubular epithelium in vivo than those of the same cells cultured in two dimensions [40]. In addition, a study that successfully created a model of the proximal tubules with blood vessels has been reported [41]. Since the patency of blood vessels is an important factor for the long-term survival of bioprinted organs [42], it is necessary to create a structure that mimics the vascular system.

Kasyanov et al. printed the arterial vascular structure of a kidney using a silicon droplet similar in 3D structure to tissue spheroids [43]. Although no cells were used, this method could be applied to spheroid-only bioprinting, and it may be possible to print human-scale kidneys using only cell spheroids without a scaffold [12].

9.3 Current Kidney Regeneration Studies

9.3.1 Kidney Organoids

Kidneys are structurally and functionally complicated and are formed through complex embryological processes; it has been considered challenging to generate artificial kidneys for these reasons. In recent years, however, there have been many

studies on kidney organoids with kidney structures and functions using ESCs or iPSCs based on embryological insights.

The mammalian kidney is derived from metanephros and proceeds through the interaction of three types of precursor cells. Nephron progenitor cells (NPCs) arise from the metanephric mesenchyme (MM) and form the nephrons, which contain the glomerulus and tubular tissues. The ureteric bud (UB) occurs from the intermediate mesoderm, forming the collecting ducts, urinary tracts, and part of the bladder. Stromal cells are derived from stromal progenitor cells, fill epithelial tissues, and differentiate into perivascular cells and other types of cells [44].

Based on these embryological findings, many studies have reported the differentiation of mouse ESCs into NPCs and UB, and the cells can produce kidney organoids [45]. Taguchi et al. successfully induced NPCs and UB from mouse ESCs, and these cells mixed with embryonic MM formed complex kidney structures [46]. Furthermore, human ESCs differentiate into renal progenitor cells, which spontaneously form 3D structures, including nephrons [47].

Human iPSCs have also been used to research ethical issues in human ESCs, and which have been successfully differentiated into multipotent NPCs and form kidney organoids with nephron-like structures [48], UB [49, 50], and collecting ducts [51]. In addition, iPSC-derived kidney organoids with all components of the kidney, such as glomeruli, stromal cells, vascular endothelial cells, proximal tubules, distal tubules, and the loops of Henle, have specific endocytosis [52]. Kidney organoids with renin-producing pericytes have various renin-angiotensin system components, including angiotensin II receptors, angiotensinogen, and angiotensin-converting enzymes 1 and 2, have been confirmed [53].

In one study, a vascular network was formed between recipient animals and kidney organoids after the organoids were transplanted, and the organoids had glomerular filtration and tubular reabsorption functions. Sharmin et al. reported that human iPSC-derived glomeruli formed vascular networks with recipient mice, and the glomeruli podocytes matured after transplantation [54]. Xiaris et al. induced kidney organoids from embryonic mouse kidney-derived cells and transplanted the organoids into a living rat host, below the kidney capsule; the organoids matured as renal tissues and capillaries with podocyte slits were integrated into the host [55]. Erythropoietin-producing cells appeared in the glomeruli, and the tubular tissues showed reabsorption functions after transplantation. Takebe et al. also reported that cocultured E13.5 mouse kidney cells with endothelial cells and mesenchymal cells produce 3D organ buds and small organ-like structures [56]. The kidney organ buds formed capillary microcirculation and glomeruli filtration after transplantation in the mouse cranium.

These kidney organoids are expected to provide new insights into kidney diseases, nephrotoxic drug screening, and kidney development. However, several problems may be encountered in the use of kidney transplantation in humans, such as size, difficulties with organically bound kidney organoids, and the need for the renal-urinary system to excrete the produced urine.

9.3.2 Fabrication of the Urine Excretion Pathway

In vivo, the glomeruli produce primitive urine, which is reabsorbed at the proximal and distal tubules through collecting ducts, and urine is excreted into the ureters and bladder. To construct the urine excretion pathways, Yokoo et al. reported the injection of exogenous mesenchymal stem cells into the kidney-budding region of the embryo and differentiated the injected mesenchymal stem cells into metanephroi with bladders by giving them the same nephrogenic signals as kidney development [57]. In this method, the transplanted graft's bladder was connected to the ureter of the host animal, and the graft-produced urine could be excreted outside the body through the host's ureter [58].

9.3.3 Decellularization

Decellularization is a different approach to kidney regeneration [59]. Organs are formed from individual cells and the extracellular matrix (ECM), which supports the organ's structure, provides a scaffold for the pericellular environment, and influences cell function. Organs are usually decellularized by infusion of a chemical detergent such as sodium dodecyl sulfate, and Song et al. successfully decellularized rat, porcine, and human kidneys via renal artery perfusion with this method [60]. The decellularized rat kidney scaffolds were recellularized with HUVECs by infusion of renal artery and rat neonatal kidney cells from the ureter, which were located at the appropriate site on the ECM after perfused organ culture. The regenerated kidneys partially recovered filtration and reabsorption functions and were successfully transplanted into rats. Seeding recipient-derived cells on decellularized kidney ECM is expected to produce kidneys with less organ rejection after transplantation.

9.4 Research in our Laboratory

9.4.1 Development of the Kenzan Method

Currently, because of the shortage of organs for transplantation, including kidneys, artificial organs are required in regenerative medicine. Biofabrication technology has advanced, but most methods to fabricate organs require scaffolds to support cells and the ECM, such as hydrogels. However, scaffold-free artificial organs are ideal for transplantation because exogenous scaffold materials may interact with tissue maturation or recipients.

We have developed a unique method called the Kenzan method, which can build 3D structures using only cells. The first step is the preparation of spheroids,

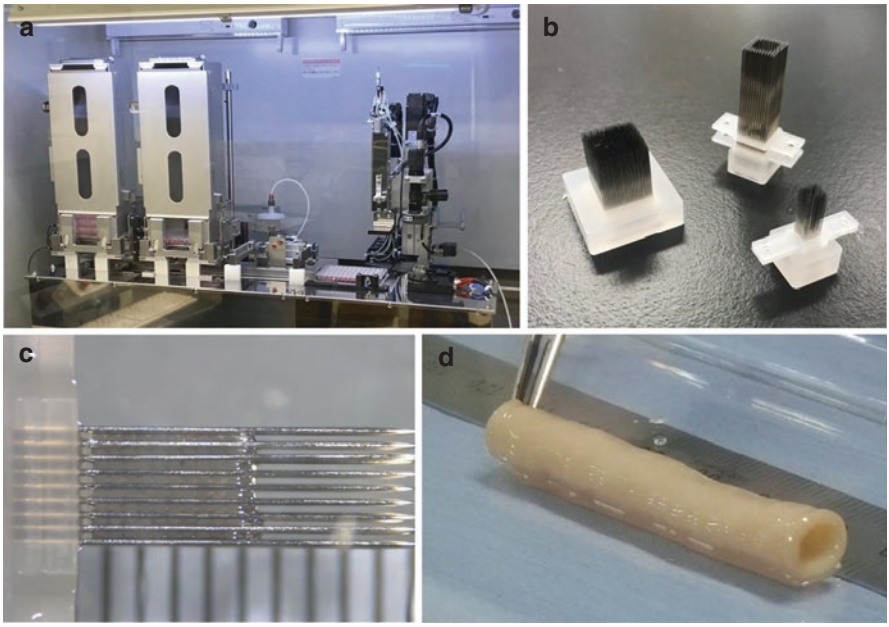

Fig. 9.2 The kenzan method. (**a**) Bio-3D printer overview. (**b**) Various sizes of the kenzan. (**c**) The bio 3D printed spheroids and kenzan. (**d**) A scaffold-free cellular tube-shaped structure for vascular grafting

aggregates 400–600 μm in diameter, consisting of approximately 20,000 cultured cells. The spheroids were then placed at a temporary support device called the "kenzan," made of stainless steel microneedles placed at 400–500 μm intervals (Fig. 9.2). The spheroids were placed in a bio-3D printer with computer-controlled robotic arms. After printing, the spheroids placed at the kenzan were reflux-cultured for a few days to 1 week; the spheroids adhered and fused with each other, and the kenzan could be removed. Furthermore, after a few more weeks of cultivation, the cellular structures showed tissue maturation and improved mechanical strength. The Kenzan method allows the creation of scaffold-free structures formed only by cells.

9.4.2 The Kenzan Method and Bio-3D Printing Have Enabled the Fabrication of Various Structures

The Kenzan method can provide arbitrarily shaped cell structures that mimic various organs by changing the cell types, depending on the spheroid arrangement at the kenzan. It has successfully generated various scaffold-free structures that mimic organs and has been used in animal transplantation experiments (Table 9.1).

Table 9.1 Transplantation of bio-3D printed organs by the kenzan method

Organs	Source cells	Transplantation period	Transplanted animals	Reference no.
Aortae	NHDFs, AoSMCs, HUVECs	5 days	Nude rat	[61]
Arteriovenous shunt graft	NHDFs	3 months	Mini-pig	[62]
Cardiac graft	RNVCMs, NHDFs, HCMECs	7 days	Nude rat	[63]
Trachea	Rat MSCs, chondrocytes, lung microvessel endothelial cells	23 days	Rat	[64]
Esophagus	NHDFs, HUVECs, MSCs, HESMCs	30 days	Rat	[65]
Liver	Human hepatocytes, HUVECs, human MSCs	31 days	Nude rat	[66]
Bile duct	Pig fibroblasts	14 days	Pig	[67]
Diaphragm	NHDFs, HUVECs	710 days	Nude rat	[68]
Urethra	Human MSCs	–	Nude rat	[69]
Cartilage, subchondral bone	Rabbit AT-MSCs Pig adipose tissue-derived mesenchymal stromal cells	3 months 12 months	Rabbit Pig	[70] [71]
Conduct for nerve regeneration	Human iPSC-derived MSCs NHDFs	8 weeks 24 weeks	Nude rat Rat	[72] [73]

NHDFs normal human dermal fibroblasts, *AoSMCs* human aortic smooth muscle cells, *HUVECs* human umbilical vein endothelial cells, *RNVCMs* rat neonatal ventricular cardiomyocytes, *HCMECs* human coronary microartery endothelial cells, *MSCs* mesenchymal stem cells, *HESMCs* human esophageal smooth muscle cells, *AT-MSCs* adipose tissue-derived mesenchymal stem cells

9.4.3 Development of Human Vascular Grafts

In recent years, the number of patients with chronic kidney disease leading to end-stage kidney disease has increased due to the aging population and the increased incidence of diseases that cause atherosclerosis, such as diabetes and hypertension. Because of this, the number of patients undergoing dialysis therapy has been increasing each year. More than 90% of chronic dialysis therapy consists of hemodialysis, which requires vascular access for blood withdrawal and return. There are several vascular access types, with arteriovenous fistulas being the most common. However, some patients have difficulty performing surgical arteriovenous shunting due to arteriosclerosis, and artificial arteriovenous grafts are used in such cases. The currently used arteriovenous grafts are made of artificial materials such as expanded polytetrafluoroethylene, polyurethane, and polyolefin-elastomer-polyester. Compared to autologous vessels, these materials are easily infected and have low patency and occlusion.

The Kenzan method can resolve these problems. Scaffold-free artificial vascular grafts for animal transplantation have been successful (Table 9.1) and have now reached the clinical research stage for human transplantation.

9.4.4 Clinical Research of Vascular Graft and Nerve Conduct

The cells used to create vascular grafts were autologous skin-derived fibroblasts, which were cultured to form spheroids, shape tubular tissues with a bio 3D printer, and mature into cellular vascular grafts (Fig. 9.2). These autologous vascular grafts have been transplanted to patients undergoing hemodialysis therapy in whom an arteriovenous shunt could not be used due to shunt occlusion, stenosis, or aneurysm.

Peripheral nerve injury can be treated by autologous or artificial nerve transplantation, although these methods are not widely used. Autologous nerve transplantation carries a risk of damaging healthy nerves, and artificial nerve transplantation has not yielded better results than autologous nerve transplantation because of the lack of cells and cytokines for nerve regeneration. Isolated from the patient's skin, autologous fibroblasts formed spheroids and produced tubular structures using a bio 3D printer. These cellular structures were then implanted to bridge the peripheral nerve-damaged site. In animal transplantation experiments, damaged peripheral nerves have been regenerated inside nerve conduits, and good recovery has been observed [62, 63].

9.4.5 Prospects for Regenerative Kidney Medicine

Since 2014, research on kidney organoids reproducing the kidney's structure and function has progressed, and organoids with all the elements of the actual renal structure, as well as those with filtration, reabsorption, and endocrine functions anastomosed with the recipient after animal transplantation, have been created and are becoming more complete. However, there is a significant technical gap, and many challenges are involved in producing artificial transplantable kidneys. One of the challenges is regenerating the kidney's higher-order structure, and various approaches have been used. By combining renal organoids with these methods, we hope to resolve the existing problems and realize the regeneration of artificial organs that can be used in human transplantation in the future.

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Chapter 10
Kidney-on-a-Chip

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10.1 Introduction

Currently, animal model experiments are gradually being restricted in terms of the three Rs (Replacement, Reduction, and Refinement of animal studies). For instance, the United States Environmental Protection Agency (EPA) has announced that it will stop requiring and funding mammalian experiments by 2035 [1]. However, conventional two-dimensional cell culture in vitro is insufficient for the expression of cell polarity, brush border, and transporters, making it difficult to evaluate nephrotoxicity [2]. Kidney-on-a-chip is an alternative, emerging technology that can be used to bridge the gap between in vivo and in vitro.

Kidney-on-a-chip, also known as kidney microphysiological system, is a technology that mimics the 3D microenvironment of tissues by culturing cells in contact with extracellular matrix (ECM) and applying physical stimuli (i.e., fluidic perfusion, shear stress, and stretch stress), to approximate the in vivo environment. Here, we introduce examples of kidney-on-a-chip and explain its feasibility.

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10.2 Examples of Proximal Tubule-on-a-Chip

Proximal tubule-on-a-chip has been realized since the early days and is the most common chip for kidney-on-a-chip.

Jang et al. [3] examined the difference in the characteristics of proximal tubular cells between perfusion and static cultures using a chip with two layers of channels vertically stacked across a collagen IV-coated porous membrane. They seeded primary human kidney proximal tubular epithelial cells in the upper channel and exposed them to a shear stress (0.02 Pa). Perfusion stimuli increased cell height and the number of cells with primary cilia compared to static culture, and albumin transport and glucose reabsorption were also higher. These data illustrate how physical stimuli using a tubule-on-a-chip can be useful for biomimicry.

The stiffness of the ECM to which cells adhere affects cell differentiation and proliferation [4]; therefore, it is important to regulate the environment of the ECM to bring cells closer to the in vivo environment.

Weber et al. [5] used Nortis ParVivo chips to fabricate a lumen in the collagen gel and seeded proximal tubular cells (PTECs) into the lumen to form a three-dimensional tubular structure. They reported that cells cultured in this system did not show KIM-1, a marker of acute kidney injury, and the inhibitory effect of dapagliflozin on expressed SGLT2 was equivalent to the clinical effect.

Imaoka et al. [6] coated Nortis ParVivo chips with collagen I and collagen IV, seeded PTECs from six donors, and evaluated the renal injury caused by ochratoxin A (OTA), a mycotoxin contaminant. The results showed that OTA dose-dependently damaged the tubules, and glutathione conjugation by glutathione S-transferase and P450-mediated oxidation contribute to the detoxification and bioactivation of OTA, respectively.

Homan et al. [2] bioprinted a tubule template on ECM containing fibrinogen and gelatin using fugitive ink, which liquefies at low temperature. After further casting of ECM, the fugitive ink was liquefied and removed, and RPTEC-TERT1 was seeded in the template to form tubular structures. When RPTEC-TERT1 was exposed to perfusion, the cell height and the length and density of microvilli were closer to those of human proximal tubules than those in 2D culture. Because the tubular structure is surrounded by the ECM, they could quantify the epithelial barrier damage with cyclosporine A by increasing protein permeability.

10.3 Glomerulus-on-a-Chip

The complexity of the glomerular filtration barrier ultrastructure makes in vitro modeling far more challenging than modeling tubular epithelial function. In vitro glomerular models have been tested using cell culture inserts; however, they are limited to static culture, and it is difficult to observe the glomeruli in real time except for the measurement of transepithelial electrical resistance. In addition, it has

been difficult to reproduce the foot process and other phenotypes of podocytes using conventional culture systems except for the organoid method.

Wang et al. [7] examined the effect of hyperglycemia on glomeruli by using a chip with five parallel channels. They consist of a capillary channel to culture primary glomerular microtissues containing podocytes and glomerular endothelial cells (GEN) isolated from rats, Matrigel channels to mimic a glomerular basement membrane, and collection channels to mimic Bowman's capsule. They cultured glomerular microtissues on Matrigel in 2D by perfusion culture of an endothelial culture medium containing 5% fetal bovine serum (FBS) and antibiotics, and then added hyperglycemic medium (5 mM, 30 mM). In contrast to 2D static culture, exposure to hyperglycemic medium (5 mM, 30 mM) decreased ZO-1 expression in GEN and disrupted tight junctions. They also showed that hyperglycemia enhanced the migration of podocytes into Matrigel, which could be due to epithelial mesenchymal transition.

Musah et al. [8] examined glomerular protein leakage by using a chip with two vertically stacked chambers flanked by a laminin-511-coated porous membrane. They co-cultured podocytes directly differentiated from human induced pluripotent stem (hiPS) cells and human glomerular microvascular endothelial cells across the porous membrane. The combination of perfusion culture with cyclic stretch (1 Hz) of porous membranes increased nephrin expression in podocytes and the number of podocyte processes that extended through membrane pores to contact the basal surface of endothelial cells. Highly selective filtration, which is different between albumin and inulin, was reproduced. Infusion of adriamycin into the vascular channel also reproduced albuminuria, podocyte delamination, and retraction of podocyte processes.

Rayner et al. [9] reproduced the 3D structure of glomeruli using multiphoton ablation. They formed a 3D patterned lumen extending from a parent vessel composed of human umbilical vein endothelial cells (HUVECs) in a collagen hydrogel by ablation. By inducing angiogenesis along this lumen, they were able to form a perfusable glomerulus with a resolution of 1 μm inside Bowman's space.

10.4 Nephron-on-a-Chip

Chips that integrate glomeruli and tubules have also been developed.

Zhang and Mahler [10] created a nephron-on-a-chip consisting of a glomerulus unit and a proximal convoluted tubule (PCT) chip. The glomerulus unit is a multilayered structure: the top surface of the polyethersulfone (PES) membrane is coated with fibronectin to grow HUVECs, and the bottom surface is coated with collagen I and heparin sulfate to grow conditionally immortalized human podocytes (CIHP-1). The PCT chip consists of a polycarbonate membrane coated with fibronectin and is capable of culturing HK-2 cells. These two units were connected by tubing as bloodstream channel, filtrate channel, and primary filtrate channel. Under their culture conditions, podocytes have finger-like projections. When the bloodstream

channel was perfused with albumin, the albumin that reaches the primary filtrate channel was only one-eighth of that in the bloodstream channel, demonstrating sufficient filtration function.

Qu et al. [11] validated the evaluation of nephrotoxicity in nephrons using a chip with vertically stacked dumbbell-shaped chambers flanked by basement membrane matrix extractant-coated porous membranes. The inlet chamber mimicked a renal corpuscle, with primary renal endothelial cells cultured in the upper compartment and primary podocytes in the lower compartment. The outlet chamber mimicked a proximal epithelial tubule, with primary renal endothelial cells cultured in the upper compartment and primary tubular epithelial cells in the lower compartment. By inducing fluorescent molecules with different molecular weights and charges into the renal corpuscle, they showed that the renal corpuscle has both size-selective and charge-selective barriers. In the proximal tubule, glucose was found to be transferred to the endothelial side of the chamber. When doxorubicin or cisplatin was induced in the upper compartment of the renal corpuscle, viability of podocytes or epithelial cells was decreased, respectively. The toxicity of cisplatin to epithelial cells was reduced by the addition of bovine serum albumin, and the toxicity was lower than when tubular cells and endothelial cells were co-cultured on a conventional chip [3].

10.5 Multi-Throughput Screening

For drug screening using kidney-on-a-chip, it is important to have sufficient throughput. Kidney-on-a-chip often has a low-throughput because perfusion pumps are connected to a single chip. To solve this problem, Vormann et al. [12] used OrganoPlate to evaluate the toxicity to tubular cells. The central channel was filled with collagen I, and the outer channel was seeded with RPTECs. By periodically tilting the OrganoPlate, 40 chips were exposed to fluidic shear stress simultaneously. Four nephrotoxicants such as cisplatin were exposed, cell viability could be evaluated using three indices such as LDH analysis and WST-8 assay, and barrier function could also be evaluated by dextran leakage. Toxicity could also be assessed from the levels of miRNAs in the culture medium.

Petrosyan et al. [13] used OrganoPlate to predict the severity of proteinuria using patient sera. After filling the center channels with collagen I, they seeded three strains of human podocytes in one channels, and then seeded human primary endothelial cells in the same channels to form two layers of cells on top of the collagen I. The cells were then perfused with medium containing the sera from patients with membranous nephropathy. In this experiment, they found that IgG deposition to podocytes and albumin permeability increases, except for immortalized podocytes, and that albumin leakage correlated with the extent of proteinuria of each patient.

Xie et al. [14] reported a rapid method for fabricating scaffolds for glomerulus chips using microfluidic spinning, a technique fabricating a microfiber using coaxial nozzles. In the chips, rat primary vein endothelial cells were cultured in tubular

channels, and murine podocytes were cultured in knots attached to the tubular channels. The chips were able to be mounted on custom-made 96-well plates and perfuse them by gravity drive. They used these chips to test for protein leakage and found that podocyte barrier injury by doxorubicin was milder than previously reported. Although it was unclear, this may be due to indirect doxorubicin exposure to podocytes or 3D tissue maturation.

As described above, by combining with the perfusion system, kidney-on-a-chip can be applicable for multi-throughput screening.

10.6 Kidney Organoid-on-a-Chip

If the cells used in kidney-on-a-chip have the similar function as in vivo cells, the results obtained from kidney-on-a-chip can be closer to those obtained in vivo. The stem cell-induced kidney organoids have the similar structure and physiology to the human kidney tissue [15], and the integration of kidney-on-a-chip and organoid model have been developed.

Homan et al. [16] performed experiments in which the lower surface of kidney organoids was partially embedded in gelatin-fibrin and perfused with medium containing 1.5% FBS at shear stresses of 8×10^{-4} to 35×10^{-4} Pa. This perfusion culture increased the vascularization (some vasculatures were perfusable) of kidney organoids to a greater depth than static culture and increased vasculature in contact with proximal tubules and vasculature invading glomeruli.

Lee et al. [15] compared kidney organoids cultured in microwells coated with 1.5% Matrigel containing 100 ng/mL vascular endothelial growth factor (VEGF) under perfusion, and they reported that the susceptibility to tacrolimus toxicity increased, and cell viability decreased in perfusion culture.

10.7 Multi-Organ-on-a-Chip

To reproduce in vivo, it is necessary to consider interactions between multiple organs; therefore, co-culturing of multiple types of cells is required.

Theobald et al. [17] seeded human hepatoma cell line (HepG2) and RPTECs in two collagen-coated chambers connected in series and perfused them with DMEM high glucose containing 10% FBS. The mRNA expression levels of vitamin D-metabolizing CYPs were increased compared to the HepG2 cells and RPTECs under static culture. Next, medium containing vitamin D3 was perfused to the chip, and the eluate was added to the myeloid leukemia cells, which enhanced the expression of differentiation markers and showed anti-tumor effects of 1,25(OH)D3.

Imaoka et al. [18] used a Nortis ParVivo chip with two collagen IV-coated lumens surrounded by collagen I and cultured HUVECs and PTECs in each lumen. They predicted the clearance of morphine and its active metabolite

morphine-6-glucuronide (M6G) from the blood vessels to the tubules more accurately than the model using culture inserts. The model was also able to predict renal clearance and plasma concentration changes over time in ESKD as well as in healthy kidneys.

Chang et al. [19] used human or rat PTECs seeded on collagen I-coated chips and human hepatocytes seeded on Matrigel-coated chips and combined with thermoplastic elastomer tubing to evaluate the nephrotoxicity by aristolochic acids I (AA-I). They showed that AA-I is bioactivated by hepatic metabolism to the sulfate conjugate of the hepatic NQO1-generated aristolactam product of AA-I (AL-I-$NOSO_3$) and causes renal injury. They also showed that AL-I-$NOSO_3$ uptake by tubular cells via organic anion transporters (OATs) is inhibited by probenecid.

Maschmeyer et al. [20] used a chip with four chambers connected by a channel capable of pulsatile perfusion with peristaltic micropump. Organotypic small intestine tissue, RPTEC/TERT-1, and biopsied skin were seeded in each chamber, and perfusion culture was performed. Even when excess glucose was administered to this chip, glucose maintained a stable concentration in the physiological range. In the tubular cell compartment, the expression of SLC5A2, the SGLT2 gene family, was increased as in diabetic patients.

These results suggest that multi-organ-on-a-chip is an effective alternative to in vivo models for the analysis of absorption, distribution, metabolism, and excretion (ADME) profiles.

10.8 Ongoing Developmental Challenges

There are still some issues to be solved for kidney-on-a-chip. Dimethylpolysiloxane (PDMS) is often used as a material for chip because it is easy to fabricate and has high oxygen permeability. However, PDMS absorbs hydrophobic drugs [21], thus it is desirable to select other materials in the future. In multi-organ-on-a-chip, some culture media may have negative effects on a cell type or may not meet specific needs for another cell type [22]; therefore, the choice of media should be made carefully. As Petrosyan et al. reported [13], the phenotype differs among cell lines; therefore, it is necessary to find a cell source that is closer to the in vivo conditions.

10.9 Conclusion

Until now, each nephron segment has been replicated as kidney-on-a-chip. Compared with conventional 2D culture, this technique reproduces phenotypes more similar to those in vivo and improves the prediction accuracy of renal injury. In addition, multi-throughput screening has been increasingly reported as an alternative to animal models. On the other hand, issues such as chip material, co-culture

conditions, and cell source remains to be solved. If these problems can be solved, kidney-on-a-chip could be a great alternative to in vivo experiments.

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Chapter 11
Innovations in Maintenance Dialysis Therapy

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11.1 Introduction

Innovation in dialysis aims to bring about clinical benefits and better outcomes when compared to current state-of-the-art treatment practices. In other words, innovation may be a novel idea, product, service, or care pathway that has the capacity to improve patient outcomes to facilitate care while containing costs and creating value in the provision of renal replacement therapy [1, 2]. In this perspective, innovation must primarily fulfill an unmet medical need and must be considered as an

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effective tool and be widely applied in clinical practice to the point it induces a therapeutic paradigm shift.

11.2 Hemodialysis Innovations from a Value-Based Care Perspective

In hemodialysis (HD) therapies, innovation includes many components that involve patient experience, scientific evidence, technical advances, skills of care givers as well as care support, and delivery practices [3]. In this section, we pointedly focus on the major innovations in HD that have been shown to improve patient outcomes and to create value in renal care [2]. These innovative tools belong schematically to five main pathways that need to be considered toward the delivery of dialysis therapies.

11.2.1 Enhance Dialysis Efficacy

11.2.1.1 Convective-Based Therapies (Enhanced External and Internal Filtration)

In prescribing HD therapy, the choice is between three main treatment modalities: low-or high-flux HD and hemodiafiltration (HDF). HDF can be delivered in different configurations, high volume hemodiafiltration (HV-HDF) being currently recognized as the most efficient blood purification method per unit time [4]. The solute removal capacity of all HDF versions is higher than high-flux HD, allowing efficient removal of a broad spectrum of uremic retention solutes that are also referred to as uremic toxins. Schematically, reduction rates of hv-HDF is 10–5% higher for low molecular weight compounds (e.g., urea, creatinine), 20–30% higher with peculiar compounds (e.g., inorganic phosphate, free light-chain immunoglobulin) [5–7], and 100% higher for middle molecular weight compounds (e.g., $ß_2$M, myoglobin) [8–10]. Furthermore, solute removal capacity is positively correlated to the total ultrafiltration volume delivered, used as surrogate of dialytic convective dose [11–13]. Therefore, relying on the law of conservation of mass within the dialysis-patient system on a weekly basis by increasing solute mass removal by HDF therapy, considering a constant generation rate, and circulating concentration of solute of interest should decrease accordingly at steady state [14, 15].

In this context, circulating levels of $ß_2$M offer a highly clinically relevant uremic marker that needs to be considered as used more widely by nephrology community [16, 17]. $ß_2$M results from cell activation (expressing HLA), is triggered by inflammation, oxidative stress, and complement activation, contributes to endothelial dysfunction, and finally is cleared by kidney functions [16]. In dialysis patients, $ß_2$M circulating level has an added importance since it reflects both efficacy of renal

replacement treatment (RRT) and toxicity risk particularly for the cardiovascular system [18–20]. Clinical interest of monitoring $ß_2M$ circulating levels is highlighted by several scientific reports [17, 21]. Using $ß_2M$ as the paradigmatic marker of RRT efficacy has been emphasized in some recent reports [22] and implemented in the guidelines of the Japanese Society of Dialysis and Transplantation (JSDT) [23]. Incorporating $ß_2M$ in the panel of biomarkers required for assessing HDF dialysis dose delivery was also proposed by the EUDIAL working group [24].

Recent studies have shown that by using more open membranes, and forcing internal backtransport phenomenon (internal filtration) through increasing flow resistance [25–27], comparable $ß_2M$ removal rates to HDF could be achieved [28, 29]. This is an interesting finding that confirms superiority of convective transport on $ß_2M$ clearances and other middle MW compounds, but at the expense risk of increase albumin loss due to higher and uncontrolled membrane stress [29].

Based on the most recent studies, it is suggested that maintaining a predialysis serum $ß_2M$ concentrations $\leq$25 mg/L is achievable with standard HDF and particularly hv-HDF and is an optimal target toward reducing mortality in dialysis patients [22, 23]. To comply with such key parameter indicator, dialytic convective dose needs to be probed and adjusted individually to patient $ß_2M$ kinetics [30]. As indicated by recent studies, using currently available high-flux membranes, that could be achieved in a majority of patients in postdilution HDF mode with 23–28 L per session of total convection volume [15]. In these cases, $ß_2M$ reduction rate per session is $\geq$80%, a value equivalent to a $ß_2M$ Kt/V $\geq$ 1.5 that provides a $ß_2M$ mass removal ranging between 150 and 200 mg/session for a thrice weekly 4 h treatment schedule [10, 31, 32]. Such treatment schedule is then able to remove 450–600 mg per week out of the 1000–1500 mg mass generated per week.

11.2.1.2 Intensive Dialysis

The conventional prescription of HD is three sessions/week, each of 4 h duration (12 h). Increasing weekly treatment time either through longer individual session (long or home HD, nocturnal) or through more frequent sessions (daily, alternate day), so-called intensive dialysis, has significant benefits on patient outcomes [33–35]. Intensive dialysis enhances clinical performances and treatment efficacy, reduces intradialytic morbidity and treatment burden, and improves mid-and long-term patient outcomes [32, 36, 37]. In this context, HDF has been shown to bring additional values on patient outcomes and patient perception [38–40].

Clinical benefits of intensive dialysis rely on different mechanisms. Firstly, it is associated with higher solute removal capacity and better homeostasis control of specific solutes (inorganic phosphate, $ß_2M$, indoxyl sulphate, para-cresol sulfate). Increased treatment time is currently the only way to overcome slowly moving intracorporeal compounds (low intracorporeal mass transfer coefficient or body clearance) during intermittent treatment [30, 41]. This is clearly shown in kinetic studies. Secondly, it is also coupled with a reduction of ultrafiltration rate, a condition that facilitates vascular refilling capacity, reduces hypovolemia, and then

improves hemodynamic response. In other words, slow ultrafiltration rate tends to minimize dialysis-induced systemic hemodynamic stress [42–47]. Thirdly, it restores a more physiological profile to short intermittent treatment schedule on kinetic and volume changes of solutes. This is confirmed and substantiated by reduction of time-averaged changes (solute concentration, fluid overload, pressure) as well as time-averaged deviations (up, down) [48–50]. Fourthly, it tends to reduce cardiovascular burden (left ventricular hypertrophy) as well as end organ damage (brain) and overall mortality [35, 42]. Finally, it is associated with a better patient perception, reduced impact on quality of life, and facilitated life and professional rehabilitation [34, 51, 52].

11.2.2 Improve Cardiovascular Outcomes

11.2.2.1 Fluid Management

Optimal fluid management has emerged over the last few years as a crucial component to minimize systemic hemodynamic stress and to reduce mortality in dialysis patients [53–55]. Fluid management has two pathways that need to be considered [56–58]: one, reflecting chronic fluid overload accumulated during the interdialytic period and not adequately or timely corrected by dialysis; the other one, reflecting fluid volume depletion induced during dialysis and the systemic hemodynamic response. Interestingly, both the factors have negative impact on the cardiovascular system through different pathways. Chronic fluid overload is responsible for hypervolemia, systemic and pulmonary hypertension, cardiac stretching with functional and structural remodeling, leading to left ventricular hypertrophy, cardiac dysfunction (systolic and diastolic), arrhythmia eventually associated with sudden cardiac death [47, 59]. On the other side, fluid depletion induced by ultrafiltration during the dialysis procedure exposes patient to various degrees of hypovolemia; too rapid ultrafiltration may induce critical hypovolemia and being likely responsible for relapsing intradialytic hypotension episodes causing ischemic insults and leading to repetitive end organ damages (cardiac stunning, brain injury, kidney injury) [47].

Fluid management, currently summed up by the dry weight probing approach, remains for clinicians a delicate between correcting fluid overload while preventing severe fluid depletion [58]. Several biomarkers have been proposed to ensure safer fluid management and reviewed recently. Multifrequency bioimpedance (BIA) has gained large clinical acceptance in supporting clinical dry weight probing [60, 61]. Recent large observational or controlled studies have confirmed the significant value of BIA in guiding more precisely and safely fluid management in dialysis patients to reduce mortality [61–63]. Lung ultrasound (LUS), by scoring B-lines number, used as surrogate marker of extravascular pulmonary edema (thickening of interlobular septa), has also been shown useful in guiding clinical decision [64, 65]. In recent controlled studies, LUS has proved its value in reducing dry weight and controlling hypertensive refractory patients [66, 67]. Further outcome studies are

required to confirm cardioprotective effect of these tools on a long-term basis. Cardiac biomarkers have been also used to evaluate fluid status either fluid overload (BNP, Nt pro-BNP), fluid depletion (copeptin), or cardiac damage (troponin) to guide clinical decision-making in fluid management [53, 68, 69]. In addition, these biomarkers used either alone or in combination may provide interesting predictive cardiac risk [70, 71]. Unfortunately, utilization of these biomarkers is associated with increased costs that often preclude their regular use as monitoring tool and are not part of best clinical practices.

11.2.2.2 Feedback-Controlled Tools

Feedback-controlled tools integrated in dialysis machine may provide additional options to facilitate dialytic fluid management and to improve hemodynamic stability. Among dialysis tools, two have been extensively studied: firstly, blood volume-controlled ultrafiltration; secondly, hypothermic or isothermic dialysis. Blood volume (BV)-controlled ultrafiltration is associated with improved hemodynamic stability [72, 73] as indicated by a significant reduction of incidence of hypotensive episodes and cardiac wall motion abnormalities [74]. However, this clinical benefit has not been confirmed in interventional large studies, meaning that volume control is not the only hemodynamic parameter to be considered [75]. Hypothermic or isothermic dialysis achieved either manually or automatically via blood temperature monitoring option has been shown beneficial in unfavorable patients (hypotensive-prone, cardiac, diabetic patients) and uniformly across all hemodynamic instability conditions as summarized in recent meta-analyses [76–78].

Automatic sodium management has been recently integrated in modern dialysis machines. Sodium control module loop incorporate dialysate sensors (e.g., conductivity cell) and processor unit that integrates conductivity data and dialysis fluid conductivity adjustment according to dialysate-plasma sodium prescribed [79, 80]. Validation studies have shown that zero-diffusive sodium or isonatremic condition could be achieved very reliably with less than 1.0 mmol/L plasma sodium concentration changes [81]. Furthermore, the sodium control module provides an estimate of the sodium mass balance and allows monitoring of plasma sodium concentration throughout the dialysis session. Further studies are needed to identify clinical benefits as well as long-term cardiovascular outcome improvement of this new tool [82].

11.2.3 Facilitate Acceptance of Alternative HD Delivery Modes

11.2.3.1 Home Therapy

Despite proven clinical and economic benefits, home hemodialysis (HHD) remains still marginal (<3% of share) as compared to in-center HD, except in few countries (e.g., New Zealand, Australia, Canada, Denmark, Finland, Sweden, UK) that have

initiated programmes to promote this therapeutic option [83]. A renewed interest for HHD is emerging due to the availability of new specifically designed, more friendly and connected dialysis machines target to meet the needs of patient autonomy and mobility, and attractivity of daily treatment and healthcare regulatory policies [84, 85]. Several manufacturers have developed new, innovative, and well featured HHD machines that includes NxStage System One, Quanta SelfCare+, Physidia S3, and Tablo Outset Medical [86]. These HHD machines have been approved by appropriate notified bodies and are being currently implemented and assessed worldwide for home dialysis treatment. Presently, it is too early to draw any conclusions on the prevalent acceptance and use of HHD, but it seems that this new technology has already triggered an upswing for home dialysis therapy.

11.2.3.2 Personalized Therapy

Incremental dialysis and more flexible dialysis treatment schedules are currently being developed not only to facilitate transitioning from end stage kidney disease to dialysis and improve patient's treatment acceptance [87–89], but also to solve renal care issues in fast-developing countries (e.g., China, India) [90–92]. Incremental or flexible dialysis relies mainly on the preservation and support of residual kidney function [93–95], or by adding an additional oral component acting on the gut and reducing uremic toxins generated [96, 97] by means of adsorber (ST120) or pro-or antibiotics mixtures [98]. In this context, several recent reports have shown potential clinical benefits, cost-effectiveness, and usefulness of such individualized approaches. However, it remains to be proved by controlled studies that such incremental or flexible dialysis schedules are applicable and generalizable to unselected populations.

11.2.4 Ensure Vascular Access Sustainability

Vascular access sustainability remains source of concern in most dialysis patients. Failure or dysfunction of arteriovenous fistula or graft is one of the first causes of hospitalization and morbidity in HD patients. In this context, tunneled central venous catheters represent an easy and comfortable alternative, although unfortunately associated with additional risks (inadequate dialysis, infection, vein stenosis) [99, 100]. Vascular access management represents a highly ranked priority in most best practice clinical guidelines [101]. Over the last few years, various new options have emerged to improve vascular access outcomes [102]: firstly, increased success of native arteriovenous fistula creation by pre-or intra-operative vasculature assessment [103–107]; secondly, percutaneous creation of proximal arteriovenous fistula [108–111]; thirdly, the use of bioengineered blood vessel in patients with exhausted vasculature [112–115]; fourthly, better handling of tunneled central venous catheter or implanted port devices and use of locking solutions [116–119].

11.2.4.1 Implement Continuous Quality Improvement Programs with Support of Digital, Advanced Analytics, and Artificial Intelligence

Outcomes of dialysis patients result from a complex equation involving individual patient profiles, renal replacement modality, multiple dimensional measures [120], skills of care givers, and therapy delivery practices. In this complex therapeutic chain involving several stakeholders with multiple sources of data, implementation of a continuous quality improvement process supported by digital and advanced analytic tools providing balanced scorecard is an attractive way to achieve targets and to improve patient outcomes in a more structured and comprehensive manner [121, 122]. The benefits of such a strategic approach has been reported in a recent study developed in a large dialysis chain provider [123]. In this study involving a cohort of 4270 incident dialysis patients, 2-year mortality was analyzed according to achievement of key performance indicators (KPI) prior (group A, 2397 patients) to and after (group B, 1873 patients) medical peer review involving continuous quality improvement (MPR CQI) onset. After MPR-CQI implementation, a significant improvement in KPI targets was achieved associated with a 30% risk reduction of mortality [123].

11.2.5 Final Considerations for HD Innovations

In the last decade, industry has developed a number of innovative technologies that support the nephrological community in monitoring and individualizing therapies toward the overall target of improving outcomes and well-being. For example, estimation of sodium levels is crucial toward maintaining fluid status and hypertension–both of which impact cardiovascular outcomes in HD patients; the automated sodium management tool of the 6008-dialysis machine (FMC, Bad Homburg, Germany) helps achieve an individualized and precise sodium prescription without adding any workload to the dialysis care staff. Such strategies that enable clinical decision-making to improve patient well-being without additional organizational effort are examples of the new trend toward value-based healthcare. This concept is graphically summarized in Fig. 11.1. In this section, we have attempted to delineate various "innovations"—grouped in five pathways—that have the potential to fulfill the aim of improving the hitherto poor outcomes associated with the dialysis patient populations (Fig. 11.1). Collectively, each of the five pathways (enhanced dialysis efficacy through convective-based therapies and intensive dialysis; improved cardiovascular outcomes through active fluid management, feedback-controlled loops and automated sodium management; facilitated patient acceptance of treatment through home-based and personalized therapies; ensured vascular access sustainability through non-invasive pre-implantation vasculature assessment, availability of bioengineered blood vessels, and better handling of tunneled central venous catheters or port devices; implementation of continuous quality

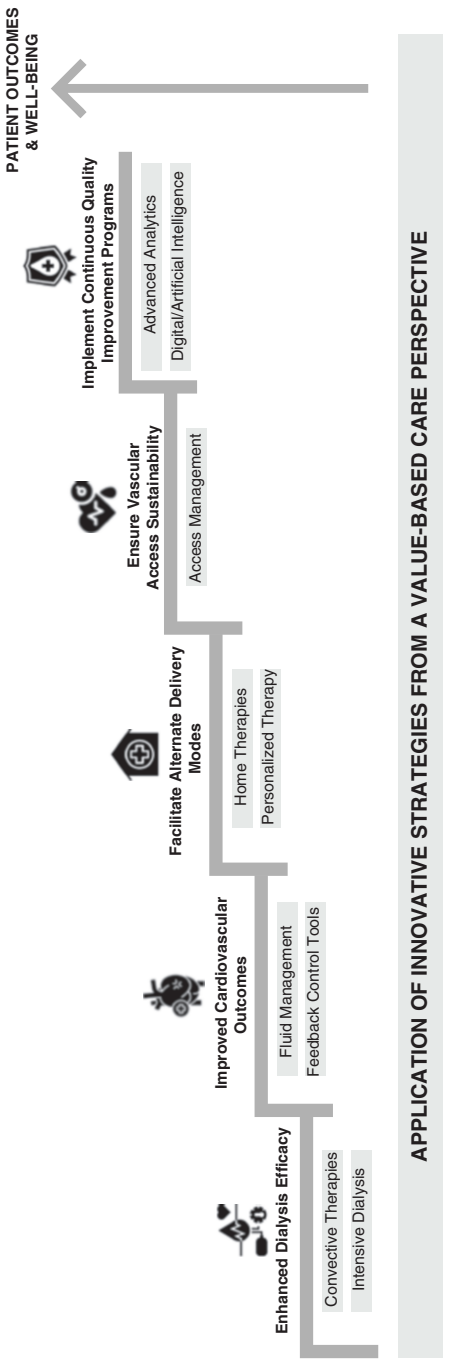

Fig. 11.1 Incremental and collective application of different strategies to target improvement of patient outcomes in terms of reducing morbidity and mortality that is still high for the dialysis population. Until now, the emphasis has mainly been on addressing issues related directly to the treatment procedure itself; today, there is evidence to suggest that a number of pathways related to the overall care of the individual dialysis patient may help achieve better outcomes

improvement program with support of digital, advanced analytics, and artificial intelligence) has a higher probability of achieving better therapy outcomes and increased patient well-being, rather than addressing each pathway separately and fragmented manner.

11.3 Peritoneal Dialysis Innovations

The advent of Continuous Ambulatory Peritoneal Dialysis (CAPD) in 1976 can be considered as a simple and fabulous innovation in the home dialysis therapy scenario [124–126], leading to a significant increase in peritoneal and home dialysis utilization. Since those days, innovations mainly in the area of peritoneal dialysis (PD) bag designs, connectivity and monitoring have kept a steady pace of development, opposite to what happens in the area of the base PD solutions and its mostly used osmotic agent, glucose.

11.3.1 Innovations in PD Access, Catheter Design, and Insertion Techniques

It is correct to say that functional peritoneal dialysis access remains the cornerstone and Achilles' heel for successful initiation and time on therapy. If catheter insertion is free of complications, then it goes to follow that patients will most likely have a successful time on peritoneal dialysis.

Catheter type and the tendency of catheter migration therefore influence failure potential. Many innovative PD catheter designs have been produced along the years. Here, we highlight two of them:

(a) The weighted catheter designed by Di Paolo [127, 128] to overcome the problem of catheter dislocation. It is a straight catheter, weighted at the end with 12 g of silastic coated tungsten, an inert biocompatible element which is denser than dialysate and gravitates into the pre-rectal peritoneal pouch (Fig. 11.2). The weighted catheter's first reported use was in 1996 with further publications in 2004 of a series of 746 patients with fewer complications and dislocations when compared with Tenckhoff catheters. In 2019, Stonelake et al. presented results that the weighted catheter was associated with lowest failure rates when compared with surgically inserted non-weighted catheters in a group of patients with increased risk factors for adverse catheter outcome [129].

(b) A new catheter design by Al-Hwiesh [130], the new triple-cuff PD catheter has demonstrated a zero rate of catheter migration, improved catheter survival, and lower peritonitis rates (Fig. 11.3)

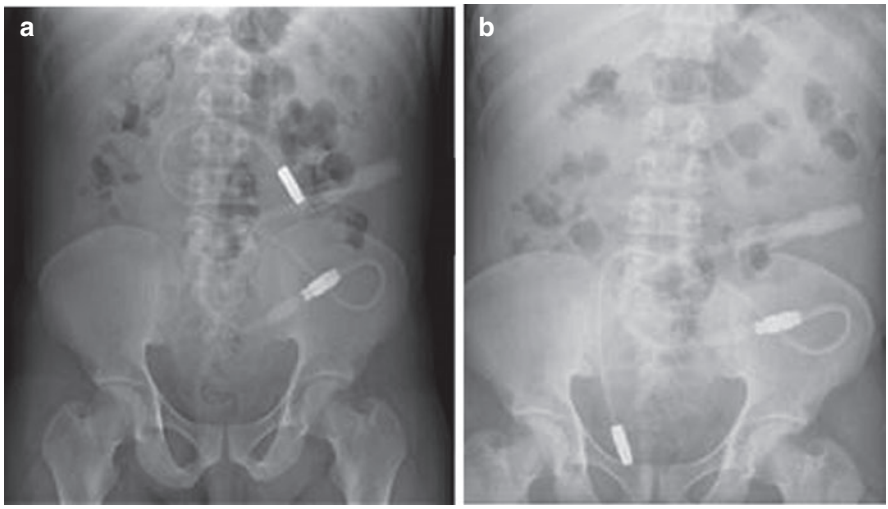

Fig. 11.2 The weighted PD catheter designed by Di Paolo. (**a**) PD catheter with weighted tip "flipped" secondary to constipation; (**b**) Same PD catheter 2 days after constipation treatment–weighted element back in satisfactory position. (Courtesy of Dr. Jyoti Baharani, Birmingham Heartlands Hospital, Birmingham, United Kingdom)

Fig. 11.3 The triple-cuff PD catheter designed by Al-Hwiesh. (Courtesy of Dr. Abdulla Al-Hwiesh, Al-Khobar, Saudi Arabia)

11.3.2 Connectology Assistance

Although assisted PD has increased the repertoire and numbers being enabled to have therapy, there are practical measures that need to be taken into consideration before an assisted service can be offered. Efforts to create devices that further

facilitate the management of the PD exchanges by patients and/or by healthcare professionals assisting the therapy are needed, for example, new connectors for PD exchanges as well as devices to manage patient training, prescriptions, therapy outcomes, and even bi-directional communication.

PeriSafe, a new connectology device, connects the transfer set and the PD bag system inside of a protected area. Manual contact of the patient with the open transfer set is prevented, and breaking the frangible or clamping the tubes can be performed by pushing a button. The PeriSafe system removes the old protective cap, and the pull-ring connects the transfer set and PD bag system, guiding the patient through the therapy with buttons. After dialysis, the transfer set is disconnected from the PD bag system and protected with a new protective cap. All the steps are performed inside of the protected device [131].

11.3.3 Peritonitis Diagnosis

Point of care (POC) devices for peritonitis provide useful, rapid, and inexpensive screening test for diagnosing peritonitis and may be helpful for patients who live in rural areas or patients who have difficulty getting to their clinics.

A leukocyte esterase strip test such as Peri-Screen/Peri-Plex may provide a point of care test for peritonitis. Preliminary results from a European study show a sensitivity of 100% and a specificity of 96%, along with the ability to detect as few as 50 leukocytes/mm^3, this test can detect as few as 100 leukocytes/mm^3 (with a neutrophil count of not less than 50%) [132].

11.3.4 Patient-Directed Therapy

In some healthcare settings, delivery of PD has focused on achieving the small solute targets suggested in the 2006 International Society for Peritoneal Dialysis (ISPD) prescription guidelines without considering the impact of increasing dialysis exchanges or hours on a cycling machine on quality of life.

However, since 2006, those in need of dialysis have changed considerably with increasing multimorbidity associated with higher proportions of people with diabetes and/or in older age groups. At the Kidney Disease Improving Global Outcomes (KDIGO) Controversies Conference on Dialysis Initiation, Modality Choice & Prescription in January 2018, it was proposed that there should be a change in terminology from "adequate" to "goal-directed" dialysis defined as "using shared decision-making between the patient and care team to establish realistic care goals that will allow the patient to meet their own life goals and allow clinicians to provide individualized, high quality dialysis care."

This approach concurs with the findings from the Standardised Outcomes in Nephrology–PD initiative (https://songinitiative.org/projects/song-pd/), which

identified core outcomes for PD chosen by patients, caregivers, and healthcare professionals, namely PD infection, cardiovascular disease, mortality, PD failure, and life participation. There is no evidence that small solute clearance on its own directly affects these outcome measures, except in a small proportion of individuals in whom transfer from PD to HD has been attributed to insufficient small solute removal.

11.3.5 Enhancing Uptake and Maintaining Numbers on PD

Innovation is not limited to material invention. A revolution happening in the last decade of translating patient experience into patient insights calls for its inclusion as the new global standard for therapeutic and device approvals as well as reimbursement decisions. This must be considered as an innovation, not earlier even imagined.

Another real-life evidence of an "abstract" innovation is the implementation of a quality improvement process. This implementation at the University Hospitals Birmingham NHS Foundation Trust successfully increased the number of incident and prevalent PD patients. PD uptake increased from 37 to 84 patients per year, giving a PD penetration increase from 8.4% to 19.1% between April 2014 and January 2018. Catheter insertions increased from 94 to 185 per year. Peritonitis rates remained stable, and PD drop out to HD, reduced from 52% to 41% during the same period.

Changes introduced as part of the QI process can take time to develop, introduce, and embed. Pathway mapping, patient education, and utilization of lean methodology can positively impact PD growth. The multidisciplinary team focus on growing home therapies can in conjunction with peer educators offers a new approach to patient education and treatment decision-making.

Pivotal to this process of enabling a philosophy of informed patient choice, resulting in the growth of home therapies, is a clinical champion "Home Therapies Lead" clinician. Continued research and audit can identify the longer-term impact of the cultural shift within renal services on growth and maintenance of home-based dialysis and more specifically PD numbers.

A key change for future state mapping is to move from thinking about patient preference for modality of dialysis, to patient preference for location of dialysis. By implementing a rapid improvement process and embedding continuous quality improvement process, an increase in the incidence and prevalence of PD should become apparent [133].

11.3.6 What About Innovation in PD Solutions and Its Mostly Used Osmotic Agent (Glucose)?

Paradigmatic of such condition is the case of one of this chapter's authors (AA), who spent most of his academic and professional medical life in researching and developing new anti-diabetic treatments, with no contact with the nephrology/

dialysis world. He was not aware that glucose, administered intraperitoneally in relatively large amounts, was the main osmotic agent used in PD therapy. This came as a surprise particularly in consideration that diabetes is the leading cause of kidney failure; the obvious next question was: *is there a way to replace glucose as the base osmotic agent?*

One possibility to mitigate the potential metabolic side effects of glucose would be to formulate PD solution containing a mixture of different osmotic agents, allowing to reduce the concentration of the single one and possibly to take advantage of a combination of favorable pharmaco-metabolic properties that may address not only the main dialytic objective of a PD solution along with a better preservation of the peritoneal membrane, but also common comorbidities such as diabetes mellitus, a disease that dramatically increases the already high preexisting risk of cardiovascular disease (CVD)/deaths in PD patients.

PD patients are potentially exposed to a constant hyperinsulinemic state because of the continuous intraperitoneal load of glucose, the most potent insulin-secretagogue [134, 135]. On the other hand, the hyperinsulinemic state in diabetic PD patients is most likely linked to insulin dipeptidyl peptidase-4 inhibitors or glucagon-like peptide 1 analog treatment [136]. An emerging concept for the etiology of CVD and the atherometabolic risk of diabetic and non-diabetic IR individuals is organ-and/or pathway-specific insulin resistance (IR), also known as selective IR [137–139]. This is based on the observation that IR does not occur for all insulin signaling pathways, with the result that those pathways still responding to insulin normally may be overstimulated because either of the concomitant compensatory hyperinsulinemic condition experienced by non-diabetic IR subjects and during the earlier stages of type-2 diabetes or in type 1 and 2 diabetics treated with insulin. This clearly indicates that glucose and insulin are the main culprits of some of the pathological sequelae occurring in PD therapy.

If insulin cannot be replaced in diabetic PD patients, it should be possible to reduce its use by improving glycemic control intervening at two levels: replacing most of the glucose present in the PD solution with osmo-metabolic agents able to improve glucose uptake and/or disposal. Two of these osmo-metabolic agents are L-carnitine and D-xylitol [140]. In addition to their good safety profile and great chemical stability, these two naturally occurring compounds are not only involved in major metabolic pathways involved in glucose and lipid homeostasis, but they may also improve insulin sensitivity and glucose uptake/disposal by modulating mitochondrial acetyl-CoA levels (i.e., L-carnitine) and via transcriptional and post-transcriptional interventions (i.e., D-xylitol) [141–144].

A combination that is currently under clinical investigation foresees the use of D-xylitol, as the major osmo-metabolic agent, along with L-carnitine and less than 70 to 85% D-glucose present nowadays in commercially available PD solutions [145]. Furthermore, in vitro studies conducted with this glucose-sparing PD solution are very encouraging as they do not seem to induce fibrosis and angiogenic effects in human mesothelial and endothelial cells, respectively [146]. Even more interesting is the recent renaissance of metabolism and metabolic reprogramming in the field of fibrosis that may see the involvement of the same osmo-metabolic agent in combatting the mesothelial-to-mesenchymal transition (MMT) too. Indeed, one

of the above osmo-metabolic agents, L-carnitine, may be extremely competent in mitigating the hyperglycolytic phenotype, also known as the "Warburg effect," commonly present in MMT [147]. The well-recognized intervention of supra-physiological concentration of L-carnitine within mitochondria in lowering acetyl-CoA and, hence, reactivating pyruvate dehydrogenase [141] is as efficient as glycolytic inhibitors in inhibiting TGF-b1 induced MMT [148]. An important implication of this finding is that to slow down the progression of fibrosis, it is not necessary to inhibit glycolysis, which may carry serious side effects, but only requires an efficient coupling of glycolysis with the Krebs cycle.

PD solution innovations such as pharmaco-metabolic intervention with a combination of osmo-metabolic agents in PD therapy may lead to beneficial effects both at systemic and peritoneal levels, improving the so-called global biocompatibility of PD.

11.3.7 Final Considerations for PD Innovations

Innovation must also be global. It is interesting to note and important to try understanding why a greater number of the innovations introduced in the PD space have actually been focused on further development of the Automated PD (APD). It seems like CAPD is the poor cousin of APD; however, sound evidence favoring APD therapy as superior to CAPD does not exist today. With innovation also focused on CAPD, expansion of assisted PD may become a perfect combination with CAPD: a simple, cheaper, and safe home dialysis option.

11.4 Future Perspectives

What can we expect from dialysis therapy in the years to come? Although there are different forms of innovation within the field, the idea of having smaller and portable dialysis devices, above all, brings hope and some excitement to the kidney community. The concept of portable dialysis is definitely not new; there are a few reports in medical literature from the 1970s and 1980s [149–151]. However, there is still a fine and little-known boundary between the dream, the myth, and effective developments in the area. Only recently, with the advent of miniaturization and nanotechnology, have we had actual innovative advances in order to possibly achieve a safe, economically viable and efficient treatment with portable devices in the near future [152, 153].

In this regard, the WAK (Wearable Artificial Kidney)–a wearable blood-based renal replacement device–is a potential innovative advance. It is a lightweight (<5 kg), battery-powered, that is used like a vest or belt (Fig. 11.4). Due to the potential risk for accidental disconnection, the WAK is more likely to be connected to an HD catheter instead of arteriovenous fistula (AVF) needles. The used dialysate

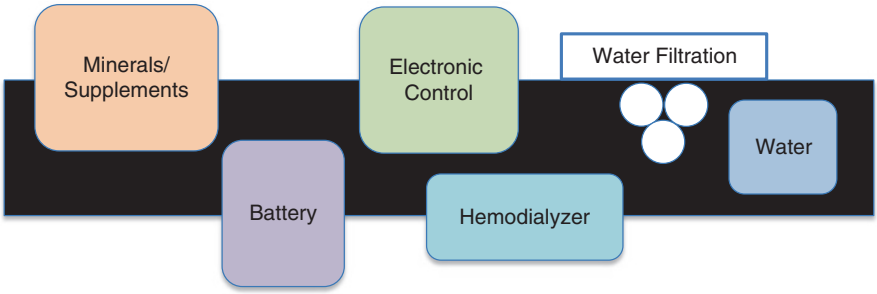

Fig. 11.4 The WAK (wearable artificial kidney). (Reproduced with permission from Salani et al. [154])

is regenerated using sorbent technology with the excess redirected through an ultrafiltration pump to a waste bag. The WAK pump has a double channel pulsatile counter phase flow, described in detail elsewhere [154–156].

A 2007 pilot landmark study by Davenport et al. showed some promising efficacy and safety results. Eight patients with end-stage kidney disease on regular HD were fitted with a WAK device for 4–8 h. Unfractionated heparin was administered to avoid coagulation, as it would be for conventional HD. Mean blood flow was 58.6 mL/min, with a dialysate flow of 47.1 mL/min, and the mean plasma creatinine clearance rate was 20.7 mL/min. There was no evidence of hemolysis, as well as no significant cardiovascular changes and no adverse changes in acid-base balance. Clotting of the vascular access occurred in two patients due to a decrease in the unfractioned heparin dose. As discussed above, AVF needle disconnection was an issue in this pilot study; one patient suffered a temporary disconnection due to his AVF needle becoming dislodged. The device's safety mechanisms ensured a quick interruption of the blood pump, avoiding blood loss, and the treatment continued with no clinical consequence to the patient [156].

A portable device also has been produced for PD; the AWAK (automated wearable artificial kidney–AWAK Technologies Pte, LTD). It is a tidal PD-based artificial kidney, battery operated, that uses regeneration of the dialysate in order to reduce fluid requirements. The system–composed of a disposable storage module, tubing set, and system controller–is even lighter (weight less than 2 kg) than the WAK and can be used like a bag [157] (Fig. 11.5). In the AWAK system, 1–1.5 L of dialysate is initially instilled into the peritoneal cavity, such as in conventional PD. Again, sorbents are used; a tidal volume of 0.5 L of equilibrated dialysate is then drained from the patient into the storage module and pumped through the sorbent cartridge for clearance. After filtration, supplementation (with glucose and electrolytes) and degassing of the spent dialysate, the regenerated dialysate returns to the peritoneal cavity. The tidal exchange lasts about 7–8 min, resulting in eight exchanges per hour. The sorbent cartridge can be utilized for 6–8 h, before being discarded and replaced–so the process can commence again [154, 157].

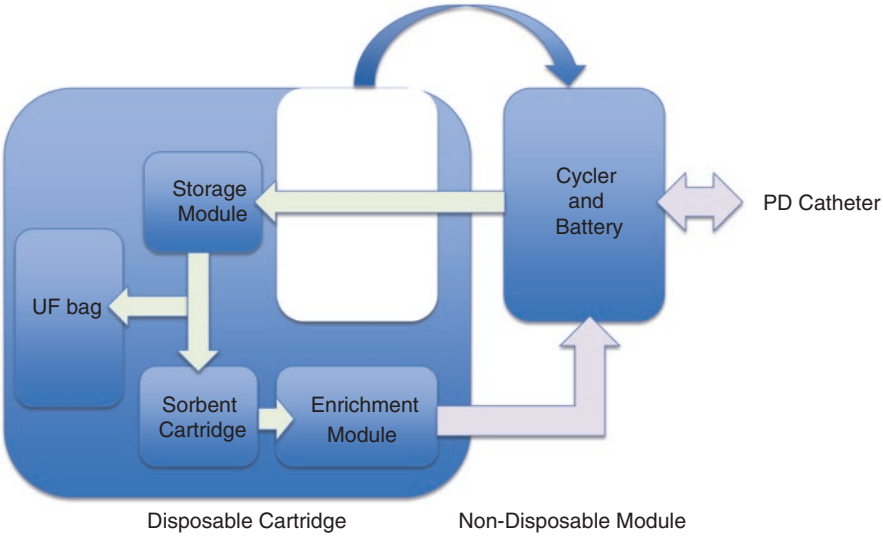

Fig. 11.5 The AWAK (automated wearable artificial kidney). (Reproduced with permission from Salani et al. [154])

A study conducted with 20 patients using the AWAK system reported a urea clearance of 31.5 mL/min. The AWAK was worn for 4–24 h, and the treatment was based on the tidal PD mode of dialysate delivery. Although no adverse event was reported, the consequences of the regenerated dialysate and the continuous fluid exchange and how this influence risk of peritonitis, membrane failure, hyperglycemia, and encapsulating peritoneal sclerosis are not yet fully understood [154, 158].

Another innovation–this one in earlier stages of research and with no clinical trial in humans so far–that arouses curiosity beyond hope to both patients and healthcare professionals is being developed by the Vanderbilt University Medical Center and the University of California, San Francisco: the IAK (Implantable Artificial Kidney). The device mimics a native kidney, incorporating tissue engineering and silicon nanotechnology, and is designed to be surgically implanted [154]. The IAK system closely replicates the nephron physiology through a combination of a high-efficiency filter (the HemoCartridge) and a bioreactor of cultured kidney tubule epithelial cells (the BioCartridge). Originally, the IAK does not require electrical pumps since it is meant to be connected to the arterial vasculature, allowing blood to be pumped by the force of the patient's own blood pressure. The ultrafiltrate produced in the HemoCartridge is processed by the BioCartridge, which returns water, salt, and glucose to the blood and concentrates toxins into a small amount of fluid similar to urine in a progressive manner (Fig. 11.6). Originally, the IAK does not require electrical pumps since it is meant to be connected to the arterial vasculature, allowing that the patient's blood pressure to pump blood through the filter [157].

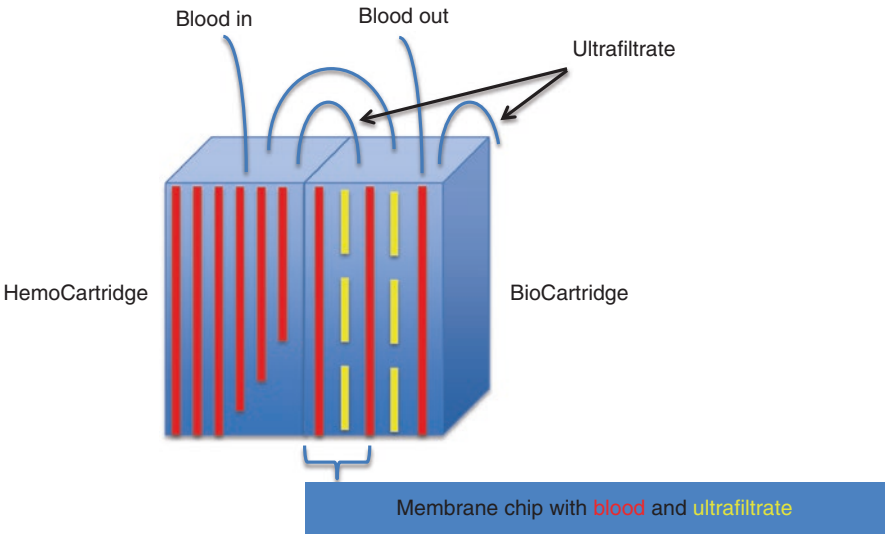

Fig. 11.6 The IAK (Implantable Artificial Kidney). (Reproduced with permission from Salani et al. [154])

In addition to research and development of the aforementioned portable (WAK and AWAK) and implantable devices (IAK), the nephrology community desires–and requires–innovations to turn dialysis more equitable and globally accessible. A few years ago, Kotanko et al. suggested a rather controversial, yet only theoretical, model in order to solve this issue. In summary, the authors proposed a model in which a healthy human being (called the "buddy") could somehow replace the function of a HD machine (Fig. 11.7). This concept, known as allo-hemodialysis (the Greek prefix "allo" means "other"), is a paradigm-breaking innovative model of renal replacement therapy. In allo-hemodialysis, blood from the "buddy" flows in counter-current direction to the patient's blood, functioning as the dialysate. Uremic toxins diffuse from the ill patient across the dialyzer membrane into the "dialysate" (which in this case is the "buddy's" blood). Then, the buddy's blood is transported back to the buddy, where solutes and fluid received from the ill patient will be finally excreted by the buddy's healthy kidneys [159, 160]. A 2019 cross-sectional survey conducted in Mexico aimed to investigate acceptance of allo-hemodialysis among caregivers and nephrology healthcare professionals. Although only 60% of the healthcare professional accepted it, this new technique was mainly accepted by caregivers–both related (87%) and non-related (90%) to kidney patients [161]. Despite several bioethics and clinical unresolved issues, allo-hemodialysis, among all potential innovation, maybe the only one that could truly make the renal replacement therapy significantly cheaper and consequently more accessible especially in the poorest settings, where dialysis is not universally available.

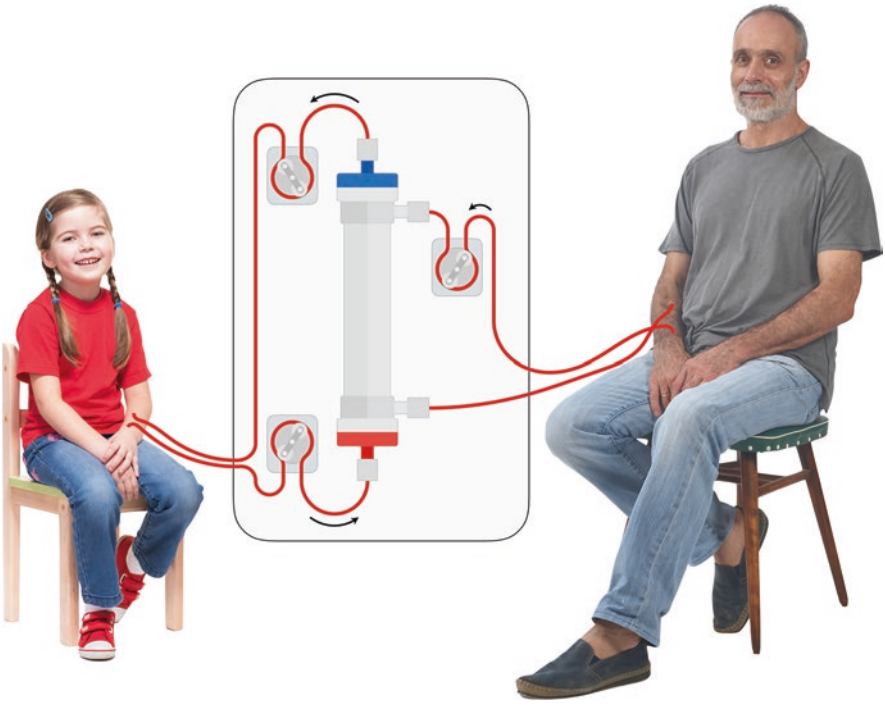

Fig. 11.7 Allo-hemodialysis model. The pediatric patient with kidney failure and the "buddy" (adult with healthy kidneys). The blood of the adult "buddy" is pumped through the dialysate compartment in countercurrent flow, serving as the dialysate. Ultrafiltration is due to speed differentials of the patient-sided pumps. (Reprinted with permission from Kotanko et al. [159, 160])

Despite all the promising research for innovations in dialysis therapy–at different stages of development, in fact–future perspectives for real breakthroughs may not lie in novel peritoneal or blood-based therapies. Otherwise, it would somehow be "more of the same" for the patient's experience and outcomes. Paradoxically, the future of dialysis may not be in dialysis therapy itself… rather, it may be related to paradigm-changing innovations that are based on demands and expectations of kidney disease patients, such as preventive (i.e., lifestyle education) or curative action on kidney disease (i.e., SGLT2i in diabetic kidney disease), or regenerative medicine to correct kidney injury, or xenotransplantation and/or biomedical engineering developing hybrid concept. Only time will tell us, and (hopefully) very soon what is the best pathway to follow.

Finally, the past and present of dialysis are filled with remarkable technical advances. Through history and looking to the future, we shall never forget the main driver of innovation: the patient. All efforts and progress are only meaningful when associated with superior outcomes, better patient perception and/or benefits for health systems. This is the only way to guarantee that innovation is a tangible reality that has the potential to change treatment paradigms and not just novelties restricted to private research or corporate interests.

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Chapter 12
Dialysis for Business and Society: From a Shareholder to a Stakeholder Perspective in Dialysis Units Management

José A. Moura-Neto

12.1 Introduction

"*The goal of the academic institution is to help improve dialysis and to study it and to hopefully prepare it for some day where it could become a widespread clinical service*." This is what Nathan Levin, one of the pioneers in nephrology, said of the dominant philosophy among the trailblazer "academic" dialysis centers back in the 1960s [1, 2]. Fortunately, this time has come and for most countries dialysis therapy is currently a widespread clinical service.

Several technological advances have occurred in the past decades, such as the hollow-fiber dialyzer, the development of safe hemodialysis machines, improvements in the water treatment, online production of substitution fluid for convective therapies, the creation of new drugs, and a better understanding of the disease and its complications as a whole. However, the economy of scale of the large dialysis facilities–those which offer therapy to dozens of patients simultaneously–really enabled dialysis to become not only viable but a widespread clinical service as predicted half a century ago. In 2020, there were 7566 dialysis facilities in the United States (US) alone [3]. Although there is still inequitable access to kidney healthcare in many countries [4, 5], more than four million people are under maintenance dialysis therapy worldwide [6].

In addition to all the technical innovations of the treatment itself, significant advances have been observed in the management of dialysis units. Business intelligence tools, novel management software, and "smart" hemodialysis machines (now even more connected and integrated) can bring additional data that can aid the clinical and managerial decision-making process. The recent development of the "green

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dialysis" concept has also brought several changes within the sector through the adoption of sustainable practices [7, 8]. Telehealth is yet another important advancement applicable to almost the entire healthcare sector including dialysis [9, 10]. However, none of these technological innovations surpass the potential impact of the (r)evolution of the mindset towards the management of dialysis units: *from a shareholder to a stakeholder perspective.*

12.2 The Traditional View

Businesses play a pivotal function in society by generating jobs, creating value, nurturing innovation, and providing essential services and goods [11]. For decades, it was believed that companies mainly existed for the benefit or interests of its owners, namely shareholders.

In the landmark book "Capitalism and Freedom," published in 1962, Milton Friedman said, "*there is one and only one social responsibility of business—to use its resources and engage in activities designed to increase its profits…"* and completes "*…so long as it stays within the rules of the game, which is to say, engages in open and free competition without deception fraud*" [12]. Friedman was indeed a harsh critic of the concept of corporate social responsibility. According to him, only people can have "responsibilities." Corporate social responsibility, then, would be nothing more than a "fundamentally subversive doctrine" [13].

In the field of business ethics, the so-called Separation Thesis suggests that morality and business can be separated in certain ways. According to this theory, business, in its best light, is "amoral" and, at worst, likely immoral. This concept was and still is frequently used by "bad" executives to legitimize their attitudes. Would it really be possible for a business decision to have moral content? Although it is possible to have different interpretations and versions of this idea, the main concept of this theory has, fortunately, been consistently rejected over the years [14, 15].

For many decades, the idea that capitalism was based on the self-interest, greed and competitive nature of mankind prevailed. However, the "cowboy capitalism"–as it is often called–cannot (or should not) be applied to healthcare, less patient care loose its value and diminish into materialism. Fortunately, this traditional and standard model of business gave way in recent years to an alternative model: the "stakeholder capitalism," which has shown to be considerably more appropriate for both dialysis and the healthcare sector as a whole [15].

12.3 The Stakeholder Framework

Although the stakeholder perspective only recently became a dominant "school of thought" in business, its roots can be traced back to the 1960s. The term stakeholder was first used in the book "Industrial Democracy and Industrial Management" by

Eric Rhenman, originally published in 1964, in Swedish, and later in 1968, in English [16, 17].

Despite its Scandinavian origin, it was 20 years later in the US that the public and academic interest in the stakeholder perspective began to flourish, especially after the publication of "Strategic Management: A Stakeholder Approach" by Edward Freeman [18, 19].

But what exactly does "stakeholder" mean? "Holders" who have "stakes" interact with the organization and make its operations feasible–a simple etymological definition [20]. The underpinning idea here is that virtually all organizations have stakeholders, which are "any group or individual who can affect or is affected by the achievement of the firm's objectives" [21]. In other words, stakeholders are individuals or groups that can directly or indirectly impact or be impacted by the organization's actions or activities [18].

As a consequence of the consistent evolution of the stakeholder perspective over the years, the non-profit organization *The Business Roundtable* modified its traditional view and published a new statement titled "Statement on the Purpose of the Corporation" in 2019. In this novel document, they declared that the interests of clients, workers, communities, and "other stakeholders" must be as important as the interests of a company's shareholders. This was a significant shift from the previous statement published in 1997 that defined the principal object of a business as "to generate economic returns to its owners" [11, 21].

This statement was originally signed by 183 Chief Executive Officers (CEO) from US and multinational companies in its first updated version, released on August 19, 2019 [21]. It had a huge media repercussion as well as a synergic effect. Following this move, *The New York Times* published an article titled "*Shareholder value is no longer everything, top CEOs say.*" The renowned *Fortune* magazine highlighted this symbolic initiative on the cover of its September 2019 issue, under the title "Profits and purpose: can big business have it both ways?" By January 2022, the original 2019 document was already signed by 243 CEOs.

12.4 Dialysis for Business… and Society

Not without controversy, the idea that the healthcare sector should not be treated or operated as a traditional business has always been prevalent [15]. The recent rise of the stakeholder perspective strengthened and even shed new light on this idea. As opposed to being antagonistic, the stakeholder perspective should be understood as an evolution of the traditional shareholder model. The company shareholders are undoubtedly still an important subset of stakeholders; they have not lost their primacy, they are simply sharing it with other stakeholders.

Dialysis fits precisely within this context and can be considered as a proof-of-concept of the stakeholder perspective in the healthcare sector. Despite the pragmatic need to be a for-profit company–this is, after all, what attracts potential investors and makes any large investment in the sector feasible–a dialysis unit must

be understood in a broader perspective as an organization with multiple responsibilities; with all of its interacting parts and within the very society in which this business is inserted. It has to be a legitimate stakeholder-oriented company: not only for corporate purposes, but for business and society as a whole.

12.5 Dialysis Unit Stakeholders

The growing importance of the stakeholder perspective is such that nowadays some might claim that the success of any business depends fundamentally on the capacity of the organization to manage stakeholder relationships [18]. The contemporary manager, therefore, is responsible for implementing the stakeholder perspective within its organization, essentially creating ways of interacting and properly "dealing" with the stakeholders. To do so, the first step is in identifying all the interacting parts and interests/expectations in order to respond to two pivotal (and "not-so-simple") questions that guide the stakeholder framework: (1) *Who are the dialysis unit stakeholders?* and (2) W*hat do they expect from the organization?*

Some articles already apply the stakeholder concept and terminology in nephrology and dialysis [22–25]. However, to the best of our knowledge, none of them clearly identify the stakeholders and their expectations in the chronic dialysis setting. In this chapter, we propose a theoretical model of the stakeholder framework as applied to chronic dialysis units (Fig. 12.1). According to this model, dialysis units usually interact with six parties: patients, healthcare workers, shareholders, government, "community," and suppliers.

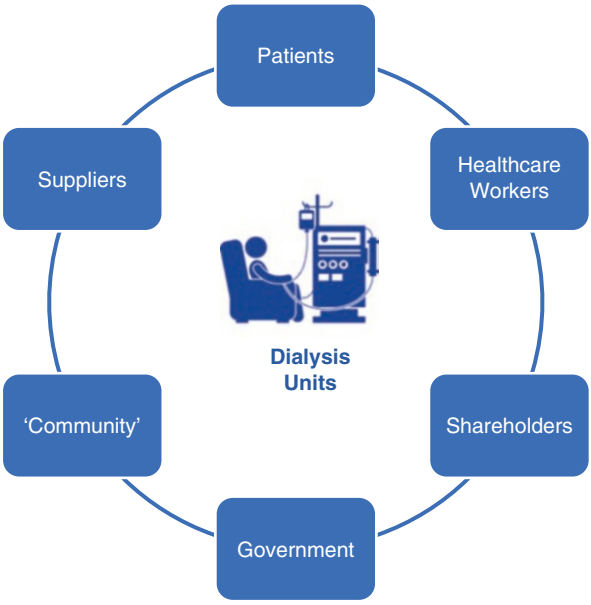

Fig. 12.1 Stakeholder framework as applied to dialysis units

12.5.1 Patients

Chronic dialysis units traditionally work in shifts, treating dozens of patients with chronic kidney disease simultaneously throughout each shift. This "organizational design" creates cost advantages that make dialysis units economically viable.

Patients are, of course, the main reason why dialysis units exist. There is still an underlying debate regarding the preferred label for these key stakeholders: patient, client, customer, or consumer? [26]. Aside from this "secondary" debate, chronic dialysis patients expect much more from the dialysis unit than just their regular delivery of a 4 h thrice-weekly therapy (on average, at least according the medical prescription). Patients expect a personalized and holistic approach, which means taking care of the "whole" person–not only renal replacement therapy, but the physical, mental, spiritual, and social needs of patients as well. In addition, they also expect an affordable dialysis service where healthcare is privatized (out of pocket costs).

12.5.2 Healthcare Workers

A great variety of employees usually work in a regular dialysis unit. Although there is a variation between countries due to cultural and regulatory specifications, most of the dialysis units' employees are by definition healthcare workers, including registered nurses, physicians, dialysis technicians, psychologists, dietitians, social workers, physiotherapists, and pharmacists. There is also a small administrative staff in the dialysis unit that functions to support the organization, members of which likewise considered as stakeholders.

Workers seek a job offering an adequate salary, respect on the part of the employer and customers, reasonable job stability, security, a decent social status, and a favorable environment for practicing healthcare. This last point is quite important. In the early days of dialysis, it was not possible to treat the majority of patients requiring renal replacement therapy–a sad reality for many regions of the world even today. In the 1960s, a committee in the US city of Seattle officially defined those patients who would be suitable for dialysis treatment. This committee, called the God Committee, "decided who lives, who dies"–as per a landmark article published in 1962 by Shana Alexander which raised a discussion on this issue [27]. The pioneering nephrologist Nathan Levin described, in the same interview in which we opened this chapter, this burden in the early days of chronic dialysis treatment: *"As a fellow I had to watch very otherwise treatable young dialysis patients die because there was no place for them in the program. I remember a young man, the same thing, the only answer was: Sorry we're full, we have no room, we have no capacity to help you and we'll keep you as comfortable as we can"* [1, 2]. Even more than a low salary, this situation has the potential to inflict a profound emotional burden on healthcare workers. As a major issue to the field, there are underlying initiatives to address with inequities in global kidney health [28].

12.5.3 Shareholders

Prior to the affluence of the stakeholder perspective, shareholders alone were considered the "owners" of the company and the primary reason for the existence of a business. Although shareholders are still an important stakeholder, we can now (and fortunately) claim that Society as a whole–which includes all stakeholders in its ranks–is the collective "owner" of dialysis units.

Shareholders expect return on investment, which can come through either profit or increasing the value of a company's shares. Furthermore, shareholders may also expect other intangible benefits beyond simple financial rewards, such as social respect, status, and recognition. To obtain this, their respective companies must strictly follow government rules and regulations and subsequently have a responsibility to actively respect the expectations of other stakeholders.

12.5.4 Government

There is an expectation for any government to oversee its domestic public health policies, as well as to ensure some viable level of investment–be it from private shareholders or general taxpayers–to the dialysis sector. Each national government may be considered a common (and powerful) shareholder, not only as it applies to dialysis, but for any business in their respective countries. They therefore expect full compliance from dialysis units regarding government rules and laws. This influence is maintained through regulatory agencies. Additionally, governments expect companies to pay any due taxes generated through its activities. In countries where healthcare is publicly funded, governments reasonably expect dialysis units to offer their services at affordable "costs"; public dialysis units, after all, have all costs paid for by the taxpayer, and even private dialysis units should maintain a reasonable rate for their services.

12.5.5 "Community"

The "community"—i.e., a neighborhood, a city, its residents, etc.–is also a stakeholder of the dialysis unit. Residents expect their neighborhood to be kept safe, clean, and organized, and thus a dialysis unit must maintain these same standards, not creating pollution, a surge in foot traffic (or actual traffic), or an increase in criminal activity in the area. Citizens expect low unemployment rates and good quality healthcare services in their region–indeed, even with healthy individuals, it is comforting to know that there are specialized health services available nearby, with low mortality rates and good health indicators.

Table 12.1 Summary of expectations from dialysis unit stakeholders

Who	What do they expect from the dialysis unit?
Patients	– Renal replacement therapy with a personalized and holistic approach – Affordable service where privatized (out of pocket costs)
Healthcare workers	– A job, with an adequate salary; respect on the part of employers and customers; reasonable job stability and security; a decent social status; a favorable environment for practicing healthcare
Shareholders	– Return on investment – Intangible benefits (social respect, status, and recognition)
Government	– Regulatory and legal compliance – Taxes – Affordable "costs" for services in countries where healthcare is publicly funded
"Community"	– Good healthcare with specialized services; low unemployment rates; a well maintained neighborhood, kept safe and clean; low mortality rates
Suppliers	– "Customers" for its products

12.5.6 Suppliers

As a whole, the healthcare supply chain is vast, and dialysis is no exception. From dialysis machines to disposable materials, such as dialyzers, hemodialysis bloodline, and manufacturers, supplies represent one of the main financial burdens to dialysis units, together with human resources. Ultimately, suppliers want a "customer" for its products.

Table 12.1 presents the questions that guide the stakeholder framework: (1) *Who are the dialysis unit stakeholders?* and (2) *What do they expect from the dialysis unit?* Noteworthy, stakeholder expectations are not static; on the contrary, it can change over the years. For example, customers and the "community" already expect (and value) sustainable practices from companies in some industries. In dialysis sector, the Green Dialysis concept is still crawling, but we might see a significant change in the coming years toward stakeholders' expectations for sustainable practices in dialysis [7, 8]

12.6 Conclusion

The traditional view that companies primarily exist to generate economic returns for its shareholders benefit is no longer the dominant philosophy today. Instead, a company is now responsible with looking after a complex network of interests. For this, they must first identify these interacting parts and their respective expectations in order to adequately manage them all. According to our proposed model, dialysis units interact with six stakeholders: patients, healthcare workers, shareholders, government, "community", and suppliers. Although this framework is already a reality, we can still expect more. There are significant inequities in access to kidney

healthcare worldwide. Moreover, patients, or other stakeholders, could still have a larger voice within the organization and its decisions–and perhaps, better representation on the board of directors for large companies. The evolution of this mindset certainly represents the principal innovation in dialysis unit management. Dialysis has, finally and fortunately, reached its manifest destiny, crafted toward a broad and noble purpose within its context of business and society.

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Chapter 13
Generation of Whole Kidney and Other Possible Strategies to Renal Replacement Therapy in the Future

Shuichiro Yamanaka and Takashi Yokoo

13.1 Introduction

Although tubular regeneration is observed in acute kidney injury, it is almost impossible to recover nephrons once they are damaged and lost in common kidney diseases. Therefore, the treatment approach for kidney diseases is primarily to break the progression of dysfunction, and chronic kidney disease is a disorder with a strong irreversible component. Moreover, some patients will progress to end-stage renal failure during their life. However, even when the kidneys stop functioning completely, renal replacement therapy (RRT) can support life. RRTs such as dialysis and kidney transplantation are known to provide life-supporting treatment to several patients. However, the supply of RRT is limited, and the waiting list for kidney transplantation in the USA exceeds 100,000 people every year (data available from URL: https://www.organdonor.gov/statistics-stories/statistics.html (accessed August 29, 2021)).

In addition, an imbalance exists in the supply of RRTs (especially hemodialysis) between developing and developed countries due to the high cost of equipment and medical resources (1). Population growth, aging of population, and increase in the number of diabetic patients are expected to increase the number of patients with renal failure worldwide (1), and the urgent task is to correct this imbalance between supply and demand for RRT [1].

Therefore, the development of novel technologies that combine various bioengineering techniques such as stem cell technology, genome editing technology, organoid technology, kidney-on-a-chip, and the use of heterologous animals is being

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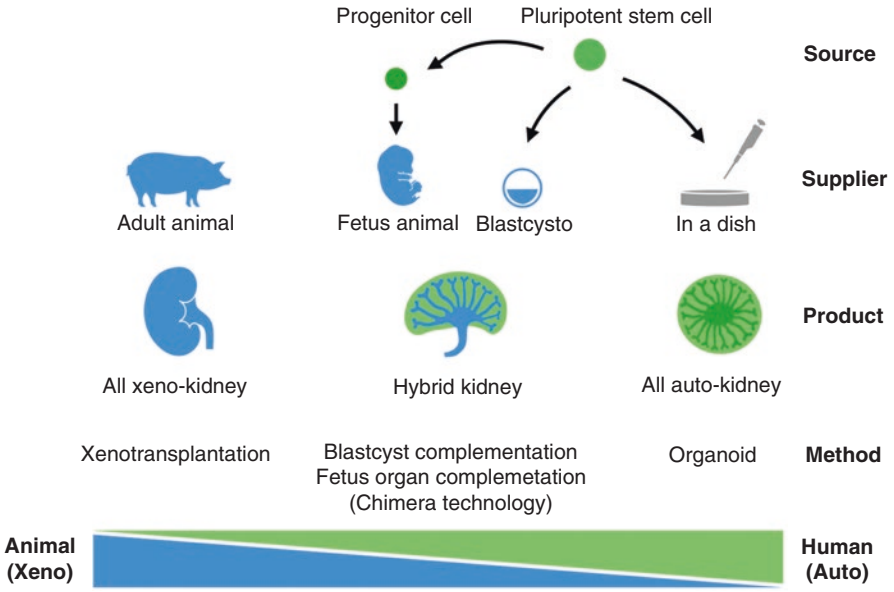

Fig. 13.1 Overview of whole kidney regeneration strategies with a focus on species components

pursued as a new approach to treat kidney diseases, which is different from current treatments.

In particular, this section focuses on (1) xenotransplantation, (2) renal organoids, and (3) hybrid kidneys using chimeric technology (Fig. 13.1). Xenotransplantation does not use human autologous stem cells, and the kidney is composed of only xenogenous tissue. In contrast, kidney cells derived from human-induced pluripotent stem (iPS) cells, such as renal organoids, are intended to be produced almost completely from autologous stem cells. Somewhere in between is the chimera technology. Each of these technologies has its advantages and disadvantages; however, their ultimate goal is to provide early rescue to patients with kidney failure.

We believe that these technologies can be inventive breakthroughs in kidney disease care. I would like to introduce them as a promising dawn of research and development, leading to the next generation of treatment.

13.2 Xenotransplantaion

It is still difficult to regenerate life-sustaining replacement kidneys from stem cells alone in vitro. However, xenotransplantation holds promise as a practical technique to address the shortage of organ donation, and protocols in the nonclinical academic research phase have yielded kidney viability of more than 12 months from pigs to baboons as life-sustaining kidney transplants [2]. Xenotransplantation has been

investigated since the early twentieth-century [3]. Xenotransplantation without the use of immunosuppressive drugs leads to violent rejection after transplantation, with several kidneys of transplanted animals failing within hours or days [4]. Immunological barriers have been the major limiting factor in xenotransplantation; however, the development of the immunosuppressive drug azathioprine in the 1960s [5] raised hopes for xenotransplantation research for clinical application. To overcome the severe rejection of xenotransplantation, further attempts were made to improve the rejection of donor tissue itself. Concerning immune rejection specific to xenotransplantation, there are three major types of xenograft rejection in a continuum, which are hyper acute rejection (HAR), acute humoral xenograft rejection, and acute cellular rejection (ACR) [6]. In addition to immunological rejection, there is a parallel dysregulation of the recipient's coagulation system.

HAR as the first xenotransplant rejection event is due to the innate immune defense mechanism in which the recipient's preexisting liquid immunity reacts immediately to the transplanted kidney. In pigs, which are the most promising donor candidates for xenotransplantation because of their size, prolific fertility, and recognition as domestic animals [7], HAR is triggered by an antigen–antibody reaction between galactose-α1,3-galactose (αGal) expressed in pigs and anti-αGal antibodies present in primates such as humans and by complement activation [8]. Although the reduction of natural antibodies to pigs by plasma exchange or antibody adsorption therapy has been demonstrated to be effective in reducing HAR [9, 10], it did not sufficiently suppress the antibody–complement response. Therefore, it was considered desirable to transform the donor tissue itself to make it suitable for pig-to-human transplantation. For the purpose of controlling HAR, a pig genetically transfected with human decay-accelerating factor (hDAF:CD55), one of the complement control factors, was reported [11]. This inhibited the activation of complement by anti-pig antibodies reacting to the transplanted organ and exhibited some effect on HAR. In 2005, a pig kidney with a knockout (KO) of α1,3-galactosyltransferase, the enzyme that generates αGal antigen, was transplanted into a baboon, and the results demonstrated renal xenograft survival accompanied with normal creatinine levels maintained for up to 83 days [12]. However, several HAR-causing antigens other than Gal antigens were subsequently identified, and it was found that countermeasures against αGal antigens alone were not sufficient to avoid HAR [13–15]. Triple KO pigs, in which three major antigens were knocked out simultaneously, were developed, but the xenografts did not survive for a long period of time, indicating that measures against the HAR-causing antigens alone were insufficient [15, 16]. It was also believed that problems in the coagulation system and inflammatory response, such as thrombotic microangiopathy and consumptive coagulopathy, were acutely involved in the abolition of graft function [17, 18]. Therefore, multiple gene transfer such as human thrombomodulin and endothelial protein C receptor was attempted as a countermeasure against excessive hypercoagulability in response to heterogeneous rejection [19]. In addition to the initial HAR regulation, it is necessary to control ACR due to cellular immunity, which appears during the initial days and weeks after transplantation. One study reported that the longest duration of life-sustaining by renal xenograft transplantation to date

was 499 days in pig donor to recipient rhesus macaque, in which the researchers used αGalKO/hDAF transgenic pigs as donor kidneys and found that blocking T-cell costimulatory signals in the CD40-CD154 pathway in the recipient rhesus led to a reduction of CD4 T-cell subsets, which was effective for the long-term survival of the xenografts [2]. However, costimulatory blockade of the CD40-CD154 pathway has not been approved for clinical use due to serious complications of thromboembolism in clinical trials, thereby making its clinical application in transplantation difficult at present [20, 21]. Hence, the development of safer anti-CD154Ab immunosuppressive agents or the application of anti-CD40 antibodies, which are expected to be equally effective, is desirable to gain approval for clinical use [22–24].

It is necessary to consider infection control for clinical applications. Methods for rearing animals in a sterile environment have been developed, and countermeasures have been implemented against retroviral activation inherent in the porcine genome [25, 26]. Furthermore, xenotransplantation raises ethical, cultural, and religious concerns that warrant further careful discussion [27]. Research on xenotransplantation as a method to develop life-supporting replacement kidneys is currently much closer to clinical application than previously believed, which is due to the development of multiple simultaneous genome editing technologies for donor tissues and novel immunosuppressive drugs, which were difficult to achieve until half a century earlier. Henceforth, the key aspect will be how to adjust the technology for clinical application. We look forward to more detailed elucidation of the underlying immune system in xenotransplantation and progress in genetic engineering for the induction of immunological tolerance [28].

13.3 Organoids

If it is possible to generate kidneys from autologous stem cells, this could become the ultimate treatment for kidney failure. The discovery of iPS cells may have paved the way for the realization of organ regeneration using autologous stem cells [29]. In this chapter, we present an attempt to generate a kidney using only stem cells.

Although direct reprogramming from fibroblasts to tubular epithelium has been investigated [30], the current mainstream approach is to construct renal tissues by reproducing developmental stages in sequence. Tissue-like structures produced by inducing pluripotent stem cells (PSCs) are known as organoids, and they have been constructed in other organs such as the liver and pancreas [31]. To regenerate kidneys from PSCs, the development of the kidney must be completely understood and reproduced. To this end, breakthroughs in genetic analysis technologies, such as single-cell sequencing technology, have contributed significantly as an approach to more precisely analyze heterogeneous populations [32, 33]. Detailed analysis of the developmental process has revealed novel insights into nephrogenesis, like unraveling a tangled thread. Previously, it was believed that the kidney differentiated into the ureteric bud (UB) and metanephric mesenchyme (MM) from a single intermediate mesoderm (IM). However, Taguchi et al. showed that the IM that forms the

kidney separates into two IMs at an early stage and that they exist spatiotemporally separately, with UB differentiating from the anterior IM of the IM located anterior to the fetus and MM differentiating from the posterior IM located caudal to the fetus [33].

The MM includes nephron progenitor cells (NPCs) and stromal progenitor cells (SPCs). As the posterior IM was identified, the induction of NPCs could be established by more accurately mimicking the development of the stepwise differentiation of NPCs through the posterior IM. Similarly, the induction of UB through the anterior IM was reported based on precise lineage analysis [34]. In the early days of organoid research, a method of organoid formation was developed to induce heterogeneous cell populations from PSCs [35]. However, we believe that the selective induction method of NPCs and UBs, which accurately mimic development, is more suitable for kidney regeneration as there were two IMs that differed significantly in time course and space. Nephron development requires the following three components of renal progenitor cells: NPCs, SPCs, and UBs. The use of NPCs and UBs induced by the selective induction method, in addition to embryonic SPCs, demonstrated the generation of renal organoids with a higher-order structure, in which the collecting ducts and distal tubules are connected in a tree-like manner, which had not been observed previously [36]. It is remarkable that cap mesenchyme (CM) formation close to the original renal tissue was observed, which has not been demonstrated in previous renal organoids. We believe that accurate CM formation is one of the checkpoints to induce organoids with higher-order structure. The CM is the starting point of nephron development and also a niche for progenitor cells to self-renew and form a progenitor cell pool [37]. Then, in the CM, the self-renewal of NPCs and their differentiation into nephrons precede in parallel and the number of CMs themselves increases as their tips branch further. In mice, approximately 3000 CMs are eventually formed from 1 CM at the initial stage [38]. From a single CM, multiple nephrons are generated in succession, all of which lead from the distal tubule to the collecting duct and then the collecting duct to the renal pelvis and ureter [39].

Interestingly, it has been previously demonstrated that reaggregation experiments using extracted progenitor cells are also capable of automatic CM reformation and differentiation from the CM into nephrons [40]. It can be seen that the renal progenitor cell population has a high capacity to self-organize. Therefore, if induction to precise renal progenitor cells (NPCs, UBs, and SPCs) can be achieved and CM capable of self-renewal and self-organization can be formed, it is possible to form organoids capable of autonomous growth. The aggregates of induced NPC (iNPC), induced UB (iUB), and embryonic SPCs derived from mouse embryonic stem cells automatically demonstrated the higher-order structure of the kidney without relying on external CHIR (GSK-3α/β inhibitor), indicating the high self-organizing ability of these cells. Therefore, the accurate reproduction of the CM, which is important in nephrogenesis, is also considered to be important for increasing the nephron number and forming a connected excretory tract. In the absence of SPCs, human iNPCs and human iUB alone did not form organoids with higher-order structure, as verified by Taguchi et al. [36]. Therefore, the most urgent issue

for the generation of human renal organoids with higher-order structures is the establishment of human SPC induction.

However, the method for the induction of human SPCs has not been achieved. Moreover, it has been pointed out that renal organoids do not have an exit excretory tract [33, 41]. As the bladder and ureter are derived from the endoderm and undergo a different developmental process from that of renal progenitor cells, regeneration of a series of integrated urogenital units may still be difficult due to their heterogeneity. For instance, Yokote et al. reported that it is possible to provide renal organoids with excretory tracts by combining them with a surgical urinary tract manufacturing method that uses a xenogenous embryonic bladder and ureter as a bridge for urinary excretion [42]. As embryonic organs have lower rejective antigenicity than adult organs, one idea is to use xenogenous tissues only partially as urinary tracts [43, 44]. The establishment of vasculature is also important in the future. Although renal organoids do not contain vascular endothelial cells, studies have demonstrated that host blood vessels invade and integrate into glomeruli when transplanted under the renal capsule [41, 45]. As larger organoids become available in the future, we suspect that there will be a limit to the sustainment of structures that depends only on the vascular invasion from the host. The establishment of a novel vascular system capable of sustaining large organoids is required. The regeneration of blood vessels by adding human umbilical vein endothelial cells to organoids has also been investigated [46]. Because the development of the vasculature still remains unclear in renal development, it would be necessary to reveal the reconstruction of the renal vasculature. However, renal organoids have a wide range of potential applications, including drug screening, toxicity testing, and pathological modeling, even in the absence of a urinary or vascular system [47–51]. It is expected that the establishment of a method for the induction of SPCs, the construction of a sustainable vasculature, and the acquisition of excretory tracts will enable the construction of a kidney composed entirely of human cells.

13.4 Chimera Technology Introduction

Chimera is the coexistence of two populations of cells with different genomes in a single individual [52]. For instance, bone marrow transplantation and kidney transplantation are chimeric conditions in which cells from another person coexist in one body. However, Gardner's experiment in which an isolated inner cell mass was injected into the blastocyst cavity of the host to produce mouse chimeras is well known [53]. This is termed as "primary chimerism." Chimerism in the embryo before organogenesis is known as "primary chimerism," and chimerism in the combination of tissues after organogenesis has begun is known as "secondary chimerism" [53, 54]. Chimerism has been applied in a wide variety of research and in the field of organ regeneration; the application of chimerism technology ("hybrid zoo") has attracted much research attention in recent years [55]. The strategy involves passive generation of organs by placing stem cells of different species on a conveyor

belt, analogous to the natural developmental process that occurs in the animal. I believe that chimera technology will be a bridge between stem cell research, such as organoids, where regeneration is completed using only autologous stem cells, and xenotransplantation, which does not rely on stem cells, and will help address the challenges of each technology (Fig. 13.1). There are two major types of organ regeneration in the animal, viz., blastocyst complementation using primary chimerism and embryonic organ complementation using secondary chimerism. These two methods are described in this session.

13.4.1 Blastocyst Complementation (Primary Chimerism, Systemic Chimerism)

Blastocyst complementation is a primary chimerism method that combines pluripotent cell populations. It is also known as systemic chimerism. PSCs such as iPS cells or ES cells are used as donor cells, and blastocysts are used as recipient cells. In the case of kidney regeneration, a blastocyst genomically deficient in nephrons (spalt-like transcription factor 1 (SALL1) knockout) is created by genome editing, and normal iPS cells are transplanted into the kidney-deficient blastocyst to complement the defect. Goto et al. succeeded in constructing a PSC-derived kidney in a rat–mouse heterologous chimera [56]. This blastocyst complementation method was originally discovered in 1993 when normal ES cells were injected into the blastocysts of lymphocyte-deficient mice to replace the missing lymphocytes with ES cell-derived lymphocytes [57]. In recent years, this method has been applied to the regeneration of solid organs and in organs other than the kidney. Kobayashi et al. regenerated a xenogenous rat pancreas from rat iPS cells using mouse blastocysts in which pancreatic and duodenal homeobox 1 (Pdx1), a master regulatory gene for pancreas formation, was knocked out [58]. Moreover, islet cells derived from mouse PSCs generated in the rat body were transplanted into a mouse model of diabetes, which demonstrated therapeutic effects by normalizing the blood glucose levels [59]. One of the challenges of the blastocyst complementation method as a chimera technology is the problem of interspecies barriers. If two organisms are very different, for example, in terms of lifespan, developmental rate, or size, can the integrated interspecies cells be aligned in a common developmental environment? One solution is to select animals that are similar to humans. Pigs are similar to humans in terms of organ size, and their developmental rate is not much different from that of humans compared with rodents. Nevertheless, in pig–human chimera experiments, the contribution of human cells was low, ranging from 1000 to 10,000:1 [60–62]. The interspecies barrier is believed to be due to differences in adhesion factors, receptors, and cell proliferation rates, although several aspects remain unresolved [63]. Although primary interspecies chimerism in rats and mice has demonstrated regeneration of the pancreas, it was reported that the chimerism rate in surviving individuals was lower than that in homologous chimerism [64]. Therefore, to obtain a high chimerism rate in heterologous primary chimeras, interventions in donor

cells was attempted. Such interventions included overexpression of Bcl2, an anti-apoptotic gene [65], and development of "intermediate PSCs" that are similar to the naive type with high chimera formation ability [66]. As an intervention in the host blastocyst, the introduction of insulin-like growth factor 1 receptor (Igf1r) was reported to improve the chimera rate in rodents [67]. Igf1r is expressed throughout the body and is involved in cell division, antiapoptosis, and transformation pathways [68]. Interestingly, the transplantation of rat iPSCs into Igf1r-deficient mouse blastocysts [67] was found to improve the chimerism rate in kidneys from a few percentage to <20% compared with those without Igf1r deficiency. However, interspecies chimeras with rats generated from the blastocysts of igf1r-deficient mice died as newborn pups due to respiratory distress, indicating that further improvement will be required if the animals are to be grown to adulthood [69]. The fact that the vasculature consists of xenogenous cells is a major disadvantage in terms of rejection during the transplantation of chimeric kidneys. Along with the challenge of overcoming the interspecies barrier, there is the ethical issue of the animals to be produced. In Japan, restrictions on human–animal chimeric embryo (HACE) research have been lifted to a limited extent, but several countries still place high restrictions on HACE research [70, 71].

The reason for the regulation arises due to concerns that human cells may integrate the host animal's brain or germ line and differentiate. The off-target effect of this primary chimeric organ regeneration method has been discussed for some time, and studies aimed at avoiding this effect have been attempted [72]. In the future, if the contribution of human cells is further improved, the problem of off-target effect may become more apparent. This issue requires more careful discussion and research to reach public consensus, for instance, establishing a rigorous test that are generalized has low variability and high detection sensitivity.

In contrast, a rapid response to organ shortages is highly desirable, and 59% of the US population has responded to a questionnaire that they would personally accept a process in which human-induced PSCs are injected into genetically modified pig embryos and human tissue generated in the pig body is transplanted into humans [73]. Despite careful discussion, it would be necessary to address various ethical challenges such as animal welfare, human dignity, and neurological humanization in the future [73], and we hope to notice progress in primary chimera technology as a highly feasible technology for three-dimensional organ regeneration.

13.4.2 Fetus Organ Complementation (Secondary Chimerism, Local Chimerism)

Among the organ regeneration techniques using animals, secondary chimerism is known as fetus organ complementation or organ niche method, which combines embryonic organs and progenitor cells during organogenesis [74–77]. As mentioned in the organoid section, the CM is the starting point for nephron development. When mouse dissociated single cells extracted from fetus kidney (metanephros) were

transplanted into the nephrogenic zone in another fetus kidney, approximately 30% of the NPCs in the host-side CM were derived from transplanted donor cells [76]. NPCs possess the ability to migrate [78], and we believe that NPCs implanted near the CM are attracted by the attraction of the UB tip. It remains to be clarified what determines the attraction of NPCs to the CM [78]. The NPCs that were implanted and attached in the CM differentiated into nephrons together with the host cells [76]. This gathering into the CM was observed not only in NPCs but also in SPCs, and it was demonstrated that transplanted SPCs could similarly attach to the CM and differentiate into mesangial cells [79]. It was found that when combined and progenitor cells during organogenesis niche (embryonic organ), a mosaic nephron of donor and host cells was formed. However, to achieve a completely transplanted NPC-derived nephron rather than a mosaic nephron, we attempted to completely eliminate the host NPCs. Specifically, we applied the diphtheria toxin receptor (DTR) system [80] and used genome-edited mice, in which only the host NPCs are ablated in a drug-induced manner. When the NPCs were transplanted at the same time as the drug-induced ablation of NPCs, it was confirmed that the donor and host NPCs were completely replaced in the CM [76]. Interestingly, the phenomenon of progenitor cell replacement observed in the nephrogenic niche was also observed between different species (mouse and rat) [76, 81]. Chimeric nephrons generated from rat NPCs and mouse nephrogenic niches also demonstrated the ability of the glomeruli to filter host blood [81]. The replacement phenomenon was highly reproducible, and the mice CM flexibly accepted the interspecies NPCs and proceeded to epithelialize them, suggesting that the developmental transcriptional signals during nephrogenesis are highly homologous. Moreover, when NPCs differentiated from human iPS cells were transplanted into the embryonic kidney of a mouse equipped with the replacement system, human NPCs gained control and formed a human renal vesicle with connectivity to the mouse UB, although in an immature state [82, 83]. This is the first chimeric kidney with a nephron with a distal relationship between mouse and human using the chimera method. It has been possible to advance epithelialization from human NPCs using mouse spinal cord in organoid experiments [34], suggesting a high degree of the homology of epithelialization signals and receptor even between human and mouse. This high homology of differentiation in the organogenesis period is consistent with the concept of the "evolutionary hourglass model," in which developmental transcription factors during organogenesis are used in a variety of ways and become difficult to replace during the course of evolution, suggesting a supportive result of high homology [84, 85]. Secondary chimerism may be the developmental period when the hurdle to cross the interspecies barrier is the lowest.

There are three advantages of creating a chimeric kidney by fetus organ complementation. First, it is possible to provide a urinary tract. In other words, the nephron differentiated from the transplanted cells is connected to the host collecting duct at the distal tubule and can directly access the existing urinary tract system. The embryonic kidney with a cloaca, which includes the fetal ureter and bladder, can be transplanted into the retroperitoneum. Then, it can connect the host ureter to the fetal bladder when the fetal bladder has grown to a certain size after producing urine

from the embryonic kidney, allowing urine to be extracted from the body [41, 86]. It may be applied as a starting point to overcome the challenges inherent in renal organoids. Second, the vasculature can be of the same species rather than xenogenic. It has been demonstrated that when an embryonic kidney is transplanted, the blood vessels on the host-side enter, and integrate with the donor kidney [87]. In xenotransplantation, HAR, in which the vascular endothelium is the mainstay, has been a challenge. As the endothelium can be theoretically composed of syngeneic tissue, it is very advantageous to avoid HAR. In addition to the nephron and blood vessels, attempts are ongoing to replace the interstitium with the same species tissue. As mentioned earlier, studies have reported that embryonic tissues themselves are less immunogenic than adult xenogenous tissues, although xenogenous components remain in the collecting ducts and some interstitium [43, 44]. The third advantage is avoidance of the off-target problem. Compared with PSCs, progenitor cells have limited differentiation potential. Differentiated renal progenitor cells normally do not stray across the blastocoel to transform into brain neurons or germ cells during kidney formation without being reprogrammed. It is necessary to avoid contamination of iPS cells in transplanted human NPCs. Secondary chimerism is also known as local chimerism [88]. Chimeric kidney generated from renal progenitor cells by local chimerism is safe in terms of off-target effect and is one method to address the ethical concerns of interspecies chimerism.

There are two models for progenitor cell replacement, viz., the DTR model, which uses diphtheria toxin as an inducer that can be used among rodents, and the diphtheria toxin fragment a model, which is a tamoxifen-driven model that can also be used for human cells. Both these systems use tissue-specific Cre mice, and we believe that they can be applied to organs other than the kidney depending on the combination of organ development niche and progenitor cells. As current removal systems take more than a day to remove progenitor cells, we anticipate that developing new models that reduce this removal time will decrease the time that transplanted cells wait outside their niche and increase the replacement efficiency.

Finally, previous attempts to generate chimeric kidneys using embryonic kidneys involved removing the embryonic kidney and transplanting the cells. However, research has also been conducted to generate organs by transplanting cells directly into the fetus without removing the kidney from the fetus. The myometrium of mice is opaque, thereby making it difficult to transplant cells into the exact region of kidney development. Therefore, by removing the myometrium and retaining only the transparent membrane, it became possible to accurately administer cells to the nephrogenic region. By combining this transplantation method with a progenitor cell replacement system, studies have demonstrated nephron generation in nephron-deficient fetuses from transplanted NPCs [89, 90]. It is still difficult to transfer the in utero-sustained organ fetus complementation to a wide range of nephrogenic zones, and the transfer technique has to be improved. Furthermore, as with blastocyst complementation, there is the problem that blood vessels remain in the xenogenous tissue.

To summarize, the regeneration of chimeric kidneys using a combination of embryonic kidneys and progenitor cells during the organogenesis stage can be

further divided into the kidney removal type (ex vivo system) and the body maintenance type (all in vivo system) due to differences in cell transplantation methods. The use of embryonic organs and progenitor cells has the advantage of being simpler and easier to perform than embryo manipulation, as well as being safer, thus making it a technology with high potential in organ regeneration.

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Chapter 14
Bioartificial Kidneys, Renal Epithelial Cell Systems, and Biomimetic Membrane Devices

Christopher J. Pino and H. David Humes

Abbreviations

AKI	Acute kidney injury
ALI	Acute lung injury
AMI	Acute myocardial infarction
ARDS	Acute respiratory distress syndrome
ARF	Acute renal failure
ATN	Acute tubular necrosis
BAK	Bioartificial kidney
BRECS	Bioartificial Renal Epithelial Cell System
CD	Cluster of differentiation
CHF	Chronic heart failure
CKD	Chronic kidney disease
CO	Cardiac output
COVID-19	Coronavirus disease 2019
CPB	Cardiopulmonary bypass
CRRT	Continuous renal replacement therapy
CRS	Cardiorenal syndrome
DAMP	Damage-associated molecular pattern molecules
ECMO	Extracorporeal membrane oxygenation

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ESRD	End stage renal disease
FDA	Food and drug administration
FiO2	Fraction of inspired oxygen
HD	Hemodialysis
HDF	Hemodiafiltration
HF	Hemofiltration
HRS	Hepatorenal syndrome
iCa	Ionized calcium
ICH	Intracerebral hemorrhage/hemorrhagic stroke
ICU	Intensive care unit
IDE	Investigational device exemption
IHC	Immunohistochemistry
IL-	Interleukin
IRB	Institutional review board
LMW	Low molecular weight
LPS	Lipopolysaccharide
MELD	Model for end-stage liver disease
MO	Monocyte
MODS	Multiple organ dysfunction syndrome
MW	Molecular weight
MWCO	Molecular weight cutoff
PAMP	Pathogen-associated molecular pattern molecules
RAD	Renal assist device
RCA	Regional citrate anticoagulation
riCA	Recommended ionized calcium
RRT	Renal replacement therapy
SAE	Serious adverse events
SCD	Selective cytopheretic device
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
SVR	Systemic vascular resistance
T2D	Type 2 diabetes
TBI	Traumatic brain injury
TNFα	Tumor necrosis factor alpha
US	United States
VFD	Ventilator-free days
WBC	White blood counts

14.1 Introduction

Several etiologies of kidney injuries may result in renal dysfunction including direct or indirect insults to the glomerulus responsible for filtration function, or renal epithelial cells responsible for many of the regulatory and secretory functions of the

kidney. Rabb et al. provides a good primer on inflammatory processes associated with acute kidney injury (AKI), including the initial response, features of acute inflammation, and reparative phases of kidney injury [1]. Other comprehensive, systematic reviews of septic shock-associated AKI discuss pathogen-associated molecular pattern molecules (PAMPs) such as endotoxin, and damage-associated molecular pattern molecules (DAMPs) such as cytokines [2–4]. AKI arising from acute renal tubule cell injury, also referred to acute tubular necrosis (ATN), may be due to direct insult from PAMPs, or through ischemic and/or nephrotoxic processes via DAMPs. Although AKI can arise from hyperinflammation, most commonly resulting from sepsis or tissue trauma, acute tubule cell injury without underlying hyperinflammation has been demonstrated to initiate and promote a systemic hyperinflammatory process that potentiates not only AKI but injury and dysfunction of other organs [5]. The resulting hyperinflammatory process is characterized by excessive activation of innate immune cells, namely neutrophils and monocytes, as well as activation of capillary microvasculature resulting in dysregulated levels of cytokines heavily weighted toward pro-inflammatory mediators. Immune dysregulation and hyperinflammatory, leukocyte-driven processes may lead to tissue damage, organ injury, and consequently, progressive organ dysfunction. Multiple organ dysfunction syndrome (MODS) may result due to inflammatory processes involving both activated leukocytes in circulation and activated endothelial cells in organ microvasculature. Poor tissue perfusion resulting from deleterious interactions of these activated cell populations has ischemic and toxic consequences, including tissue edema, systemic hypovolemia, hypotension, and cardiovascular instability a negative impact on organ function. In the kidney, sequestration and aggregation of activated neutrophils in the peritubular capillaries and tissue infiltration of these cells can lead to necrosis of renal tubule cells, which promotes AKI. Conventional therapies such as hemofiltration strategies have been used to replace the reduced or lost filtration function of the kidney, while some sorbent therapies have focused on the removal of pathogens and secondary immunological mediators such as cytokines thought to be responsible in MODS. This review centers on a review of emerging extracorporeal device treatment interventions using renal epithelial cell-based devices, bioartificial kidneys, and leukocyte processing therapies.

14.2 Overview of Conventional Devices in the Treatment of Kidney Disease: Dialysis, Filtration, and Hemopurification

The most established device-based strategies in the treatment of AKI are filtration technologies including hemodiafiltration and peritoneal dialysis, which attempt to replace lost filtration function of the kidneys. The aim of these filtration-based renal replacement therapies (RRT) is the removal of low molecular weight (LMW) uremic toxins, middle molecular weight cytokines, and other inflammatory molecules through porous membranes via diffusive (dialysis) or convective (filtration)

processes, while retaining beneficial molecules such as albumin through size exclusion. Dialysis is commonly utilized for ESRD to maximize uremic toxin clearance during intermittent treatments. For a thorough, systematic review of peritoneal dialysis in AKI, see Chionh et al. [6]. Extracorporeal blood purification using devices to capture and/or remove inflammatory mediators and both endogenous and exogenous toxins particularly during sepsis and sepsis associated AKI is an evolving area that has been comprehensively reviewed [7]. Extracorporeal cytokine removal using extracorporeal devices is a mature concept focused on the belief that reducing peak cytokine serum levels during the hyperinflammatory period would ameliorate detrimental actions of these molecules [8], potentially also altering tissue cytokine gradients and facilitating restoration of immunologic homeostasis. Many cytokines fall in a molecular weight (MW) range of 8 kDa to 70 kDa (Table 14.1), and endotoxin fragments associated with sepsis are 1 kDa to 15 kDa, which are generally removed by conventional hemodialysis (HD) and hemofiltration (HF) membranes, with a MW-cut off (MWCO) of between 45 and 65 kDa. While measurable cytokine removal is achieved using various modalities including use of high molecular weight cut-off membranes and high volume hemofiltration schemes [8], therapies solely aimed at reducing cytokine load have been largely ineffective in improving clinical outcomes [9–13]. Ineffective filtration treatments may be due to in part, failure to remove blood protein bound toxins, cytokines, and inflammatory molecules [14]. Higher MWCO membranes which

Table 14.1 Molecular weights (MW) of various PAMPs and DAMPs, potential therapeutic targets

Class	Molecule	Molecular weight (kDa) or size range (nm, μm)
PAMPs	Bacteria/LPS fragments	Various, generally 0.2–30 μm/1–15 kDa
	Viruses	Various, generally 5–100 nm
	Fungi	Various, generally 2–25 μm
	Parasites (*T. gondi*)	Various, generally 2–150 μm
DAMPs	Complement (C3a, C4a, C4b, C5a, C5b)	Various, generally 9–190 kDa
	Heat shock proteins	Various, generally 8–100 kDa
	Hyaluronic acid	Various, generally kDa to MDa
	IL-1	17–25 kDa
	IL-1β	17 kDa
	IL-4	30 kDa
	IL-6	21–26 kDa
	IL-8	8 kDa
	IL-10	17 kDa
	IL12p70	70 kDa
	MMPs	Various, generally 50–95 kDa
	Non-proteins: Nucleic acids	Various, generally kDa to MDa
	sIL-6r	55 kDa
	sTNFr-I	60 kDa
	TNF-α	17 kDa
	TNF trimer	51 kDa

may remove protein bound cytokines also result in blood protein loss [15, 16] and require relatively expensive protein administration, entailing albumin supplementation at a minimum. Additional hemopurification strategies using newly developed membranes and alternative techniques such as apheresis or selective plasma exchange have seen some utility for cytokine removal, but still have limited data regarding efficacy in sepsis associated AKI [17]. Devices such as plasma fractionators with precise MWCO along with methods such as double/cascade filtration and coupled plasma filtration adsorption have been used for very precise removal of molecules within a specific MW window [18, 19], but have not seen widespread adoption.

14.3 Introduction to Sorbents and Immunomodulatory Devices for AKI and Multiple Organ Dysfunction Syndrome (MODS)

Citing the difficulty in removing middle molecular weight PAMPs and DAMPs via filtration, the use of sorbents attempts to specifically adhere these molecular targets with high potential therapeutic impact. Use of sorbent materials in conjunction with hemofiltration or as stand-alone devices are relatively mature technologies that attempt to adhere inflammatory molecules to specialized device surfaces through adsorption or through binding via various mechanisms. Sorbent technologies to date have mainly focused on removing specific PAMPs such as endotoxin, or DAMPs including a myriad of cytokines, and are exemplified by devices such as Cytosorb®. Sorbent technologies have been well reviewed by Winchester et al. [20–22]. In brief, sorbent columns or cartridges can be packed with various media in order to adsorb specific molecular targets, many times utilizing immobilized antibodies, functionalized surfaces, or specialized porous resins for molecular interaction and capture. PAMP targets have included endotoxin, also known as lipopolysaccharide (LPS), typified by devices utilizing Polymixin B [23]. Clinical studies involving these devices have been heterogenous, lacking adequate randomization, blinding and incomplete outcomes [23]. Due to the low quality of evidence provided to date, therapeutic use of Polymyxin B-based devices may only be conditionally recommended for very high-risk patients [23]. DAMP molecules have been targeted by sorbent systems such as Cytosorb® [24], finding utility in sepsis, cardiac surgery, organ transplant, and liver failure among other contexts. Specific DAMPs targets of sorbent technologies have included β2-microglobulin, angiogenin, complement factor D, leptin, IL-1, IL-6, IL-8, IL-10, IL-13, IL-18, interferon-α, TGF-β, and TNF-α [22, 24]. However, to date, Sorbent-based blood-purifying technologies have not shown selectivity and durability in lowering blood cytokine levels nor robust efficacy to improve clinical outcomes [2]. Like many technologies in the AKI/sepsis field, additional large, randomized, controlled, clinical trials are still required to provide evidence of clear therapeutic benefit [23].

With the limitations of filtration and sorbent technologies, there is an unmet medical need to develop advanced therapies that address hyperinflammation in kidney disease. Immunomodulatory device treatments in development cover a wide range of approaches to recapitulate the immunologic functions of the kidney from bioartificial kidney and extracorporeal cell bioreactors to leukocyte processing devices. These emerging technologies, which utilize admittedly less well-understood mechanisms of action, attempt to harness the innate characteristics of cells and their cellular feedback mechanisms involved with immune system regulation in order to correct immune dysregulation. Exogenous, extracorporeal bioreactor strategies such as the renal assist device (RAD) and the bioartificial renal epithelial cell system (BRECS) employ metabolically active cells maintained in an extracorporeal circuit in order to process body fluids such as blood or plasma, to both remove inflammatory molecules and supplement beneficial molecules such as secreted factors, leveraging both anabolic and catabolic functions of exogenous renal cells. Leukocyte processing devices, such as the selective cytopheretic device (SCD), attempt to ameliorate immune dysregulation through a multifactorial approach of sequestration of activated leukocyte subpopulations including neutrophils and monocytes, while modulating the circulating leukocyte populations.

14.4 Exogenous Cell-Based Devices: Renal Assist Device (RAD), Bioartificial Kidney (BAK), and Bioartificial Renal Epithelial Cell System (BRECS)

In order to replace renal tubule immunomodulatory activity during AKI, an extracorporeal device containing human renal tubule cells, the RAD, was used in intensive care unit (ICU) patients with AKI requiring continuous RRT (CRRT). Incorporation of the bioreactor into the CRRT circuit was associated with a decrease in the high plasma levels of cytokines in these patients, amelioration of the AKI promoted hyperinflammatory condition and improved survival [25]. This cell-based strategy replaces some of the cellular functions lost or compromised during AKI by supplementing with exogenous renal cells grown in an immuno-isolating device with blood access, which allows for metabolic and secretory support from the applied cells. The premise of the RAD was based upon perceived shortcomings of established renal replacement therapies: HD, HF, HDF, etc., which are centered on small solute and toxin removal of molecules below the MWCO of porous membranes and volume control. However, these therapies fail to replace the many additional aspects of renal function outside of small toxin clearance, including the secretion of hormones: renin, prostaglandins, angiotensin, endothelin, bradykinin, erythropoietin and calcitriol, as well as ignore the important immunomodulatory role held by the kidney, which is still being elucidated. Part of the regulatory strategy in development of a fully implantable bioartificial kidney (BAK), was based on proving the safety and efficacy of extracorporeal cell-based therapy while concurrently working on the miniaturization of required technology and biocompatibility

of components [26, 27]. Different BAK technology approaches, engineering challenges, and related regulatory hurdles are presented in several full-length reviews [28–30].

In the RAD IIa clinical study, safety and initial efficacy was demonstrated in subsets of patients that were treated with a cell containing RAD over a sham device without renal cells [31]. However, in the Phase IIb RAD clinical study, additional cohorts were added, allowing the enrollment of patients being treated with regional citrate anticoagulation (RCA). Surprisingly, improved patient survival rate was demonstrated in the sham device group receiving RCA, in addition to the RAD cell treatment group, in comparison to the sham acellular device during systemic heparin anticoagulation [32]. These cohorts were scrutinized in a retrospective analysis, and the 28-day survival rate in the RCA sham group was observed to be 75% vs. 50% for the heparin anticoagulation sham group (n = 12 for each treatment arm). Similarly, at the 90-day mark, survival rate was 67% for sham device with RCA vs. 25% for sham device with heparin anticoagulation. Demographics for the two patient subsets were comparable, having similar Sequential Organ Failure Assessment (SOFA) scores (12.2 ± 0.9 vs. 13.4 ± 1.1), organ failure number (3.93 ± 0.36 vs. 4.17 ± 0.46), and identical incidence of sepsis (58%) for the heparin versus RCA sham groups, respectively [32]. This unexpected clinical result identified the potential benefit of using an acellular fiber-based device in conjunction with RCA to treat AKI. This approach later became known as selective cytopheretic device (SCD) therapy, which is detailed below.

The halted RAD clinical trial effectively stopped the development and commercialization of the RAD, but led to the development of both the acellular, SCD and a second generation cell-based device called the bioartificial renal epithelial cell system (BRECS), which was designed to be a cryopreservable bioreactor populated with allogenic renal cells for potential "off-the-shelf" use in an extracorporeal therapy for AKI. BRECS cell device recapitulated many aspects of the metabolic support of the RAD in a more practical form factor for on-demand, acute use. Unfortunately, the regulatory hurdles facing the BRECS, including requirement of an extensive Pre-Market Approval (PMA) study and expensive manufacturing requirements led to prioritize the development of the SCD, an acellular device with fewer regulatory hurdles. Review articles of the medical device development process recognize the slow clinical translation process moving from benchtop to bedside, especially so for FDA Class III devices with added regulatory requirements, which tend to take longer to develop [33, 34].

14.5 Leukocyte Processing Strategies: Selective Cytopheretic Device (SCD) in the Treatment of AKI

Mounting evidence for clinical benefit from targeting modulation of the leukocytes themselves to directly modulate hyperinflammation, utilizing innate characteristics of cellular machinery which are effectors of secondary factors (e.g., cytokines), has

arisen from serendipitous discovery of the acellular SCD during the RADIIb trial. The SCD is an extracorporeal, immunomodulatory, device containing hemocompatible fibers enclosed in a housing with inlet and outlet blood flow ports. SCD can be incorporated into a patient's established CRRT circuit (Fig. 14.1), where the blood flow is directed along the outer surface of the fibers where blood cells can interact with membrane surfaces under a low shear stress blood flow and low ionized calcium environment. When SCD were examined microscopically after patient treatment, a significant population of leukocytes were found to be adhered on to the outer membrane surface of the fibers at the interface with the blood flow path [31]. Sequestered leukocytes were dominated by cells of myeloid lineage, namely neutrophils and monocytes. Leukocyte adhesion is not routinely observed in the inner lumen of hollow fiber membranes, the blood flow path for standard hemofilters and dialyzers. This is likely due to the high shear stresses involved at the fiber interface with blood, with blood flowing at high flow rates through small-diameter hollow fibers (on the order of a hundred to hundreds of dynes/cm^2). However, in SCD, with blood flow directed along the outside surfaces of fibers, the shear forces are on the same order as capillary force (<1 dyne/cm^2), favorable dynamics for LE–material interactions such as adhesion are achieved. Some leukocytes are adhered and sequestered over long periods of time in SCD, while some leukocytes may adhere and subsequently release from SCD [35]. Capture and release after potential alteration within the SCD microenvironment and return of these cells to the patient, seems to have biofeedback implications that results in amelioration of many deleterious effects of hyperinflammation during AKI.

The precise mechanism of action of the SCD is becoming better understood and appears to be an immunomodulatory process which inhibits leukocyte activation, a critical component of the systemic inflammatory response syndrome (SIRS) leading to multi-system organ failure. The modulation of the pro-inflammatory state also

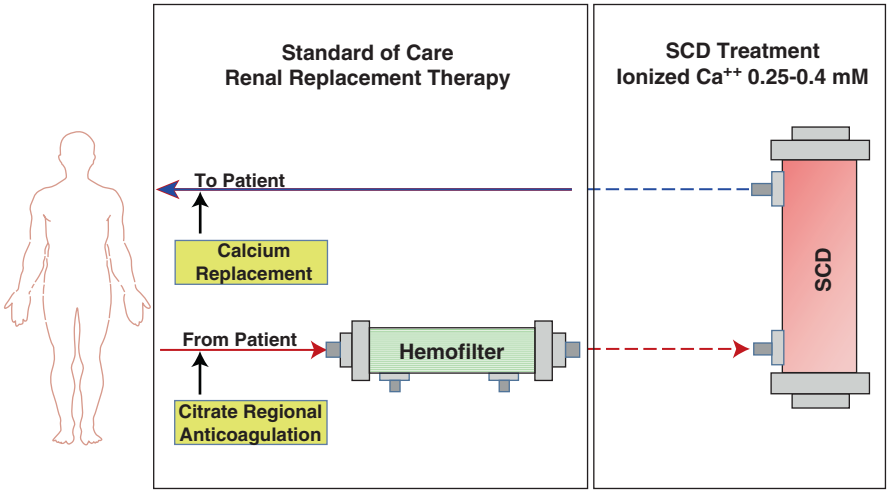

Fig. 14.1 Circuit diagram of SCD therapy incorporated into a standard RRT circuit

allows recovery of renal function in AKI and other associated organ failures. The cartridge in the presence of citrate anticoagulant acts as a selective cytopheretic device to bind and immunomodulate potentially damaging circulating leukocytes. This perspective is based upon evolving data from *in vitro* bench studies, preclinical animal models, and human clinical trials (Tables 14.2 and 14.3) utilizing measurements of biomarkers and leukocyte cell sorting by cytometric analysis.

Table 14.2 SCD clinical trial history for acute indications

Clinical trial	SCD clinical trial description	ClinicalTrials.gov identifier	Key findings
Phase I/II	AKI receiving CRRT, 15 patients (China), SCD treatment arm only [36]		No device-related serious adverse events (SAEs). Reduction in mortality: Case-matched controls was 77.78%, SCD group was 22.22% ($p < 0.027$) [36]
Phase II	FDA/IDE AKI receiving CRRT, 35 patients (USA), SCD treatment arm only [37]	NCT01072682	No device-related SAEs. Expected mortality was >50% based on contemporaneous literature review. At day 60, death from any cause was 31.4%, and all survivors did not require dialysis [37]
Phase III	FDA/IDE protocol SCD-003, AKI receiving CRRT, randomized controlled, multicenter, clinical trial, 134 patients [38]	NCT01400893	Study suspended due to injectable calcium shortage. Ad hoc analysis of SCD group with per protocol circuit recommended ionized calcium (RiCa) levels during RCA demonstrated improved 60-day mortality rate in SCD-treated subjects compared to controls treated with conventional CRRT therapy: 16% (3/19) vs. 41% (11/27). The 60-day dialysis dependency was improved with 0/16 survivors in the SCD-treated group versus 4/16 (25%) in the control non-treated group. A composite endpoint consisting of 60-day mortality or dialysis dependency between the two groups of patients was statistically significant ($p < 0.01$) [38]
Phase I/II	FDA/IDE, pediatric AKI, 16 patients, SCD treatment arm only (SCD-PED-01)	NCT02820350	Case study on the first treated pediatric patient was published [39]. 19 patients enrolled and 16 pediatric patients treated with SCD. Favorable results have been observed with 12 of the 16 SCD-treated patients surviving at 60 days. A favorable reduction compared to historical controls with mortality rates above 50%. All 12 surviving patients were dialysis independent. No SCD-related SAE were recorded [40]

(continued)

Table 14.2 (continued)

Clinical trial	SCD clinical trial description	ClinicalTrials.gov identifier	Key findings
Phase II	Up to 35 COVID-19 patients with AKI and/or ARDS will be treated with SCD, with contemporaneous control group available via CRRT.net	NCT04395911	Recently completed. Outcome measures: 1. (a) mortality at day 60. (b) dialysis dependency at day 60. (c) ventilation free survival at day 28 2. (a) dialysis dependency at day 28. (b) mortality at day 28. (c) urinary output change. (d) pO_2/FiO_2 change. (e) safety assessments including SAEs. (f) device integrity. 3–6 assessed in 10 days of treatment
Phase I/II	FDA/IDE, pediatric AKI, 10 patients, SCD treatment arm only (SCD-PED-02)	NCT04869787	On-going: 1. (a) number of SCD-related AEs. (b) number of unanticipated adverse device effects 2. (a) mortality at day 28 and 60 post treatment. (b) renal recovery: % patients free from chronic dialysis treatments at day 28 and 60. (c) hospital stay from enrollment to day 60. (d) ICU length of stay from enrollment to day 60
Phase III	FDA/IDE, adult AKI, 175 patients, randomized controlled, multicenter clinical trial, supplemental IDE approved		Not yet recruiting, supplemental IDE approved with composite endpoint consisting of 60-day mortality or dialysis dependency

The low ionized calcium (iCa) environment during regional citrate anticoagulation and the low shear stress along the blood pathway within the SCD promotes a selective binding of the most activated neutrophils and monocytes to the membranes of the device [35, 39, 41]. This selectivity is due to the calcium dependency of leukocyte binding processes. It is postulated that once bound, the activated neutrophils are promoted in the low iCa environment to transition from delayed apoptosis to an apoptotic program and released back to the systemic circulation [44–46]. The transition of these neutrophils to apoptosis and release from the SCD results in the clearance of these previously highly activated inflammatory cells via well-described pathways of phagocytosis and digestion within macrophages in the bone marrow and liver [47]. A continuous process of binding, apoptotic conversion, release, and clearance from the circulation of the most activated circulating neutrophils results in immunomodulation of the systemic inflammatory process to a less proinflammatory state [41]. For monocytes, the most activated, proinflammatory circulating monocyte pool is selectively bound to the SCD. The binding and sequestration of this monocyte subset promotes a shift of the circulating pool of the proinflammatory

Table 14.3 SCD preclinical animal disease models and related publications

Acute models		Key findings
SCD ICH	Porcine Model of Intracerebral Hemorrhage [41]	Reduction in edema and leukocyte infiltrate at site of intracerebral injection of thrombin in SCD treatment group [41]
SCD SSMOD	Porcine Model of peritoneal E. coli induced SSMOD [35]	Survival time advantage in SCD treatment group in this severe, non-survival model of sepsis, as well as hemodynamic index improvement [35]
SCD IRI	Canine Model of Ischemia Reperfusion Injury [41]	Reduction in extent of damage, edema and leukocyte infiltrate in peri-infarct zones for SCD treatment group [41]
SCD ALI	Porcine Model of Acute Lung Injury [41]	pO_2/FiO_2 ratio tended to be higher for SCD-treated group, with suggestion of fewer $CD11b^+$ ($CD11R3^+$) leukocytes in lung tissue [41]
SCD CPB(b)	Bovine Model of Cardiopulmonary Bypass [42]	Reduction in immature neutrophil influx, and maintenance of low total leukocytes, neutrophils and monocytes in circulation for the SCD group during the follow up period post-CPB [42]
SCD CPB(p)	Porcine Model of Cardiopulmonary Bypass [43]	Maintenance of low total leukocytes in circulation during the follow up period post-CPB, in the SCD-treated group [43]
SCD ARDS	Porcine Model of Acute Respiratory Distress Syndrome [41]	Reduction in resuscitation fluids and vasopressor requirements for SCD treatment group
Chronic models		
SCD T2D	Ossabaw porcine model of Metabolic Syndrome/Type 2 Diabetes [44]	Improved insulin resistance via HOMA-IR scores up to 2 weeks after SCD therapy [44]
SCD CHF	Canine Model of Chronic Heart Failure [41]	Increase in heart contractility for SCD treatment group assessed by ejection fraction [41]

monocytes to a patrolling, reparative phenotype. This shift thereby promotes immunomodulation of circulating monocytes from a degradative phenotype to a reparative, recovery subset [35, 48], enhancing tissue repair and functional recovery.

Leukocytes that adhere more avidly to the SCD appear to be more highly activated based upon analysis of the expression of the CD11b integrin on the surface of these cells. In an animal model of sepsis associated AKI, an immunomodulatory shift in the leukocyte population to a less inflammatory state is observed with SCD treatment, evidenced by reduced expression of the CD11b integrin marker on the surface of neutrophils in circulation, lower serum levels of myeloperoxidase, lower secretion of proinflammatory cytokines by isolated mononuclear cells and reduced emergence of immature neutrophils in the circulation [35]. Furthermore, in SCD-treated animals, the degree of both cardiovascular and renal dysfunction during sepsis was significantly reduced [35]. SCD therapy aims to treat AKI by ameliorating the degree of renal tubule cell injury through these immunomodulatory processes, whether the damage stems from a non-renal process (e.g., sepsis) or a renal derived hyperinflammatory condition initiated and potentiated from primary ischemic or

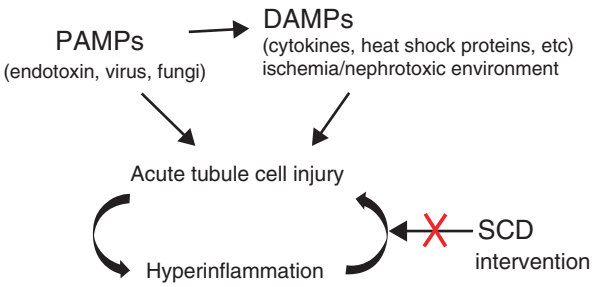

Fig. 14.2 Acute tubule cell injury promoted by any process: hyperinflammation or ischemia/nephrotoxins promote a worsening hyperinflammatory condition. SCD intervention diminishes the feedback cycle of continuing inflammatory injury to the kidney

nephrotoxic insult (Fig. 14.2). SCD intervention diminishes the feedback cycle of continuing inflammatory injury to the kidney, interrupting worsening hyperinflammation. This process elicits a systemic immunomodulatory effect different than simplistic leukocyte trapping and removal achieved by leukoreduction filters.

First in man studies for SCD were unintentionally completed during the acellular cohort testing in the RAD IIb study. However, since then, SCD has been used in several clinical studies in patients undergoing dialytic support for severe AKI as well as other acute indications (Table 14.2), including a Phase I/II study in AKI patients in China [36] and a Phase II multi-center trial in the USA [37]. Once integrated, SCD therapy is administered continuously with sequential replacement of the SCD every 24 h, and generally used for up to 7 consecutive days, but to date, has been used safely as long as 17 consecutive days. This continuous treatment is required to maintain the immunomodulation of circulating neutrophils due to the short half-life of circulating neutrophils, which is less than 24 h [49].

In a Phase III, multi-center, randomized, controlled, pivotal study to assess the safety and efficacy of SCD. In patients with AKI, the study was designed with two-arms: SCD-therapy integrated into CRRT circuits compared to contemporaneous patients being treated with CRRT alone (control). Both groups received RCA. The primary outcome measure was 60-day all-cause mortality. Secondary endpoints included RRT dependency at Day 60, and ventilator free days at Day 28. This trial enrolled 134 patients at 21 U.S centers. Each clinical site used their approved RCA protocol for anticoagulation of the extracorporeal circuit. The per protocol recommended ionized calcium (RiCa) intracircuit levels of 0.25–0.4 mm. Unfortunately, before the study had enrolled only 134 patients which was substantially less than half of the planned target, a national injectable calcium shortage occurred in the United States due to FDA concerns regarding the manufacturing procedures in the major supplier. This shortage resulted in some sites reducing the infusion rate of citrate to minimize calcium solutions to prevent hypocalcemia in patients, thereby resulting in RiCa above per protocol requirements below 0.4 mm and losing efficacy in SCD therapy. The calcium shortage was so severe that nine sites were not able to enroll due to lack of injectable calcium. Accordingly, the clinical trial was

paused, and an interim analysis undertaken on 134 enrolled patients. This analysis demonstrates no significant difference in any of the primary or secondary endpoints between SCD-treated subjects and controls. A post hoc analysis was undertaken to see if the key variable of RiCa had an impact on the endpoints of the study. Those patients whose circuit RiCa below 0.4 mm 90% of the therapy time had a substantially improved 60-day mortality rate in SCD-treated subjects compared to controls treated with conventional CRRT therapy: 16% (3/19) vs. 41% (11/27). The 60-day dialysis dependency was also improved with 0/16 survivors in the SCD-treated group versus 4/16 (25%) in the control non-treated group. A composite endpoint consisting of 60-day mortality or dialysis dependency between the two groups of patients was statistically significant ($p < 0.01$). Dialysis dependency following dialysis requiring AKI is considered a poor outcome due to the high probability of progression to end stage renal disease. In fact, large prospective trials in these types of patients have demonstrated an incidence of greater than 20% of survivors being dialysis dependent after 60 days or more of follow-up [50, 51]. In this regard, a supplemental IDE has been approved by the FDA for a follow-up on pivotal clinical trial with this composite endpoint as the primary outcome.

SCD has also been tested in pediatric patients with AKI (>15 kg, age up to 22 years) in a Phase II clinical trial. A multi-center trial of the SCD therapy to treat children with AKI and MODS receiving CRRT as part of standard of care was initiated under FDA-approved IDE G150179. In pediatric patients, mortality rates have historically approached 50% for those with AKI and MODS requiring CRRT [40, 52, 53].

The first adolescent patient treated with SCD was written up as a case study, which reports on the treatment course for an 11-year-old female with a severe reaction to propofol during an elective surgery that resulted in MODS [39]. After SCD treatment for only 24 h, improvements were seen with regard to the degree of liver injury and hematologic failure. After only 4 days of SCD therapy, lung function improved markedly, allowing for extubation. After SCD therapy for 7 days, kidney function was significantly improved. The patient was later discharged from the hospital with normal renal function [39] and did not require any follow-up dialysis treatments.

In this pediatric clinical trial, 16 pediatric patients have been treated with SCD. Favorable results have been observed with 12 of the 16 SCD treated patients surviving at 60 days. No deaths were associated with device treatment but were due to underlying illness or treatment interventions (post-op complications, extracorporeal membrane oxygenation (ECMO), viral myocarditis). All 12 surviving patients were dialysis independent. Treated patients ranged from 5 to 20 years old; admission diagnoses included: severe rhabdomyolysis (case study presented below), shigatoxin-associated hemolytic-uremic syndrome, community acquired pneumonia, multiple patients with AKI and/or septic shock. Patients generally received 3–7 days of SCD therapy, with one patient withdrawn from care after 11 h of SCD therapy. No SCD-related SAE were recorded [54]. Of importance, the FDA has recently designated the SCD as a Humanitarian Use Device with a pathway for Humanitarian Device Exemption for the treatment of pediatric patients with AKI.

In summary, the SCD has been tested in a number of clinical trials in adult patients with AKI requiring CRRT [32, 36, 37] and pediatric patients [39, 54], demonstrating an excellent safety profile with strong efficacy signals. However, despite promising preclinical data and early clinical data in pilot studies, clinical translation of the SCD has been hampered by the setback encountered during the Phase III multicenter, randomized, controlled, pivotal trial where injectable calcium shortage impacted the intended RCA protocol. This underscores an acknowledgment of the need for better designed clinical trials in the AKI/sepsis field including functional outcome measures that may be decoupled from mortality in heterogenous patient populations, and secondly, the importance of understanding the mechanism of action for proper device function, to ensure the proposed therapy may be beneficial to the sub-group of sepsis patients treated; common issues in clinical trial design in sepsis have been emphasized in reviews by Vincent et al. and Gomez and Kellum [55–57]. In the case of the SCD, maintaining a low iCa environment in the SCD therapy circuit in order to immunomodulate hyperinflammation and achieve efficacious treatment was demonstrated by mortality reduction and elimination of dialysis dependence at 60 days in the subset of patients maintaining RiCa. The combined functional outcome measure including dialysis dependence at 60 days is a more robust clinical measure and is already approved in the SCD-004 IDE protocol as an IDE supplement for Phase III trial. The immunomodulatory effect of the SCD on neutrophils and monocytes appears to have a critical role in reducing acute inflammation resulting in more rapid improvement of cardiovascular, pulmonary, and renal functions in SCD-treated multiorgan failure patients compared to controls. This effect also translates into a regulated repair and recovery of renal function as reflected in the lack of 60-day dialysis dependency in surviving patients after SCD therapy in trials to date. These clinical results, when viewed together with preclinical large animal studies (Table 14.3), reviewed more comprehensively by Pino et al. [41], suggest a beneficial treatment effect in multiple acute inflammatory conditions, as well as chronic inflammatory conditions involved with chronic organ dysfunction, suggesting utility of leukocyte processing in several other inflammatory disease indications.

14.6 Beyond AKI: Potential Impact of Immunomodulatory Devices for Other Kidney-Related Diseases

A focal non-renal inflammatory process, such as acute MI or pneumonia or pulmonary embolism, results in a localized inflammatory response but generally not a systemic inflammatory process with far-reaching sequelae. In contrast, when an AKI insult occurs, it results in loss of tubule immunoregulatory function so that a localized kidney injury develops into a systemic process due to a vicious cycle of incremental worsening inflammation. If a non-renal injury or infectious process is severe or secondary complications occur, such as developing hypotension, AKI often results. The progression to AKI requiring RRT is a reflection of failure of

standard medical care to limit a local inflammatory process such that peripheral organ injury ensues and continues propagating with extension to MODS. An immunomodulatory therapy may then be required for more complete RRT, attempting to halt the downward spiral of progressive inflammation, and worsening renal injury. Despite clinical observations that generally AKI patients have demonstrated a predisposition to go on to develop CKD, clinical trials utilizing the SCD in ICU patients with AKI have demonstrated that all survivors treated with the SCD were dialysis independent 60 days after treatment. In an Investigator-initiated trial of SCD used in ESRD patients for safety and bio-inflammatory assessment, no device-related SAEs were found due to SCD. The bio-inflammatory assay portion of testing showed SCD therapy promoted a shift in circulating monocyte population from predominantly $CD14^{hi}$ expressing MO at baseline/pre-SCD therapy to $CD14^{low}$ expressing MO post-SCD therapy [48].

Treating the hyperinflammatory process with the SCD has been demonstrated in preclinical large animal models (Table 14.3) to reduce the degree of AKI as hyperinflammation develops from various insults, including septic shock and cardiopulmonary bypass (CPB), common disorders associated with AKI [36, 43]. In CPB, leukoreduction filters have been previously utilized to help reduce cell-based hyperinflammation [58]. In SCD preclinical studies, reduction in injury biomarkers during treatment of organ dysfunction including heart and lung failure have been observed [35, 43] and have prompted several investigator initiated pilot clinical trials to assess impact of SCD therapy in patients with various types of organ dysfunction such as cardiorenal syndrome and hepatorenal syndrome. Related chronic indication clinical trials are listed herein (Table 14.4).

Recently, the FDA approved an IDE for SCD treatment of ICU patients with AKI and/or acute respiratory distress syndrome (ARDS)-associated COVID-19 infection. Evidence suggests that hyperinflammation with high concentrations of cytokines plays a critical role in the development of respiratory insufficiency and ARDS in COVID-19. SCD immunomodulatory therapy has been used in emergency/expanded use treatment of patients with refractory COVID-19 ARDS requiring ECMO. Two severely ill patients were selected for treatment based upon their declining clinical criteria and IL-6 levels greater than 100 pg/mL, a biomarker used to assess severity of hyperinflammation. In patient 1, the elevated IL-6 level before treatment was 231 pg/mL, which was reduced to 3.32 pg/mL within 52 h of SCD treatment initiation [59]. For patient 2, cytokine profile was greatly elevated with a pretreatment IL-6 level of 598 pg/mL, which was reduced to 116 pg/mL within 50 h of SCD therapy initiation [59]. Improved IL-6 levels corresponded with improvements in other inflammatory indices in both patients, including procalcitonin, D-dimers, lactate dehydrogenase, ferritin, C-reactive protein, and IL-10 [59]. Pulmonary edema was rapidly reduced, and vasopressors were discontinued within 30 h for both patients, after the start of SCD therapy. Patient 1 received a total of 17 days of SCD therapy and was taken off ECMO 20 days after initiation. Patient 2 was taken off both SCD therapy and ECMO after 16 days of therapy [59]. Both patients were subsequently extubated and discharged alive from the hospital.

Table 14.4 SCD clinical trials in chronic indications of organ failure

Clinical trial	SCD clinical trial description	ClinicalTrials.gov identifier	Key findings/outcome measure(s)
Phase I/II	Safety and bio-inflammatory assay study of SCD treatment in 15 ESRD patients. IRB approved as non-significant risk [48]		No device-related SAEs. Bio-inflammatory assay showed that SCD therapy promoted a shift in circulating monocyte population from predominantly CD14hi expressing MO at baseline/pre-SCD therapy to CD14low expressing MO post-SCD therapy [48]
Phase I/II	Myocardial stunning in ESRD patients requiring chronic hemodialysis with intradialytic hypotension or large intradialytic weight gain, recruiting	NCT03539861	1. Change in regional wall abnormalities identified on echocardiogram 2. (a) number of participants with an adverse event based on iCa measurement. (b) number of participants with an adverse event based on hemoglobin
Phase I/II	SCD treatment of patients with cardio-renal syndrome (CRS). The study will enroll eligible patients in the ICU with acute or chronic systolic heart failure and worsening renal function due to cardiorenal syndrome while awaiting LVAD implantation, recruiting	NCT03836482	1. Percent of patients with reversal of worsening renal function 2. 15 measures including urine production and urine analytes and respective clearances
Phase I/II	SCD treatment of patients with cardio-renal syndrome (CRS), no LVAD, recruiting	NCT04589065	1. Improvement in cardiac function—Left ventricular ejection fraction 2. (a) improvement in renal function by serum creatinine. (b) improvement in renal function by blood urea nitrogen
Phase I/II	SCD treatment of patients with hepatorenal syndrome (HRS), recruiting	NCT04898010	1. To evaluate the safety of daily 24-h SCD treatment in conjunction with CRRT and RCA for up to 7 days in ICU patients with AKI requiring dialysis due to HRS type 1 2. To evaluate the effect of SCD treatment to improve renal function, urine sodium excretion, and net volume removal. Liver function coagulation parameters along with MELD score will also be followed as per standard medical practice. The MELD score will be assessed before, immediately after and periodically after discontinuing therapy

14.7 Summary

Conventional filtration and sorbent therapies will continue to see utility in the management of AKI and MODS, including treating a vast number of cases that arise from sepsis, viral and toxic causes. However, emerging technologies, such as extracorporeal renal cell bioreactor-based bioartificial kidneys, and leukocyte-processing devices such as the SCD may see increased utility as they are developed further and become commercially available. Immunomodulatory therapies aim to treat AKI by reducing the degree of renal tubule cell injury, whether this damage stems from the non-renal hyperinflammation process or a renal derived hyperinflammatory condition, initiated and potentiated from primary ischemic or nephrotoxic renal tubule cell injury. Despite clinical observations that generally AKI patients have demonstrated a predisposition to go on to develop CKD, clinical trials utilizing the SCD in ICU patients with AKI have demonstrated that all survivors treated with the SCD were dialysis independent 60-days after treatment. This is in sharp contrast with the observed 25% incidence of ongoing renal support requirements in survivors receiving intensive dialytic therapy without SCD during these trials. Mechanistic details of immunomodulatory device impact on inflammatory diseases are still being fully elucidated; however, SCD has been demonstrated to impact the activity of neutrophils, which are key cellular players in acute inflammatory processes. Clinical data is mounting and suggests an effect of SCD to diminish ongoing organ injury and speed the recovery of organ function. This effect is most likely related to SCD-induced modulation of the immunologic responses controlling hyperinflammation as well as the regenerative repair processes responsible for functional recovery of organs, and specifically, kidney function. However, like many developing technologies for the treatment of AKI and sepsis, additional large, randomized, controlled, clinical trials with functional outcome measures are still required to provide evidence of clear therapeutic benefit.

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Chapter 15
Artificial Intelligence in Nephrology

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15.1 Introduction

AI plays a critical role in almost every area of our daily lives and academic disciplines; medicine is not an exception. According to one of the individuals who co-authored the document that coined the term "artificial intelligence," McCarthy [1], AI is defined as the science and engineering of creating intelligent machines that behave in a way that could be considered intelligent if it was a human being. However, the possibility of machines being able to simulate human behavior and actually think was raised earlier by Alan Turing who developed the Turing test in order to differentiate humans from machines [2, 3]. In the past decades, the growth of computing power, advances in methods and techniques, and the explosion of the amount of data hugely expanded the capacity of AI in resolving a broader spectrum of tasks.

Since Deep Blue's victory over Garry Kasparov in chess in 1997, International Business Machines (IBM) had been on the hunt for a new challenge and developed the Watson, a question-answering computer system capable of answering questions posed in natural language. A successful experience from AI/Watson in nephrology has been developed by Società Italiana di Nefrologia (SIN) in collaboration with Amgen Company (AMGEN) and IBM. It is intended to support the user in learning activity through advanced document research, and it specifically applies the latest ML Model to analyze scientific literature on calcimimetics. It has been trained on almost 100 scientific documents on calcimimetics and is periodically updated in collaboration with SIN scientific experts. Formulating the question in a proper way, it will receive an answer from Watson for Clinical Evidence in Nephrology, and

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belonging to the knowledge base domain, concerning calcimimetic drugs and their use in the treatment of secondary hyperparathyroidism in dialytic patients [4].

One of the major principles of AI is machine learning (ML), which is defined as the study of algorithms and statistical models that computer systems use to learn from sample data and past experience without being explicitly programmed to perform specific tasks. With the capacity of identifying patterns in the data, ML can be used to solve various problems, such as finding associations of two variables, classifying subjects by certain criteria, making predictions based on baseline characteristics, and recognizing objects with similar patterns. Generally, ML algorithms include support vector machine, random forest, gradient boosting trees, and artificial neural network (ANN) [5].

Two main changes have taken place in this field in the last decade. Firstly, the digitalization of medical information through the use of electronic health records (EHRs), which store patient medical reports electronically. This facilitates automatic information processing through specialized software [6]. Secondly, medical Big Data phenomenon (Fig. 15.1), which is characterized by the availability of vast

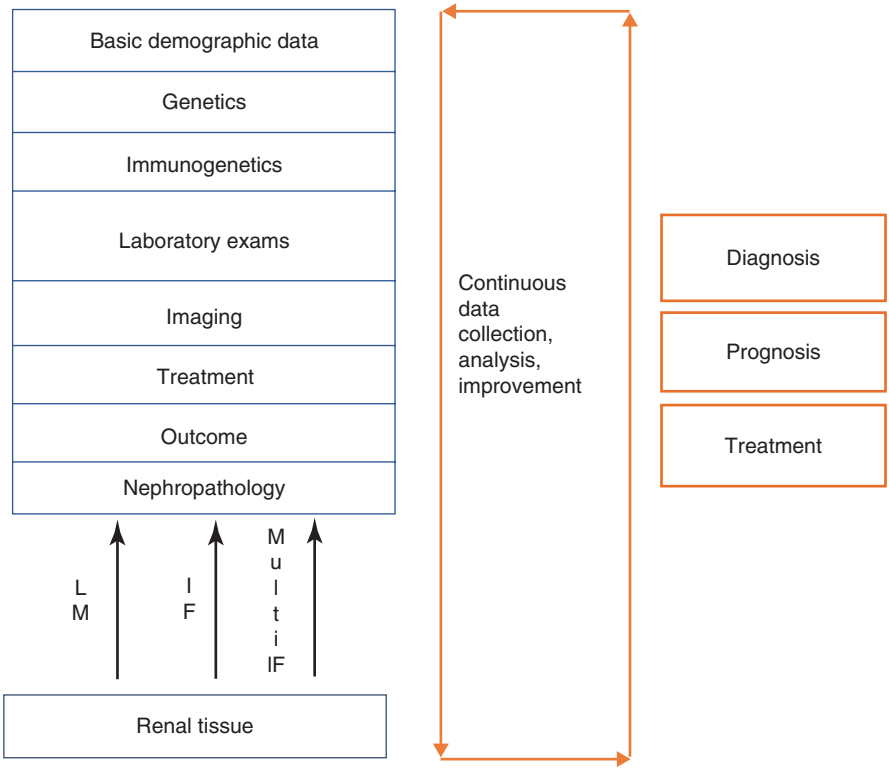

Fig. 15.1 Nephropathology in the context of other disciplines contributing to big data in precision medicine. AI can be used in all steps of tissue analysis and during the integration and analysis of data from other disciplines for the big data-based determination of diagnosis, prognosis, and treatment of an individual patient. *IF* immunofluorescence, *LM* light microscopy, *Multi-IF* multichannel immunofluorescence. (Adapted from Becker JU et al. (2015))

amounts of data in various formats, generated at an exponential speed [7, 8]. This phenomenon has affected medicine and made available vast amounts of data on various digital formats on the medical and healthcare activity of the patients who come to health centers or undergo a healthcare procedure (blood tests, X-rays, treatments, operations, organ transplants, among others) [6].

15.2 Impact of AI on Medical Reasoning (MR) and Decision-Making (DM)

Medical reasoning (MR) refers to the study of cognitive processes in medical practice, such as perception and action, understanding and problem solving, memory, and knowledge representation. There are two types of MR: Type 1 (intuitive) faster and more commonly used by specialists and Type 2 (rational), slower but more reliable, that focuses on hypotheses and deductive clinical reasoning (hypothetical ratifier–deductive). Repetition of Type 2 reasoning leads to Type 1 consolidation, meaning: as you see more clinical cases and use Type 2 reasoning effectively, you create your own disease scripts and your ability to use the Type 1 process [9].

Decision-making (DM) is the planning and making of diagnostic and therapeutic decisions by the physician, based on information processed from medical reasoning [10].

Traditional MR is based on qualifying the patient's experience by looking at a specific pathogen, prognosis, and treatment group. More than a century ago, this qualification was based almost entirely on empirical observation, that is, what we saw, what we heard, and even what our intuitive thinking conditioned based on our previous knowledge and experience.

Progressively, there was the incorporation of paraclinical tools that could help the diagnostic procedure, however, without discarding inductive thinking. Obviously, modern medicine has increasingly incorporated technology into diagnosis, often making diagnosis based on it.

However, this is essentially clinical diagnosis, and the armed propaedeutics essentially remained based on cognitive medical practice in which the human figure remains essential. And, at this moment, the recent entry of AI into the medical scene includes a completely new reality to clinical practice, as it offers a tool that is neither astute nor intuitive, but capable of analyzing with a natural language process to read the current scientific evidence, collect information from medical records, providing a diagnosis at a speed and accuracy superior to specialists in the field [11]. This impact on patient care can be measured, in the future, in evaluating the reduction of medical errors related to the cognitive biases of the human mind that, for obvious reasons, are impossible to completely eliminate.

AI can be a valuable tool to assist physicians in clinical judgment, particularly as disciplines dedicated to medical decision and problem solving are rare or non-existent during the medical course in most countries. In this way, the AI could not only guide physicians in all their clinical reasoning but also help them to be more aware of different aspects in the decision process, without interference from their cognitive and affective prejudices.

15.3 Machine Learning Techniques

These are the ML algorithms used in the analysis of survival in kidney transplant. The use of decision trees, ensemble methods, neural networks, and support vector machines is described.

15.3.1 Decision Tree

Decision trees are a segmentation method that tries to achieve a classification into homogeneous groups of the observed sample, gradually segmenting them in accordance with the variable of interest or segmentation variable. A division process is carried out in a tree form, where nodes represent the features of the sample to be classified, and each tree branch represents a possible value of that feature. A sample subject is allocated to a specific segment by selecting the features that best discriminate and by building a decision rule that makes it possible to select the best possible division at each of the division process levels [12]. This feature will define the first division of the sample into two segments. Then, the previously created segments are segmented again, and division continues successively until the process end, using a stop criterion previously established or voluntarily halting the process [6] (Fig. 15.2).

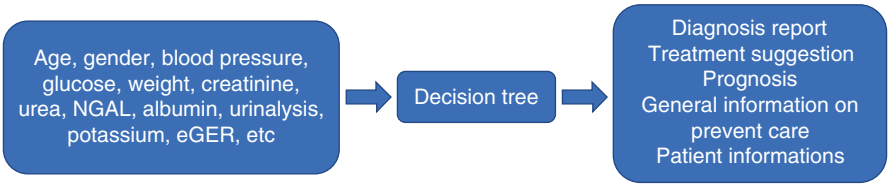

Fig. 15.2 Decision tree

15.4 Artificial Neural Networks (ANNs)

ANNs are a regression or classification model inspired in biological neural networks (NN). The structure of a neural network is composed of nodes, layers, and synapses. A node or neuron is the basic element of a computation. It receives the entry data from an external data source or another node. Every entry has an associated synaptic weight that is modified through the learning process. A neuron consists of a set of entries, a propagation rule (which determines the potential result of the neuron's interaction with its neighboring nodes), an activation function (which determines the neuron current activation status), and an exit function (which yields the neuron's exit value). These functions depend on the neural network used. The layers are structural units that group nodes. Nodes are interconnected by synapses with an associated weight (synaptic weight). The network's behavior is determined by the structure of the synaptic connections [13].

One of the NN architectures most frequently used in the study of survival analyses in the area of kidney transplants is the multilayer perceptron (MLP). MLP is a neural network that contains one or more hidden layers and uses the backpropagation (BP) algorithm to train. This type of network is used to directly model the survival function as well as to classify graft and patient survival [14, 15].

15.5 Support Vector Machines

The ML technique known as support vector machine (SVM) uses entry data represented in a space of features that can have multiple dimensions and generates an optimal function for the separation of the values of the variable to be modeled [16]. If the population is separable or quasi-separable, a linear function that can separate the data in the original space of the entry samples is used. When the population is not linearly separable, a kernel function is used to transform the original entry data and take them to a new space of features where the hyperplane can achieve linear separation of the classes in the variable to be modeled [14, 17]. The support vectors are the observations on the border of these margins [6].

15.6 Clinical Applications of ANNs in Nephrology

15.6.1 Nephropathology

A kidney biopsy contains a wealth of information from conventional staining to single or multi-channel immunohistological stains. Over the past 100 years, nephropathology has become a powerful diagnostic tool integrating these data into a structured report, indispensable for the management of most native and transplant kidney

diseases. However, even in a standardized report, many of these data are filtered and are not available for assessment [18].

Research on pathological glomerular images using ML, including the CNN, involves the detection and classification of glomeruli. As examples, Gallero et al. reported that their CNN detected glomeruli from a periodic acid−Schiff (PAS) with an F score (a measure of a test's accuracy) of 0.937, Kawazoe et al. reported that they detected glomeruli from PAS, periodic acid-methenamine-silver (PAM), and Masson trichrome, with F scores ranging from 0.876 to 0.925 using a modified CNN, Bukowy et al. reported that they constructed a CNN to detect glomeruli from trichrome stained with an average precision and recall of 96.94% and 96.79%, respectively, and Hermsen et al. constructed a CNN to segment WSIs into 11 classes such as "glomeruli" "interstitium" from the PAS-stained whole slide image (WSI), and it detected 92.7% of all glomeruli [19].

A computational system designed to assist pathologists in teaching about and researching kidney disease is PathoSpotter-K. This system is the version that was developed to detect nephrological lesions in digital images of kidneys, which uses classical image processing and pattern recognition methods to detect proliferative glomerular lesions with an accuracy of 88.3 ± 3.6%. Such performance is only achieved by similar systems if they use images of cell in contexts that are much less complex than the glomerular structure. The results indicate that the approach can be applied to the development of systems designed to train pathology students and to assist pathologists in determining large-scale clinicopathological correlations in morphological research [20].

Success examples of integrating nephropathology into big data are the risk score for IgA glomerulonephritis [21] using histopathological parameters from the Oxford Classification [22] and the iBox risk score for transplant kidney survival using Banff Lesion Scores [23]. Reproducibility of nephropathology parameters is crucial for both the development and clinical application of these tools.

In lupus nephritis (LN), nephropathology is still the standard criterion for diagnosing and the best predictor of end-stage kidney disease (ESKD) [24]. Nephropathologists are expected to assign a class from I to VI for LN, including activity and chronicity indexes [25]. These three parameters (class, activity index, and chronicity index) inform prognosis and treatment decisions [26]. Because LN has a much wider histopathological spectrum, it is not surprising that reproducibility of these key parameters is suboptimal. The interpathologist reproducibility for class I–VI is between 0.19 and 0.60, with a median of 0.33, being qualified as poor to moderate [27]. Likewise, the interpathologist reproducibility for the activity and chronicity indexes ranges from 0.4 to 0.7 [28–30]. The situation is similar in transplant nephropathology. Although educative efforts in nephropathology might be helpful, technological AI-based solutions could provide a long-term solution, delivering perfectly reproducible results.

Applications of machine learning in nephropathology have been published for automated recognition of sclerosed glomerular transections in tissue sections in

rodent and human kidneys [31, 32]. A recent publication has shown that ANNs are capable of classifying diabetic nephropathy with sufficient precision [33]. Similar pipelines have been created that enable the rapid instruction of a CNN by nephropathologists for tasks such as the segmentation of glomeruli, glomerular cell types, and cortical tubular atrophy and interstitial fibrosis in mouse and human biopsy tissue [34].

Recently, CNNs have shown better performance in fibrosis staging of native kidney biopsies than pathologists [35]. Additional training data will clearly improve the already remarkable good accuracy of 91.3% (vs. 0.5 for a coinflip for this binary classification), considering that only a fraction of the glomeruli show lesions considered as indicative of antibody-mediated rejection by a human nephropathologist. It will be interesting for nephropathologists to review the decisive image hotspots from this semisupervised training and compare them with traditional diagnostic features [18].

However, there are some obstacles in this area: integration, standardization, data availability, and annotation. Considering the relatively high number of routine paraffin stainings required for nephropathology, staining variability poses a serious problem for ML algorithms [36]. A solution to this problem is offered by color normalization techniques, whereby the color distribution of the target and the source images are matched in different ways. Another limitation is the current lack of standard data sets as well as data sets including all possible variations relevant for a certain classifier [37]. Ultimately, the effectiveness of any CNN will depend largely on data available for training. Basic autoencoders trained to identify, for example, glomeruli on HE or periodic acid–Schiff stain can be used as a starting point and re-trained with relatively little effort for other or more specific classifiers, such as the distinction between entities of glomerulonephritis [38].

The major bottleneck for the creation of suitable training sets is the time-consuming manual annotation by a highly trained nephropathologist. For specific tasks, this bottleneck could be avoided through nonexpert annotation, provided that basic features can be explained effectively through an example. Of course, the usual caveat in research about the quality of input data determining the quality of the results applies and should inform the project strategy [39].

Therefore, at minimum, a nephropathologist must integrate multimodal tissue diagnostics, which include conventional light microscopy with special stains, immunohistology for Ig and complement split products, other special immunostains [40], transmission electron microscopy, and, among others, serological, immunogenetical, and genetic data [41]. Each of these modalities poses challenges regarding standardization and reproducibility within and between laboratories, introducing additional barriers to AI integration that must be controlled. Of course, one cannot expect AI to instantly offer some miraculous full diagnostic solution, emulating a skilled nephropathologist. We rather expect a gradual introduction for selected tasks and diagnostic scenarios into routine practice [42]. Even some simple tools for, for example, automated counting and presentation of glomeruli might already be

helpful in daily practice. It is not expected that AI makes nephropathologists redundant in the foreseeable future. AI will rather transform the nephropathologist work profile, for example, offering solutions for repetitive work that requires high levels of attention [18].

15.6.2 IA in Glomerulonephritis

In 2019, Chen et al. [43] published results showing precision nephrology by developing a machine learning that aided risk prediction model for immunoglobulin A nephropathy (IgAN)–Nanjing IgAN Risk Stratification System. Compared with the previous prediction models for IgAN which used standard modeling with a small number of predefined variables, Chen et al. employed supervised machine learning methods to capture the useful information under the big data. They used the eXtreme Gradient Boosting (XGBoost), an ensemble learning method which generates a series of iteratively constructed decision trees on the previous ones, approach to learn the regularity directly from the 36 possible candidate features without presteps of feature selection. The method has both precise prediction of performance and generalization ability in various amounts of risk prediction tasks, such as achieving C statistic of 0.84 for the prediction of ESKD or a 50% reduction in estimated glomerular filtration rate within 5 years after the biopsy diagnosis. Another advantage of the XGBoost model is that it can handle the missing values automatically. Thus, the model is applicable in practice even when some of the variables are missing for a given patient (Fig. 15.3).

Based on this XGBoost system, baseline characteristics of a patient typed in the risk calculator automatically predicts the 5-year prognosis of this patient and classifies in high-, moderate-, or low-risk category. Based upon the personalized risk stratification, nephrologists are able to identify patients who suffer from a higher risk of deterioration, and thereby improve care for them by scheduling more frequent visits and monitoring their risk factors closely.

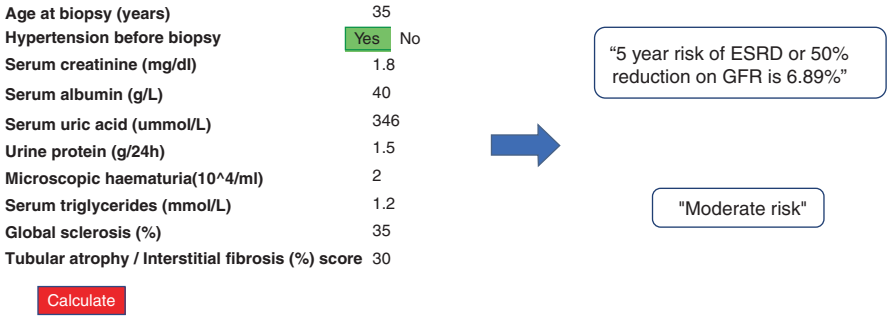

Fig. 15.3 Nanjing IgAN Risk Stratification System. *GFR* glomerular function rate, *ESRD* end-stage renal disease. (Modified from Chen et al.)

15.6.3 Kidney Transplant

Some projects are looking to solve the problem that donation and transplantation success rates have not kept up with an increasing number of donors over the years. To help improve donation success rates, a tool is needed to better predict the donation window for a potential donor so that limited healthcare resources can be used more efficiently and effectively. To help improve transplantation success rates, a tool is needed to standardize and personalize assessment of donor and organ suitability to assist with decisions about organ retrieval, as well as a tool to help organ recipients make the complicated decision of whether to accept a donor organ or wait for a better match.

In modeling the patient survival function, this approach tackles the problem of estimating the distribution function for the time between transplant and graft failure considering data censoring. Yoo et al. [44] focused on patient survival after a kidney transplant and evaluated the survival decision tree technique with respect to a decision tree and a Cox regression model. The study used as explanatory variables the 3-month serum creatinine level post-transplant, which is known to offer a high degree of discriminating capacity in the field of kidney transplants. The result obtained was that the survival decision tree offers greater predictive power than conventional decision trees and Cox regression models. The study also examined other tree-based models: bagging and RF. However, it provides no results on the performance of the models examined to compare the different techniques.

In 2019, a study titled "Predicting Deceased Donor Kidney Transplant Outcomes: Comparing KDRI/KDPI with Machine Learning," analyzed 10 years' worth of data and hundreds of variables about kidney transplant with AI. The primary outcome was to better predict outcomes of graft survival in kidney donation. The machine learning methods (MLM) were compared to kidney donor risk index (KDRI) for the ability to predict graft failure by 12, 24, and 36 months after deceased donor kidney transplantation (DDKT). The MLM model, an ensemble of thousands of randomly generated decision trees, was trained with the same data initially used to develop KDRI. The MLM trained with the readily available recipient and donor variables performs significantly better than KDRI/KDPI when predicting graft failure during the follow up after DDKT. When comparing equal prediction failure rates of 10%, MLM successfully predicted 126% more successful DDKTs than KDRI/KDPI from 1995 to 2005. Therefore, using MLM, kidneys can then be allocated more efficiently, and with less waste [45].

15.6.4 Acute Kidney Injury

AKI is diagnosed using Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines, based on serum creatinine and urine output [46]. However, since renal impairment typically precedes increases in creatinine, staging guidelines only detect AKI after renal injury or impairment has already set in [47].

There are many ways that AI is helping to move the care of patients with AKI forward. AI has been used to of state-of-the-art AKI risk scores and implement clinical decision support tools for patients at risk for or with early AKI [48].

At the Acute Disease Quality Initiative (ADQI) consensus conference in 2015, AKI was recognized as an ideal disease state to apply ML [49]. This is because AKI affects a large portion of hospitalized patients and is associated with high morbidity and mortality, making the early detection of disease extremely valuable to patient outcomes [50]. Numerous traditional risk assessment models have been created to predict AKI in various patient populations: general surgery, pediatric and adults in critical care, and iodinated contrast [47, 50, 51]. However, just because it can predict AKI risk does not mean you can prevent AKI from happening. While several risk scores have been developed and validated, there is much less published data around the implementation and pitfalls of the modern risk scores. Much of what we know about implementation has been learned from clinical trials [48].

The first study to compare logistic regression models and ML algorithms was a retrospective study by Kate et al. (2016), who analyzed EHRs of 25,521 hospital stays of elderly patients and aimed to predict within the first 24 h of admission whether a patient would develop AKI during hospitalization. This study demonstrated only modest performance in all ML models, with an area under the receiver operating characteristic curve (AUROC) ranging from 0.621–0.664, and better performance of logistic regression with an AUROC of 0.743 [52].

Overall, ML models identify patients at risk of AKI nearly a day and a half earlier than the current criterion standard (serum creatinine concentration), have high discrimination for the more severe forms of AKI (i.e., stage 3 AKI and the need for renal replacement therapy). However, model accuracy often decreases during external validation. These clinical tools are associated with early nephrology care in the setting of increased AKI risk or early AKI and is associated with improved patient outcomes [54, 55]. Selby and colleagues demonstrated that this strategy improves quality of kidney care, with shorter length of stay, improved AKI recognition, medication optimization, and fluid assessment [56].

Some ML studies on AKI prediction are summarized in Table 15.1.

15.6.5 Ethics and Safety in the Use of AI Technologies

Aside from simply being put into use with superior efficacy and performance, AI entering the field of nephrology must adapt with ethical and safety concerns. Even though machine-learning and deep-learning models always achieve high performance in retrospective studies, these models do not guarantee wide applicability in different scenarios and may be subjected to automation bias [57]. Indiscreet or overuse of AI in healthcare may lead to misleading diagnosis and treatment recommendation for patients as well as decreased vigilance and sense of responsibility of clinicians, which obviously violates the principle of “do no harm.” Privacy of

Table 15.1 ML studies on AKI prediction

Field, *n*	Design	External validation	AUROC (range)	Results	Study
To compare four ML models for AKI prediction and detection tasks. *n* = 25,521	Retrospective	No	0.621–0.664	Modest performance in all ML models to predict AKI	Kate et al. (2016) [52]
To analyze prediction of noncardiac postoperative AKI. *n* = 42,615	Retrospective	No	0.817 (0.80–0.83)	EMRs data may be used to accurately stratify patients at risk of perioperative AKI.	Lei et al. (2019) [53]
To develop a deep learning approach for the continuous risk prediction of future deterioration in patients, using AKI as an exemplar. *n* = 703,782	Retrospective	Yes	91.8 (91.6–92.1)	This model predicts 55.8% of all inpatient episodes of AKI, and 90.2% of all AKI that required subsequent administration of RRT, with a lead time of up to 48 h and a ratio of 2 false alerts for every true alert	Tomasev et al. (2019) [54]
To analysis the accuracy of a single-center ML algorithm for predicting AKI when internally and externally tested. *n* = 495,971	Retrospective	Yes	0.86 (0.86–0.86)	Predicted at least stage 2 AKI in the next 48 h (AUC = 0.86 and receipt of RRT within 48 h (AUC = 0.96)	Churpek et al. (2020) [55]
AKI in pediatric critical care patients. *n* = 16,863	Retrospective	No	0.89 (0.88–0.090)	The model predicted 70% of subsequent RRT episodes, 58% of stage 2/3 episodes, and 41% of any AKI episodes	Dong et al. (2021) [47]
Development and external validation of an AKI risk score for use in the general population. *n* = 273,450	Retrospective	Yes	0.80 (0.80–0.81)–the development cohort 0.71 (0.70–0.72)–UK external validation cohort 0.76 (0.75–0.76)–Canadian validation cohort	A risk score including four variables (older age, lower baseline eGFR, diabetes and HR) had good predictive performance, with a C-statistic of 0.80 in the development cohort and 0.71 UK external validation cohort and 0.76 in the Canadian validation cohort	

ML machine learning, *AKI* acute kidney injury, *AUROC* area under the receiver operating characteristic curve, *EMR* electronic medical records, *eGFR* estimated glomerular filtration rate, *HF* heart failure, *UK* United Kingdom, *ML* machine learning, *AKI* acute kidney injury, *RRT* renal replacement therapy

patients, data security, and data ownership are other major issues, given that current laws and regulations are insufficient to address the issues [5].

Privacy of patients, data security, and data ownership are other major issues, given that current laws and regulations are insufficient to address the issues [58].

About nephrology jobs, it will be expected that nephropathologists will have to acquire skills to manage the information derived from AI applications during the diagnostic workup, which will require a new, different set of skills. Therefore, the claim that medical professionals supported by machine learning could lose skills reflects only the negative part of the constant advancement of medicine. Indeed, we urge more optimism and point out that so far the gains through new technologies have been far greater than the loss of human skills they replaced. Understanding of the functions, benefits, and limitations of AI and its classifiers will become crucial for nephropathologists. Moreover, procedures and rules will have to be devised and implemented to identify uncertainty and override questionable AI outputs when deemed necessary by the human nephropathologist who will ultimately be held accountable. To this end, AI developers must ensure that the decision-making process remains transparent. AI applications should complement traditional nephropathology in the hand of nephropathologists, eliminating human flaws and weaknesses. They should not be expected to deliver a substitute for nephropathology or skilled nephropathologists.

Therefore, an international consensus on ethics and safety use of AI in nephrology needs to be built by the whole community.

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Chapter 16
PathoSpotter: Computational Intelligence Applied to Nephropathology

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16.1 Introduction

Kidney is among the human organs with the most clearly demarcated compartments at the histological level of glomeruli, tubules, interstitium, and blood vessels (arteries and veins). These structures are organized in functional units, called nephrons, based on the flow of the blood through afferent arterioles, glomeruli, efferent arterioles, and the flow of the liquid ultra-filtrated from the blood, through the tubules (proximal convoluted tubule, Henle's loop, and distal convoluted tubule). Diseases affecting any of these compartments progressively extend to the other structures. Most of the known nephrologic diseases affect primarily the glomeruli with inflammation, fibrosis, anomalous deposits, or degenerative changes. A

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continuous effort has been made by pathologists toward establishing a consensus on histological markers to be used in renal disease classification and to predict the outcome of renal diseases. Although most of these markers have shown to be useful in defining diagnosis and prognosis of renal diseases, the criteria adopted to define these markers are time consuming, labor intensive, and sometimes have poor agreement among pathologists. Automatizing the process of renal disease classification would contribute to improve the accuracy and reduce the time spent with this process.

The dissemination of digital image acquisition systems, for collecting either snapshot images or large whole slide image (WSI), opened possibilities to facilitate the transit of the information about the biopsies among pathologists. In this sense, a new field of research has arisen, which was coined as digital pathology. With advances of hardware and software to digitize WSIs, large histological image libraries emerge to improve diagnostic performance and the course of consensus. One perceived soon that the information contained in these images would remain largely unexplored without a proper effort to automatize the analysis of the histological images. Such large collection of digital histological images, gathered in different laboratories with different hardware devices, provided resources for supervised learning, directly considering the training of increasingly accurate intelligent systems able to recognize lesions in WSIs. This made possible the emergence of a new area of research called computational pathology. Computational pathology systems rely on the extraction and recognition of visual patterns from images and are used in multiple tasks. In the nephrology context, intelligent systems are applied, for instance, in the segmentation of renal structures and lesion classification.

16.2 Digital × Computational Pathology

The use of computers as a tool for assisting in cell analysis is not new. Tolles [1] introduced some basic tools and techniques for image acquisition and image analysis. Henceforth an expressive evolution of computational techniques for this field has driven the rise of **digital pathology**, which is a broad term that encompasses tools and systems to digitize pathology slides and associated metadata, their storage, review, analysis, and infrastructure [2].

The increasing application of artificial intelligence (AI) and machine learning (ML) techniques for image analysis in digital pathology drove the emergence of a new field called **computational pathology**, which guides its efforts to the pattern recognition from digitized histology images and their associated meta-data. Although in the pathology community there are no formal definitions neither for digital pathology, nor for computational pathology, we could define the main tasks for the first as to acquire, process, storage, and distribute digital histology images,

while for the second as to analyze digital histology images with the purpose of recognizing image patters and ultimately aid pathologist with the diagnosis. From the information technology perspective, digital pathology infrastructure and methods are related to the quality of acquired images, the reliability of the stored images, and the image distribution and security. Besides, methods for computational pathology are related to the detection, segmentation, labeling, retrieval, and classification of structures presented in the images.

Figure 16.1 illustrates the digital and computational pathology procedures and tasks. Activities in digital pathology encompass the tissue sampling and slide preparation, which is usually followed by scanning to turn them into high-resolution images that must be stored in secure repositories. Complementarily, the computational pathology exploits the image collections to perform the many artificial intelligence tasks for medical decision support. A common method relies on visual feature extraction and representation through image descriptors.

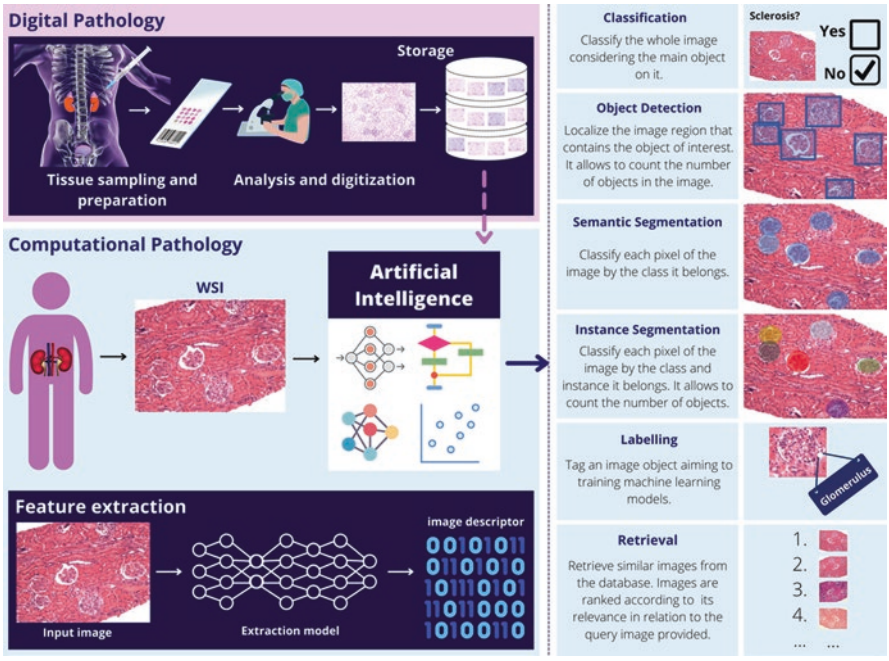

Fig. 16.1 Digital and computational pathology procedures. Digital pathology is a broad term that encompasses tools and systems to digitize pathology slides and associated meta-data, their storage, review, analysis, and infrastructure. Computational pathology guides its efforts to the pattern recognition from digitized histology images and their associated meta-data across tasks of image classification, object detection, image and per-instance segmentation, labeling and retrieval

16.3 How Do Computers Find Patterns in Histology Images?

The importance of finding patterns in several human activities has stimulated the development of computational systems that have the goal of automatizing tasks, ultimately reducing classification errors prone to be achieved when done exclusively by humans.

Computational techniques have been a commonplace to aid in diagnoses based on radiological images [3–5] and dermatology [6–8], while the use of such techniques on biopsies is rapidly increasing, particularly in nephropathology [9–14].

A computational system used for histology image classification is usually built upon the pipeline depicted in Fig. 16.1. Although image acquisition takes place via any proper device to the considered task, the pre-processing stage is specifically responsible to make the image appropriately uniform to the next stage, where features sufficiently representative to help the image classification task are extracted. Broadly speaking, image classification should be understood here either as object classification where an image is labeled to pertain to a specific class, or as semantic or per-instance segmentation where a pixel or a set of pixels are classified to belong to a specific class, respectively. Each stage presents its own set of challenges to be overcome, and classification error may occur individually or as a result of interaction among all phases.

In computational nephropathology, the acquisition phase captures an entire slide that is converted into a digital image using some type of optical-electronic transductor. Aspects such as image resolution (number of pixels), noise filtering, scale adjustments, and color spectrum are strongly influenced by the quality of the lamp used to light the slide, as well as the accuracy of the transductor. The result of this phase is a digital colored image composed by the combination of three channels (R-red, G-green and B-blue), computationally represented as a matrix of integer numbers associated to the color intensity at each image point (pixel), which was captured by the transductor. Once the image has been digitized, the next step is to guarantee image uniformity regarded lighting, bright, and other essential aspects inherited to histology images. Image classification can be regarded to the level of a pixel, a set of pixels, or an object. The first two are related to semantic or instance segmentation, respectively, while the last one is the traditional object classification where a box is labeled as a specific known class. Image segmentation is the process of partitioning a digital image into multiple segments in order to separate the parts of interest from all other parts. For the sake of computer algorithms, image segmentation is the process of assigning a label to every pixel in an image, such that pixels with the same label share certain characteristics. Figure 16.2 illustrates an example of how image segmentation works over a cropped image containing a glomerulus. Feature extraction and image classification are very interdependent and critical to the overall quality of the system. Given a specific image classification problem, the most difficult challenge is accurately selecting and extracting the best features, and then choosing a classification supervised model that is capable of correctly separating the classes. Due to the complexity in translating which features are the most

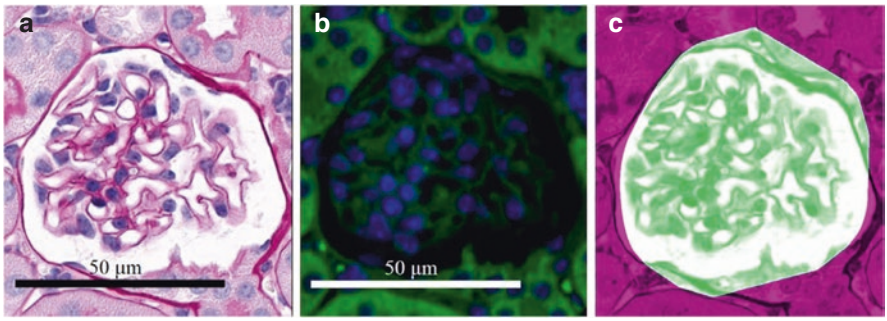

Fig. 16.2 (**a**) Original image, (**b**) preprocessed image, (**c**) segmented image containing a glomerulus

appropriate and how they best represent the object of interest in computer language is that the whole task is challenging. Several algorithms have been developed to extract features based on color, edges, corners, shape, and textures when analyzing images with different performance levels.

To be able to make predictions, ML-based algorithms must first rely on data in order to build a mathematical model [15]. This stage is called training, which is by itself a complex, time-consuming task, as several models and parameters must be tuned to reach the best classifier. Each model built must be "trained" with a sample data set in order to "learn" how to classify samples with an acceptable degree of error, in the prediction phase. For each context, a specific set of features will be relevant, and a certain model of the classifier will yield optimal results when analyzing these features. Since there is no way to a priori determine either the best feature set or the best classifier model, for many years, computer scientists struggled with the development of mathematical functions that could represent images as features in order to obtain the best result possible in the classification stage.

For several years, computer scientists attempted to tackle those limitations until a major breakthrough occurred in the field of the computer vision: The use of convolutional neural networks (CNN) to automatize the feature extraction process during the training stage. A CNN is a combination of convolutional filters designed to achieve trainable feature extractors, followed by a fully connected artificial neural network (ANN) to perform image classification. Figure 16.3 illustrates an earlier form of a CNN architecture, which is comprised of a backbone and a top layer, the latter formed by a multi-layer perceptron (MLP). In the 1990s, this architecture was shown to work well for recognizing hand-written digits [16]. In recent years, thanks to the increasing use of graphics processing units (GPU) to speed up computational processes, and the rise of large image data sets [17], it has been possible to develop larger, more complex computational vision models based on CNNs. These models favor the creation of a new ML approach, called deep learning (DL) [18], which led to the widespread use of deeper networks [2] in computer vision, mainly after the success of the AlexNet model [19], which won the 2012 edition of ImageNet Large Scale Visual Recognition Challenge [20].

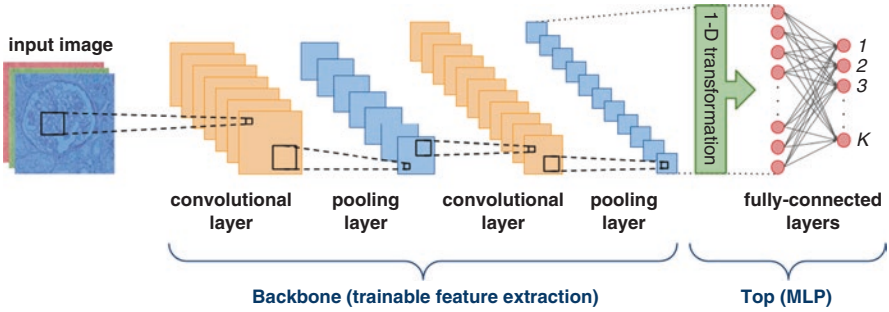

Fig. 16.3 An example of an earlier CNN architicture [16] that is comprised of a backbone and a top layer. The backbone is formed by convolutional layers followed by pooling layer, this latter is in charge of dimensionality reduction and consequently control of the parameter degree-of-freedom in the process of training. The top layer is usually laid out by a multilayer perceptron (MLP). Before feeding the fully connected (FC) MLP, data from backbone is vectorized through 1D transformations; after passing through the MLP, K neurons are outputted according to the number of classes to be classified

In computational pathology, the conception of CNN architectures in association with the increasing availability of digital biopsy data sets, stimulated by the adoption of slide scanners, has created momentum in the development of computational systems capable of assisting pathologists. A very useful feature of CNN is the possibility of transfer learning [21]. In this technique, a CNN is initially trained with as large data set as possible, enabling it to adjust its filters to correctly extract the best features from images. Then, using the previously trained feature extractor, a new data set of interest (usually with a few number of training image samples) is submitted to the network, which will then quickly converge on the classification. Transfer learning has proven to be a useful technique for developing effective CNN models even using small training data sets.

There has been a steady increase in the use of computational image classification systems in computational pathology, mainly in the detection of cellular lesions to assist in diagnosis [22–24]. Every day new techniques and methods are being developed to improve the quality of these systems. We believe that in the near future, computational systems capable of performing automatic image analysis will become an important tool for pathologists, and these will foster significant evolution in evidence-based medicine.

16.4 The PathoSpotter Project

Histological images bear a variety of information about patient disease. Frequently, patients with similar forms of clinical disease have different histological presentations and differing prognoses (see Fig. 16.4). Take, for example, a situation frequently faced by pediatricians: A child with high proteinuria leading to

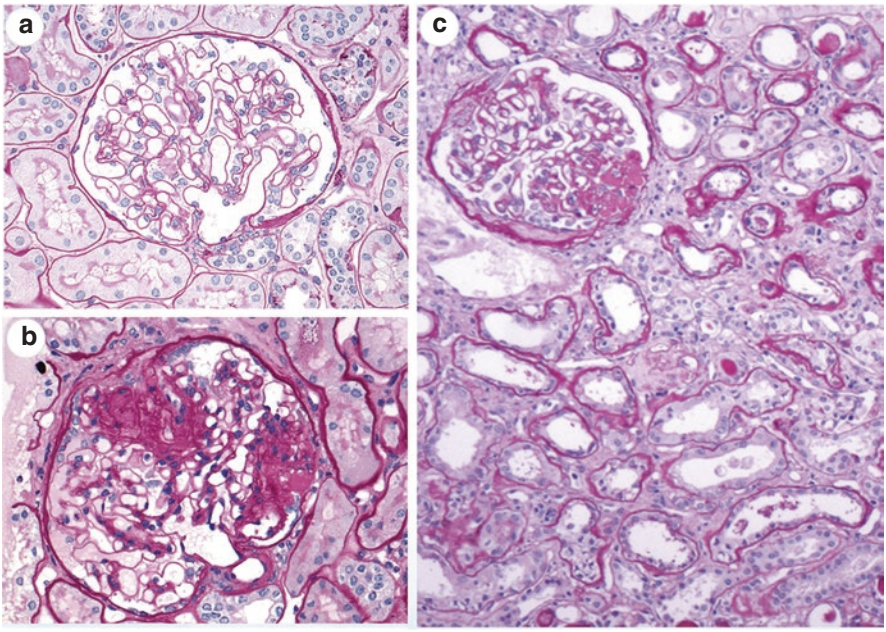

Fig. 16.4 Examples of glomeruli with lesions. (**a**) Minimum change disease, (**b**) glomerular sclerosis, (**c**) diffuse interstitial

hypoalbuminemia, edema and dyslipidemia presents a condition known as nephrotic syndrome. If the renal biopsy of this patient reveals normal glomeruli, as shown in Fig. 16.4a, the pathologist knows that child has a benign disease and will completely recover under treatment with corticosteroids [25]. However, if a child with similar clinical presentation shows scars in the glomeruli, as seen in Fig. 16.4b, this patient has a progressive disease that will not respond to corticosteroid treatment [26]. Then histological lesions contain information that is relevant to defining a diagnosis and, consequently, to disease prognosis. Fig. 16.4c depicts a kidney with diffuse interstitial widening due to fibrosis, as well as tubular atrophy. Interstitial fibrosis and tubular atrophy are considered among the most relevant histological markers of renal disease chronicity [27]. Histological images also contain information regarding the activity, chronicity, and progression of diseases [25].

Although one is able to recognize many signs that predict the course of disease, pathologists are constantly revising the histological criteria used to define disease activity, chronicity, progression, and even diagnosis [28]. It is not uncommon that pathologists find variations in the characteristics of ordinary lesions in their day-to-day practice or encounter lesions that are rare or of unknown relevance [9].[1] Due to the relevance of images used in anatomopathological diagnoses, pathologists typically will maintain records of the images of lesions received in their routines. The

[1] https://pathospotter.bahia.fiocruz.br

emergence of digital image collection and storage has allowed for the creation of huge data sets consisting of histology images. Despite that, no tools have been fully developed that permit the exploration of the information contained in these images. Accordingly, the elaboration of a system capable of automatically detecting and comparing histological lesions would be of great interest and could be used to:

- Support pathologists in their routines, mainly those residing in remote areas with limited access to specialists in different areas of anatomical pathology;
- Teach young pathologists by providing access to a variety of images of the same histological lesion across different cases;
- Perform large-scale clinical-pathological correlations, accelerating research into new treatments and the definition of consensuses.

Hence several research groups around the world have been working in the development of intelligent systems for automatic classification of histological lesions of breast, prostate, skin, and other organs using different approaches [3, 10, 23].[2] In 2014, a group of pathologists and computer science experts started the PathoSpotter project [3] in an effort to build a system to perform automatic identification of histological lesions. The most significant developments produced by PathoSpotter to date have been in the area of renal pathology, due to the availability of a robust digital image library of histological renal lesions. This library, stored at the Gonçalo Moniz Institute of the Oswaldo Cruz Foundation, [4] began in 1997 and contains all biopsies received for diagnosis from all public referral nephrology services in the municipality of Salvador (Bahia, Brazil). The digital image library was built by Dr. Washington LC dos-Santos. Unlike most of the data sets built for experimental research, this library possesses a diversity that likely reflects that found in everyday life of pathologists. The library contains more than 110,000 images of more than 3000 biopsies, mostly of native kidneys stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), periodic acid-methenamine silver stain (PAMS), Mallory's trichrome (AZAN) or picrosirius red stain, as well as immunofluorescence for IgA, IgG, IgM, kappa and lambda chains of immunoglobulins, C1q, C3, and fibrinogen, in addition to images obtained by transmission electron microscopy. Lesions are the main focus of the images, which employ different magnifications. These images were generated using at least five different digital image capture systems. Most are in JPEG format with a resolution of 1024 × 768 pixels. Additionally, the library contains 400 WSIs. The clinical characteristics of the patients from whom the biopsies originated have been previously reported [29]. Most patients were adults, with similar male vs. female distribution, and 50% had nephrotic syndrome. The main biopsy diagnoses were focal and segmental glomerulosclerosis, lupus nephritis, and membranous glomerulopathy. The library also contains about 2000 images donated by colleagues from four other laboratories at the Federal Universities of Piaui (Teresina, Brazil) and Minas Gerais (Belo Horizonte, Brazil),

[2] The Brazilian Health Ministry Research Agency. https://portal.fiocruz.br/en/

the Kidney Hospital of the Federal University of São Paulo (São Paulo, Brazil), and Imagepat, a private service in Salvador, Bahia.

16.4.1 A Map of Kidney Histological Lesions

As mentioned before, histological images bear a variety of information about the patient's disease. Having a way of organizing and visualizing the images under different points of view is essential for pathologists and computer science specialists to glance through the histological patterns of renal diseases and to grasp the distinctive presentation of the same category of the lesion in different diseases. To solve this problem, we developed an interactive web-based visualization tool that accesses a database of images and displays the information in a circular hierarchical form (see Fig. 16.5).

The compact visualization of the hierarchy is built on the fly based on the available information for each image: Staining, nosological diagnosis, pathological anatomical diagnosis, renal compartment, and morphological changes. By using this tool, the pathologists can navigate the hierarchy, including zooming and panning, collapse and expand branches, search for a specific keyword, and change the hierarchy order. Additionally, the pathologists can see and change the details of each image displayed on the right panel. This flexibility allows the pathologists to reorganize the images based on different aspects and explore how the images are related.

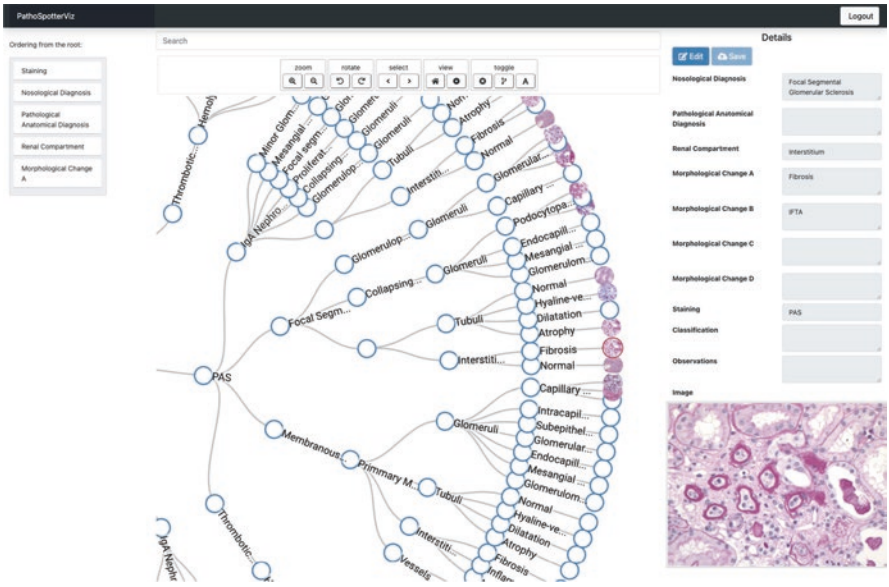

Fig. 16.5 Visualizing kidney histological lesions as a tree

16.4.2 A Content-Based Image Retrieval System

Anatomopathological diagnosis is performed through visual findings of lesions in WSIs. A type of lesion can emerge with variations in visual presentation, which are diagnostically relevant. Quite often, the pathologists need to find previous cases that are similar to the one under analysis. This process is usually conducted by manually inspecting the previous cases to search for similar lesion variations, which is a laborious, time-consuming task, especially considering large image repositories. Therefore a system capable of automatically retrieving histological images that present similar characteristics to a target image is of great importance for the refinement of the anatomopathological diagnosis and for the research of histological markers for diagnostic purposes. In computer science, such task is known as content-based image retrieval (CBIR). A typical CBIR system performs two primary tasks: (a) extraction of visual features for the representation of the images and (b) computation of the similarity between the query image and the other images in the database using their feature descriptors, which is followed by ranking them accordingly (Fig. 16.6).

The effectiveness of a CBIR system strongly bears in the quality of the features adopted to describe the images [30]. To select and extract representative features, traditional CBIR systems have to deal with several specificities of image objects, such as scale and rotation variances. Some algorithms like SIFT [31], SURF [32], and HOG [33] have been traditionally used to deal with these issues. For medical purposes, the development of effective feature extractors has been considered a challenging problem [4, 34].

The evolution of the CNN-based architectures facilitated the feature extraction by automatizing the learning and extraction of invariant features [35], while allowing its use as feature extractors for CBIR systems [34, 36–39], including some works in histopathology [40, 41].

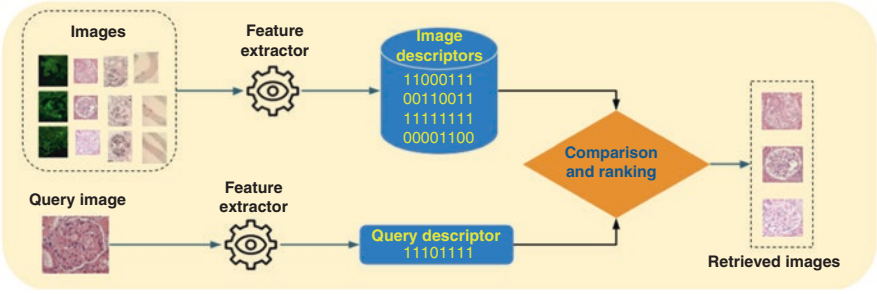

Fig. 16.6 Typical CBIR system. A feature extraction method creates image descriptors that are stored in a database. Such descriptors allow comparison and indexing of th images. When a query image is submitted, a descriptor is built and compared to the stored ones. Finally, the images are ranked according to a similarity score and the most similar are presented to user

In CBIR systems applied in nephropathology, the images may be indexed by their visual content (color, texture, shape) and by metadata representative of the histopathological findings (characterization of renal structures). Both information can be used for image indexing and retrieval. Although many systems used in medical practice are capable of comparing images based on their global appearance, subsequently list them according to some similarity score, they are not capable of finding relevant images based on the particular features presented in specific nephrological findings. A system able to perform such specialized retrieval depends on the development of methods for extracting specific discriminative visual patterns in images from renal biopsies.

The PathoSpotter Search[3] service is the first initiative for a CBIR system devoted to nephropathology. It uses a feature extraction algorithm based on CNNs (trained with the nephropathology images) to compute visual descriptors for the set of images that will be used as the retrieval repository and save them in a database for future querying. To search for images in this set that are similar to a query image, the pathologist submits this query image to the system and select the database to be used for retrieval. The system uses the same feature extraction algorithm to extract a descriptor for the query image and compare such descriptors to all descriptors in the database. Each comparison yields a number that express the similarity between the query image and the compared image (similarity score). Finally, the system ranks the images in the database according this similarity score and shows the ones that present the higher similarity score with the query image.

16.4.3 How PathoSpotter Finds Patterns in Histology Images

Detection of renal lesions in histology images is the ultimate goal of nephropathology. When considering the analyzed renal structure along with the clinical data, the pathologist and the nephrologist are capable to provide a nosological diagnosis as accurate as possible. Likewise, the main goal of PathoSpotter is to pursue precision in the detection of lesions over biopsy images by using ML and computer vision techniques. The PathoSpotter system is fed with WSIs or cropped images, and to accomplish that goal, our team has been working with two fundamental tasks: image classification and segmentation. We consider segmentation as pixel classification when dealing with the semantic or the per-instance forms of this task. Here however we differentiate both tasks as to label an object (considering a bounding box) or to label a pixel, respectively, inside the specific concepts of object classification and image segmentation.

The first classification system developed in PathoSpotter was based on handcrafted features and a K-nearest neighborhood classifier to label images of no-lesion or with hypercellularity glomerulus [9]. The input is a cropped image containing a glomerulus, and the data set was comprised of digitized images fixed in formalin (to

[3] https://pathospotter.bahia.fiocruz.br/pathospottersearch

preserve their histological structure), embedded in paraffin, cut into 2–3 μm thick sections, and finally stained using one of the following techniques: H&E, PAS, PAMS, AZAN, or picrosirius red stain. In total, 811 images were used, considering 300 images of no-lesion (normal) glomeruli and 511 images of glomeruli from kidneys with hypercelullarity. A binary classification was performed, yielding 88% of accuracy in small image samples used to assess the performance of the system.

Lately an evolved system was conceived, [11] grounded on an own CNN architecture and support vector machine (SVM). As mentioned before, CNNs provide features that are found in the process of training the classifier. After training, these features are appropriated to be used in any other classifier rather than the MLP on top of the CNN; in our case, an SVM was used. Over the same data set used in the work of Barros et al. [9], using a tenfold cross-validation evaluation procedure, this new classification method found near perfect results in hypercellularity/normal binary problem. Considering four classes—endocapillary, mesangial, both, and normal–this new classifier achieved 82% of mean F1-score and accuracy.

CNN-based classifiers commonly output the probability of classifying an object via a softmax function placed in the last layer. The softmax function assures that the scores of all classes sum up to 1, then guaranteeing that the highest score is given to the "most probable" class. Since these softmax scores are also nonnegative, by definition, we can say they represent probabilities, although these cannot be interpreted as reliable confidence scores. Hein et al. [42] showed that CNNs almost always return high confidence predictions even for inputs far away from the training distribution. Since the scores tend to be high regardless of the input, they are not a suitable measure of uncertainty.

An uncertainty metric should indicate whether the model is "certain" or "uncertain" about its prediction, being mostly useful for high-risk applications such as autonomous driving or computer-aided diagnoses (CAD). Ideally, when evaluating a model, the incorrect predictions should have higher uncertainty scores than correct predictions. This way, when a model is uncertain about a given image, we can consider that the prediction is probably wrong. As the classification cannot be fully trusted, the sample should be properly assessed by specialists. Recently research on uncertainty estimation is gaining more relevance [43]. Specifically for CAD applications, Begoli et al. [44] highlight the need for uncertainty measurements in machine-assisted, medical systems. Indeed several works studied how uncertainty can be applied in medical imaging classification approaches [45, 46] even for nephropathology [47].

Diving into this issue, Chagas et al. [12] proposed to evolve the PathoSpotter classification system by combining CNN architectures with an uncertainty estimation method for membranous nephropathy classification. Besides achieving competitive classification results (average F1-score of 93.6%), their uncertainty scores showed high relation with correctness on predictions (higher uncertainty scores represented mostly incorrect predictions). For practical applications, the uncertainty score works as additional support information for the pathologist. If a model returns results with high uncertainty, the pathologist can reevaluate, ignore the prediction, or mark the image for further inspection.

Starting from the uncertainty estimation, Chagas et al. [13] also increased PathoSpotter potential by tackling the problem of out-of-distribution (OOD) detection for membranous nephropathy classification. OOD detection aims to determine whether the input image belongs or not to the training distribution. Considering the overconfidence problem of neural networks, it might be troublesome to analyze the output of a model when the target image belongs to a different domain (e.g., a non-glomerulus image) or to an unknown or novel class of the same domain (e.g., a glomerulus with an unknown or novel lesion). This OOD detection was based on an unbounded open-set setup, i.e., when there are no constraints to the unknown classes (novel glomerular classes and non-glomerular classes are considered OOD in the same way) [48]. OOD detection ensures the safety and robustness of the evaluation pipeline, as OOD samples might represent an outlier or noisy data.

A whole pipeline can be depicted considering uncertainty estimation and OOD detection, as Fig. 16.7 suggests. An ideal pipeline is based on a model that performs class prediction, uncertainty score, and OOD detection. Figure 16.7 details how we propose using these model outcomes. Firstly, one must determine whether the input image is an OOD sample or not. Depending on the OOD method and implementation, this step could be achieved automatically or via threshold. If the image is classified as OOD data, the sample could represent a non-glomerulus or a novel class, thus should be assessed in another pipeline defined by specialists. We propose discarding the image, reevaluate it, or mark it for further analysis, but this phase depends on the application and specialists involved. Alternatively, if the sample is not classified as OOD data, one must determine how reliable the class prediction is. With the uncertainty score, we can define (automatically or via threshold) whether the model is "certain" or "uncertain" about the class prediction. If the model is "uncertain," the sample should be investigated, using an assessment similar to the OOD case. If the model is "certain," we can trust the class prediction and use it as our final classification label. Knowing when the model "does not know" is of

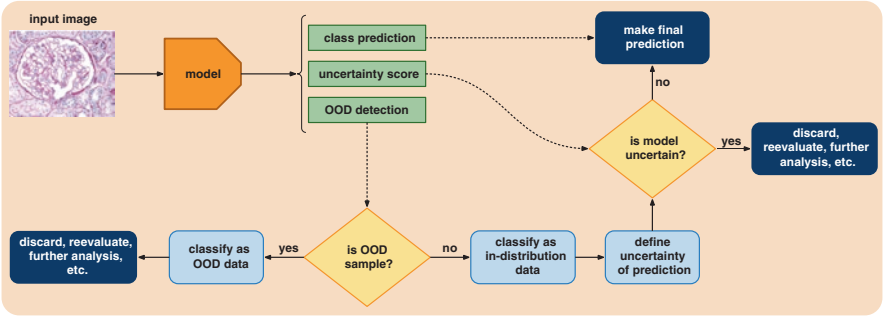

Fig. 16.7 Uncertainty-aware open-set classification pipeline proposal. Instead of predicting a single label, the model performs class prediction, uncertainty score, and OOD detection. Each model outcome is used as input (represented by dashed lines) for other steps. Depending on whether the input image is an OOD data and whether the model is "uncertain," a different end for the pipeline can be reached (represented by dark blue boxes): Discarding the image or further assessment or using regular class prediction for glomerular classification

underlying importance because not only the image might not be related to the data domain, but also the model could return an overconfidence score for any given image. Regardless, in a safe evaluation pipeline, the pathologists should be aware of when any of those situations occur.

In the classification works by Barros et al. [9] and Chagas et al. [11], the glomeruli were cut manually by the pathologist, making the classification task still with some degree of human interference. Thus, with the aim of improving the injury classification capabilities of the PathoSpotter system, Rehem et al. [14] propose a glomerulus detection method on renal histology images. For that, we evaluated two state-of-the-art deep-learning techniques: single-shot multi-box detector with InceptionV2 (SI2) and faster region-based convolutional neural network with InceptionV2 (FRI2). As a result, we reached 0.88 of mAP and 0.94 of F1-score, when using SI2, and 0.87 of mAP and 0.97 of F1-score, when using FRI2. On average, to process each image, FRI2 required 30.91s, while SI2 just 0.79s. In the experiments, we found that SI2 model is the best detection method for the particular task as it is 64% faster in the training stage and 98% faster to detect the glomeruli in each image.

16.5 Achievements, Challenges, and Future Prospects

We have seen several groundbreaking achievements in computational pathology and nephropathology in the literature, mainly due to the advances in the DL methods to classify and segment images. Some examples can be found in detecting skin cancer [23] or classifying glomerular lesions in WSIs [11, 49]. Although much has been done toward solving fundamental problems in the field, unfortunately very few studies exploit large and heterogeneous data sets with substantial cohorts that might validate the clinical usefulness of some pre-clinical prospective works. This situation may result in some skewed opinions from what is hype and what is effectively promised in the state-of-the-art works. This is so because many challenges are involved in the development of systems capable of automatically integrating expert knowledge pertaining to specific histological lesions, such as:

- The number of existing histological lesions in a given organ. It is very difficult to precisely define the exact number of existing lesions in a given organ. For instance, in renal pathology, the concept of a histological lesion is vague. These are considered to be discrete, specific, and defined as elementary changes in a histological structure (e.g., mesangial hypercellularity), but can also be a combination of distinct lesions, also referred as a lesion pattern (e.g., membranous, membranoproliferative). The denominations used are sometimes misleading, due to associations with nosological entities [50]. In some cases, due to the origin and progression of a structural change in a tissue, it will not always be possible to stratify a complex pattern of structural changes into different elementary lesions. Some elementary lesions may become evident only through the use of histochemical techniques that highlight specific structures or chemical com-

ponents [50]. Although this strategy is helpful for the pathologist to perform a diagnosis, it also expands numerous possible representations of a given lesion. Additionally, the histochemical staining technique used to highlight specific structures varies greatly among laboratories. Although H&E is the most widely used stain, it is also used together with various other stain combinations. Thus, efforts to establish a comprehensive classification of all lesions within a given organ may prove unsuccessful.

- Lack of agreement in lesion definition. In their daily routine, pathologists must decide whether a given histological structure is normal or if it presents a lesion. Most of the time these decisions are easily made, and most pathologists would share the same opinion. Difficulty emerges when faced with early stage or not fully-developed lesions. Decision-making can be further complicated by the existence of lesions that, at early stages, bear some resemblance to artifacts that appear during histological preparation (sectioning or staining for instance). In fact, these issues are a frequent topic of debate among specialists, with consensus generally achieved through the exclusion of borderline lesions. For instance, the definition of glomerular mesangial hypercellularity varies between the MEST-C classification of IgA nephropathy and the lupus nephritis classification revised by Bajema et al. [27] and Markowitz [51]. Furthermore, the definition of mesangial hypercellularity used in MEST-C classification excludes nonclustered adjoining mesangial cell nuclei [52].
- Low frequency of lesions. Reports on the frequency of biopsy-confirmed glomerular diseases attribute more than 50% of cases to focal and segmental glomerulosclerosis, membranous glomerulopathy IgA nephropathy or lupus nephritis, while amyloidosis and Alport syndrome are less frequent (about 1% each) and fibrillary glomerulopathy and fabry glomerulopathy are rarely observed [29, 53]. Consequently, the elementary lesions associated with these diseases are represented differently in most histological image libraries. Since the current supervised approaches used in computer vision require large amounts of images, it may prove difficult to find suitable sets of images for analysis.
- The emergence of new histological lesions. One of the most interesting observations in pathology meetings is shifting relevance attributed to structural changes in tissues. These fluctuations profoundly impact disease classification and patient treatment. Great effort is expended in the identification and validation of the relevance attributed to a given lesion. For instance, tubulitis attained high relevance in the context of kidney transplant rejection [28].
- Tissue processing. Although a trend exists toward the use of phosphate-buffered formalin as a preserving medium for renal biopsies, the choice of fixative varies among pathology laboratories. In practice, formalin acetic alcohol and Bouin's fluid are used by many laboratories. These fixatives preserve molecular residues differently, affecting tissue morphology and staining properties. At least four different staining techniques are used for highlighting different structures. Although most pathology laboratories commonly use H&E, PAS, and PAMS, staining and counter-staining techniques vary widely among pathology laboratories [54].

- Image capture and processing. Although much emphasis has been given to whole slide scanners, systems based on portable image capture devices, such as smartphones, could facilitate rapid consultations among pathologists. This practice requires an adequate normalization step prior to conducting image analysis.
- The obtainment of a diversified, annotated data set of images, validated by pathologists, presents a major challenge for computational pathology. This is largely due to a shortage of professionals with high levels of expertise in specific areas or diagnostic pathology.
- One of the expected uses of a system capable of identifying histological lesions is to perform clinical-pathological correlations on large scale. This approach may require flexibility in order to quickly learn new lesions as well as the capability to combine images acquired by different sources and methods (immunofluorescence, electron microscopy) with meta-data obtained clinically.

Based on the challenges cast before, we can dig in some future prospects to the field of computational nephropathology until pathologists are able to effectively use CAD-based systems in their daily laboratory routines. They are:

- Classification systems must provide true reliability when examine WSIs in the wild. For that, methods should provide a consistent uncertainty score.
- Supervised methods must provide appropriated generalization either to accomplish any kind of classification or even to segment renal structures. Due to limited availability of data for all types of renal lesions, it is hard to guarantee broad generalization in the process of training supervised methods. An interesting topic of research that is gaining enough attention lately is the self-learning methods, which should be capable to recognize patterns even if it was not trained for this purpose.
- AI/ML-based methods should work on stain-free biopsy images or learn how to generalize from one stain over the others. Each stain highlight characteristics of the image edges, which usually skew the generalization performance of an ML technique. Working on stain-free biopsy images, it is possible to subsequently stain the histology image according to generalization purposes; another way could be to conceive powerful methods that could generalize from a unique stain to rule images stained by other methods.
- Integrate clinical data with histology images. One way to provide more information to ML-based systems is across specialized text, which would create more powerful workflows in computational nephropathology. Broadly speaking, textual data would bring large possibilities to build systems to recognize timely patterns, rather than spatial patterns as it is commonly done by DL methods, nowadays.
- The computational nephropathology would benefit considerably if there was an integration of data from clinical sources and images, but particularly with data from genomics and proteomics. These two types of information would favor, for instance, the identification of new histological markers of disease previously unrecognized by pathologists.

To reach these future milestones, there is an avenue to improve current research in computational nephropathology, considering since data set gathering to how researchers measure the performance and generalizability of the proposed methods.

16.6 Concluding Remarks

Computational pathology is a fast-developing area with a wide scope and research agenda full of opportunities and challenges that must be overcome to allow its full adoption by pathologists in their daily practice. The new techniques and tools yielded by computational pathology may improve the way that the pathologists perform their tasks, allowing them to dedicate more time to the integration and analysis of clinical, morphological, and molecular information collected from tissue specimens. Even though much has been done in the field, the proposed solutions in the scientific literature are far from the point where they can be applied in preclinical trials. Weaknesses in terms of analytical and clinical validation still need to be bridged so that such solutions are robust and reliable enough to be deployed to be used by the pathologists in their daily routine.

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Chapter 17
Internet of Things and Wearables for Kidney Diseases

Wendley Souza da Silva, Leila Maria Rodrigues de Sousa, Gustavo Marques Fernandes Bezerra, Leonardo Pontes Andrade, and Geraldo Bezerra da Silva Junior

17.1 Introduction

With the advent of globalization, several new technologies have emerged every decade, and many of them can be used in the health area, such as the Internet of Things (IoT), a paradigm that integrates physical objects with processing and communication capacities, such as smart home appliances, software, and people connected into a network that allows interaction, communication, collection, and exchange of data with each other [1]. Therefore, the IoT can be understood as a type of network to connect anything to the Internet based on specific protocols on sensors that collect information to perform intelligent identification, location, tracking, monitoring, and administration, among other functions [2].

The integration of IoT with medicine makes it possible to increasingly apply the concept of P4 (Predictive, Preventive, Personalized, and Participatory) medicine, which proposes a systemic approach considering the most recent technological advances and interdisciplinary work. Based on the paradigm, aspects directed to the health area were generated, such as the Internet of Medical Things (IoMT), which uses implantable and wearable devices connected to a smartphone or smartwatch connected to the Internet. The Internet of Nano Things (IoNT) is about developing

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IoT applications in nanomedicine to implement proactive monitoring, personalized diagnosis and treatment, preventive health, chronic disease management, and medical follow-up. The Internet of Health Things (IoHT) allows the combination of mobile applications and wearable devices connected to medical devices with professional-grade sensors that are always on and are sensitive to the context [3].

To enable the communication between equipment that stays with the patients or other users, there is currently a wide variety of wireless communication protocols that allow the interconnection of health monitoring systems. Current technologies used for this purpose include WiFi for local networks, WiMax for wireless broadband networks, third- (3G), fourth- (4G), and fifth-generation (5G) mobile phone communication, infrared (optical wireless communication based on the point-and-shoot principle), Lora, ZigBee (provides low power consumption with low transmission rates), and Bluetooth Low Energy (BLE). The choice of which wireless technology will be used in an IoT system will depend on the specific need of that application, that is, if a system needs to save as much electricity as possible for a short-distance transmission, it is recommended to use the Bluetooth; if, however, one needs low power consumption and long transmission range, one may need to use ZigBee or Lora. Moreover, there is the massive use of technologies based on electromagnetic field communications, including Radio-Frequency IDentification (RFID) and Near Field Communication (NFC) [4].

One of the main examples applied to medicine are wearables, which have been increasingly studied due to their diverse functions that may revolutionize health care (1). Mobile devices have been hierarchized into three different categories: portable devices (bracelets, watches, clothing), attachable devices (contact lenses, skin patches) and implantable devices (pacemakers, biotints, tattoos), and ingestible devices (pills) (2) (Fig. 17.1).

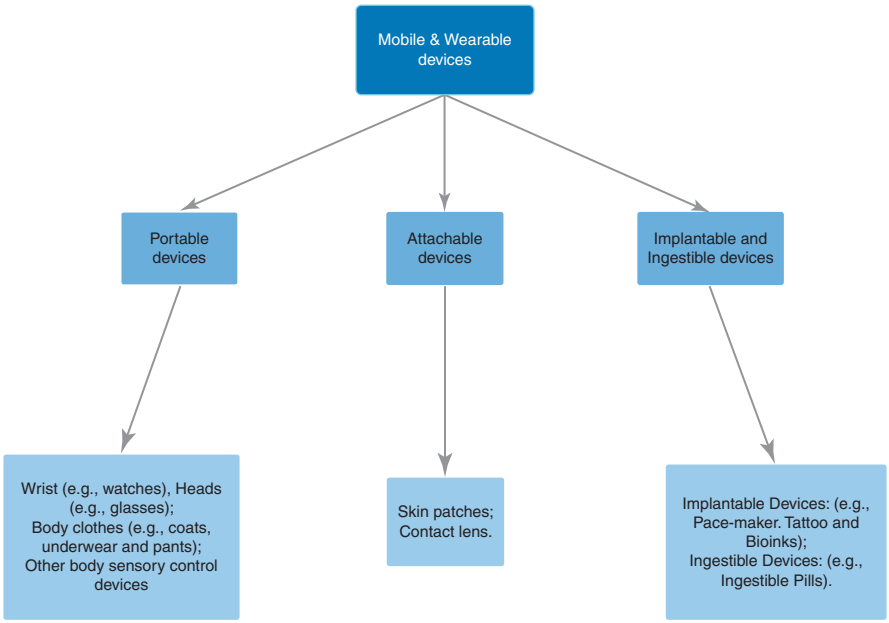

Fig. 17.1 Wearable device categories applied to Medicine. (Adapted from Syed-Abdul et al. [6])

Wearables can detect several variables, including movement, location, cardiac activity, outside sounds, photoplethysmography (PPG), among others. Therefore, they can be used to assess, for instance, the quality of sleep and/or physical activity, excessive exposure to noise and important vital signs in real time, such as heart and respiratory rates, blood pressure, body temperature, oxygen saturation, among others, which can be used to monitor patient health status and predict possible morbidities, based on artificial intelligence (AI) [5, 6].

17.2 The Use of Wearables for Health Monitoring

Portable devices are the most frequently used ones nowadays. However, attachable devices, such as skin patches, have been studied, and it has been recently discovered that they can obtain more accurate and reliable information about the individual, as they are not affected by their movements. Moreover, other examples, such as contact lenses, can monitor important parameters such as patient blood glucose. Implantable devices also have the ability to detect important parameters of body changes, such as transplant rejection and arrhythmias, which pacemakers can treat using low-frequency electrical pulses. Biotints are able to monitor some electrolytes in the human body, such as sodium. Ingestible pills can send signals to patches applied to the body when in contact with gastric juice and can, for instance, be used to assess the time of medication digestion [6].

In fact, wearables have the ability to obtain different types of user data associated with health, either directly or indirectly. Physiological data, such as heart rate, blood glucose, oxygen saturation, among previously mentioned others, can be used in different ways. One of them is in preventive medicine, as the devices, through AI and machine learning (ML), are able to detect possible abnormalities, such as cardiac arrhythmias or hyperglycemic states [5, 6]. Therefore, they notify the user through a report and, based on the latter, the user can make an appointment with their physician or use an AI physician, plus the wearable can recommend medication to the user [6]. Some of these monitoring methods are able to outperform the traditional clinical methods, as they continuously monitor the data [5].

Also regarding the treatment of diseases, the device can also perform the function of reminding the user of the time and doses of medication to be administered, through alerts. Based on the ingestible devices, the individual can more precisely monitor the time of medication intake. In the treatment of chronic diseases, in addition to these functions, the devices can use the detected abnormalities to improve the treatment of individuals with chronic diseases, such as diabetes or chronic kidney disease (CKD) [6]. Apps with this purpose already exist, aimed at patients with chronic kidney disease, which help in the self-monitoring of the treatment [7].

Many wearables, such as smartphones, bracelets, and watches, have movement and location sensors, thus being able to monitor different types of data related to physical activity. These devices collect data such as the number of steps, amount of calories used, traveled kilometers, rotation speed, and acceleration, among others. Thus, they are able to use these numbers to detect the user's pattern of sports activity

behavior and, therefore, help improve performance or prevent lesions and other adverse sports-related conditions, such as cardiorespiratory arrest [6].

Based on the physiological data, wearables can also, albeit superficially, attain a parameter of the user's mental health status, associating certain physiological states with certain emotions. These data can be useful to help individuals deal with their mental health, thus preventing important conditions such as depressive disorder [5, 6].

Currently, one of the best known and most often used wearables on the planet is the Apple Watch, manufactured by Apple Inc. company (California, USA) [8]. This device is characterized as a smartwatch, capable of performing several functions, including the measurement of some health indicators, mainly related to the cardiovascular system. In 2018, Apple Watch Series 4 gained Food and Drug Administration (FDA) approval as a heart rhythm and atrial fibrillation (AF) medical device [8].

Starting with the Apple Watch Series 1, cardiac arrhythmias can be detected through an algorithm based on photoplethysmography (PPG). The watch has an optical LED light sensor that detects changes in blood flow in the wearer's wrist and based on that generates a photoplethysmogram that estimates the heart rate. To detect variations in heart rate, the Apple Watch uses the intervals between pulses to create tachograms—graphs that measure HR at a given time—every 2–4 h [8–10].

A more recent model, the Apple Watch Series 7, has some increments in its structure that allow it to detect cardiac electrical impulses from the user's fingerprint and pulse, which allow the creation of a closed circuit and, therefore, obtaining an electrocardiogram (ECG) similar to a single-lead ECG (D1), thus promising revolutionary innovations [11]. From this, an application was developed, called the ECG app, which the individual can open on the watch, and to generate an ECG, they must insert the contralateral finger on the wrist where the watch is worn. From the ECG, an algorithm can detect a sinus rhythm, atrial fibrillation, or be inconclusive [8–10]. An Apple company study with 588 participants detected, for the classification provided by the algorithm, a sensitivity of 98.3% and a specificity of 99.6% [9, 10]. The demonstration of the electrocardiographic tracing obtained by the Apple Watch can be seen in Fig. 17.2. The monitoring of the electrocardiographic tracing using smartwatches seems to be promising, especially for the early detection of cardiac arrhythmias [12]; however, one must be concerned with issues related to the high cost of this technology, accessibility, data protection, and other issues [12].

In this regard, the company financed an observational study, called the Apple Heart Study, one of the largest studies on atrial fibrillation to date and which was carried out in a completely virtual manner [9, 13]. The study included 419,297 Apple Watch users and lasted 8 months. Of the participants who returned the ECG with arrhythmia notification, AF was present in 34%, and of those who were notified with irregular pulse, the positive predictive value was 0.84 for AF [13].

Another interesting use of wearables is in diabetes mellitus (DM), a condition that affects a significant number of individuals globally, affecting more than 500 million adults in 2021 and is the leading cause of CKD worldwide [14, 15]. Currently, there are continuous real-time glucose monitoring devices (rt CGM),

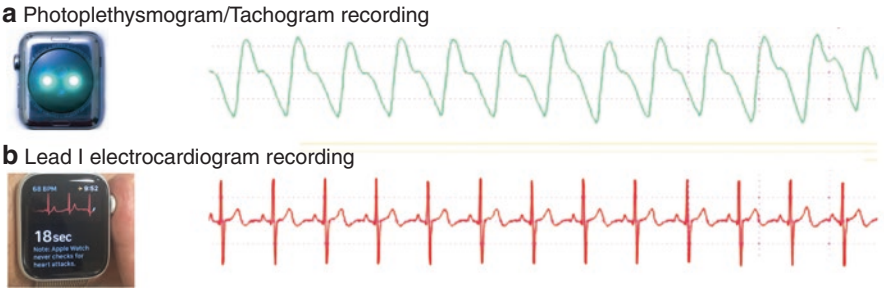

Fig. 17.2 (**a**) Demonstration of the photoplethysmogram/Tachogram recording using Apple Watch utilizing light beams to record changes in the blood volume passing through the wrist (caused by peripheral pulse) (Apple Inc.). (**b**) Demonstration of the Lead I electrocardiogram recording through a circuit between the detector on the watch back and the digital crown using an Apple Watch (Apple Inc.). (Reproduced with permission from Isakadze & Martin [12]. © 2020 Elsevier)

with the first one being authorized by the FDA in 1999. Currently, some brands lead the rt. CGM market, such as the North-American companies Dexcom, Medtronic, and Abbott, although these devices are relatively invasive ones. However, there are noninvasive alternatives, mainly for pre-DM, aiming at secondary prevention, or for non-insulin-dependent type II DM, aiming to chronically monitor the patient. For type I DM, there are wearables integrated into insulin pumps, creating an "artificial pancreas" [5].

Although limited, there are useful wearables for monitoring the gastrointestinal system, of which diseases generate great costs, both directly and indirectly, for the health system—one example is that by the company G-tech (USA), which uses electrodes to monitor the electrical activity of the stomach and intestines [5]. In the field of neurology, there are devices that quite effectively monitor sleep and detect prevalent disorders, such as sleep apnea. The smartwatch manufactured by the Empatica company, called Embrace, can identify seizures and notify emergency services and may be useful for people suffering from epilepsy. In the respiratory system, there are useful wearables for the detection and monitoring of important lung conditions. The ADAMM device, worn on the patient's chest, can monitor asthma and differentiate it from other diseases, such as pulmonary fibrosis and chronic obstructive pulmonary disease based on audio data [5].

Thus, these are some examples of the different body systems and the diverse ways in which wearables can monitor patient health and, therefore, acute and chronic health conditions, inside and outside health facilities [5]. Hence, they can contribute to creating a multidimensional view of the patient and help in the screening, diagnosis, and treatment of several important conditions, which can increase patient adherence to preventive measures at their levels and, consequently, contribute to the population's quality of life [5, 6]. Despite regulatory and technical obstacles, wearables constitute promising equipment that have the potential to impact the health panorama in different areas [5].

17.3 Application of Wearables in the Context of Kidney Diseases

Regarding the use of wearables in nephrology, especially in CKD, first it is important to offer a brief contextualization of the disease. CKD consists of an alteration in kidney function and/or structure, with a progressive and slow evolution. When related with adult patients, they must have a persistent glomerular filtration rate <60 mL/min/1.73 m^2 for a period ≥3 months. If the filtration rate is >60 mL/min/1.73 m^2, evidence of damage to the renal structure is required [16, 17].

Therefore, CKD is often asymptomatic in the early stages, subsequently developing into several complications, including cardiovascular ones. At advanced stages, it may require more specific treatments, such as kidney transplantation or dialysis, which cannot always be carried out due to the lack of donors or even the lack of resources and which require great commitment by patients and the healthcare team [16, 18].

From this perspective, wearables and other technologies can be useful in minimizing the progression of CKD [19]. However, it is noteworthy that they have several interactions that allow these devices to effectively contribute to health monitoring [16].

First, when considering the aesthetics, it is known that for efficient health monitoring, wearables should not become a complication in people's daily lives, considering that if this hindrance becomes something significant, adherence by users will be reduced. However, the wearables' operation and technology must be sufficiently accessible for all ages, especially the elderly, as this age group is the most affected by CKD [16].

As explained above, CKD can have cardiovascular complications, such as resistant hypertension. Wearables used to measure blood pressure can be divided into two groups: those with a pressure cuff and those without it. The methods that do not involve the use of pressure cuffs usually have better portability and convenience for users, and the main techniques used are Pulse Transit Time (PTT) and Pulse Arrival Time (PAT) [16].

Additionally, the use of wearables to perform an ECG can also satisfactorily contribute to the monitoring of patients with CKD [16]. An example of these devices is the Apple Watch Series 4, which has mechanisms that allow performing an ECG similar to a single-lead ECG (lead I), aimed at screening for arterial fibrillation [10], as shown above.

Regarding the self-management of other chronic diseases, such as diabetes mellitus, the main cause of CKD worldwide, especially with behaviors that involve changes in lifestyle, such as dietary and physical exercise changes, the wearables have shown great effectiveness [16]. Through wearables and their corresponding apps, monitoring exercises and activities and data such as heart rate are useful in the self-management of these chronic diseases, in addition to, in many cases, increasing the stimulus to the practice of exercises [16, 19]. No less important, bioimpedance aims at measuring the body composition of individuals, which can prevent obesity, for instance [16].

In a community study with 6568 individuals in Japan, a significant association was found between sleep disorders (respiratory and hypertensive), measured through the wearable actigraph and pulse oximeter devices, and albuminuria. In Taiwan, a group of 60 patients with CKD were instructed to use wearable devices that collected exercise-related data and connected them to social networks, showing improved quality of life and slower eGFR decline in people with CKD at stages 1–4 [20]. Arrhythmias are common in CKD, and for this reason, the use of wearables can be very helpful. A study of 608 individuals with diabetes followed in the mHealth Screening to Prevent Strokes (mSToPS) trial was carried out to investigate the use of an ECG patch for 2 weeks, twice, over a 4-month period, and followed clinically through claims data for 1 year [21]. Among these patients, 15.8% had CKD, and atrial fibrillation was detected in 19 cases over a 1-year study period using the wearable, even before the development of any symptoms [21].

An example of a wearable specific for the field of nephrology, but which is not yet a reality, is the wearable artificial kidney (WAK) [22]. The idea of using renal replacement therapies, which are often necessary in patients with chronic kidney disease, with the advent of better aesthetics, portability, and convenient transportation, would be extremely useful in the daily lives of these patients. Many studies have shown progress in the use of these devices. The main treatment strategies are peritoneal dialysis and hemodialysis [23]. In a pilot clinical trial carried out in Singapore with 21 patients, the "automated wearable artificial kidney" (AWAK) device was tested for 1 month, and no serious adverse effects were observed [24]. The main adverse effects observed with the use of the AWAK device were pain/discomfort (60%) and swelling (47%). The median estimated peritoneal weekly K_t/V_{urea} was 3.0 [24]. There are other ongoing clinical trials with portable peritoneal dialysis equipment, of which results are expected for the next few years [25]. There are also projects for the manufacturing of implantable devices that carry out the filtration function as performed by the kidneys, which would be an implantable artificial kidney [26]. The most advanced of these devices is the one developed by researchers at the University of California in San Francisco, in partnership with Vanderbilt University, in Nashville, USA, called "The Kidney Project" [26]. However, more clinical trials with a larger number of patients are required, and moreover, regulatory issues are also important and must be taken into consideration before implementing its use in clinical practice [27].

17.4 Conclusion

Wearables are extremely useful tools in many health contexts, including the treatment of kidney disease. Devices that monitor vital signs, electrocardiography tracings, sleep quality, and other physiological parameters can and should be used, especially with the aim of detecting as early as possible any dysfunctions that can predict potentially fatal conditions and that can provide a specific treatment aimed at improving the prognosis of CKD patients. More sophisticated devices, such as

wearable dialysis equipment, still need larger and more detailed clinical studies, as well as evaluation and approval by regulatory agencies in several countries, before they can be actually implemented in daily clinical practice. However, these devices will certainly bring benefits to people with advanced CKD, especially regarding mobility and quality of life.

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Chapter 18
Conversational Assistants and their Applications in Health and Nephrology

Elizabeth Sucupira Furtado, Francisco Oliveira, and Vládia Pinheiro

Abbreviations

AF	Atrial fibrillation
AI	Artificial intelligence
CA	Conversational agents
CKD	Chronic kidney disease
DRP	Drug-related problem
ECA	Embodied conversational agent
ECG	Echocardiogram
ESKD	End-stage kidney disease
HC/HIT	Health communication and health information technology
IoT	Internet of things
IPA	Intelligent personal assistant
IVA	Intelligent virtual assistants
LHL	Limited health literacy
NLG	Natural language generation
NLP	Natural language processing
NLU	Natural language understanding
PDA	Personal digital assistants

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PGT	Pharmaceutical guidance table
PPG	Photo plethysmo graphy
VCA	Voice-based conversational agents
VUI	Voice user interfaces

18.1 Introduction

At a time when healthcare professionals are in short supply and the demand for low-cost treatments is increasing, virtual caregivers have been designed with Artificial Intelligence (AI) concepts to provide guidance and health suggestions [1]. These virtual caregivers are commonly implemented as a Health CA and aim to maintain a dialogue with the user, engaging and motivating them and thus promoting their health.

A CA is a computer program that applies Natural Language Processing (NLP) techniques to understand users' intentions, and thus maintain an adequate conversation that responds to or guides a solution to the human user's needs.

A CA can be a type of Embodied Conversational Agent (ECA) that emulates a human personality, using gestures, looks, and human speech for communication [2]. An ECA take on different forms, including virtual avatars (see Fig. 18.1 [3]) or physical agents, such as a humanoid robot [4]. It does not have to be a human body, it can even be pet body [5]. The important thing is that it has a body and can be used to transmit "embedded" information. For this reason, an ECA can also implement assistive technologies (through an attention monitoring algorithm) to observe user's behavior (as their attentional disorders) and react using its synthetic voice or emotion to call the attention of the user [6].

Intelligent Virtual Assistants (IVA), or just Virtual Assistant [7], also use conversational interfaces and are intended to respond to a specific need according to the

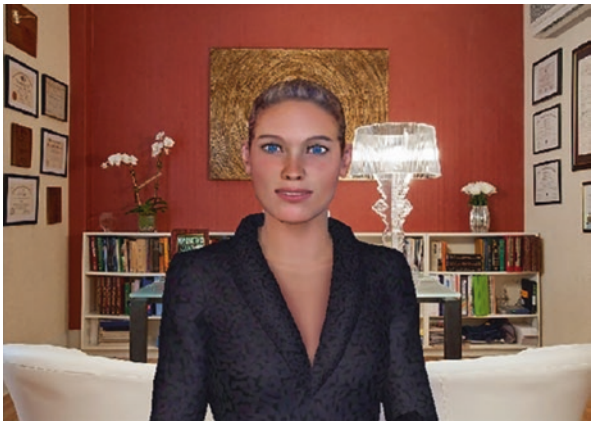

Fig. 18.1 Multimodal ECA Interface used as virtual counselor regarding alcohol problems

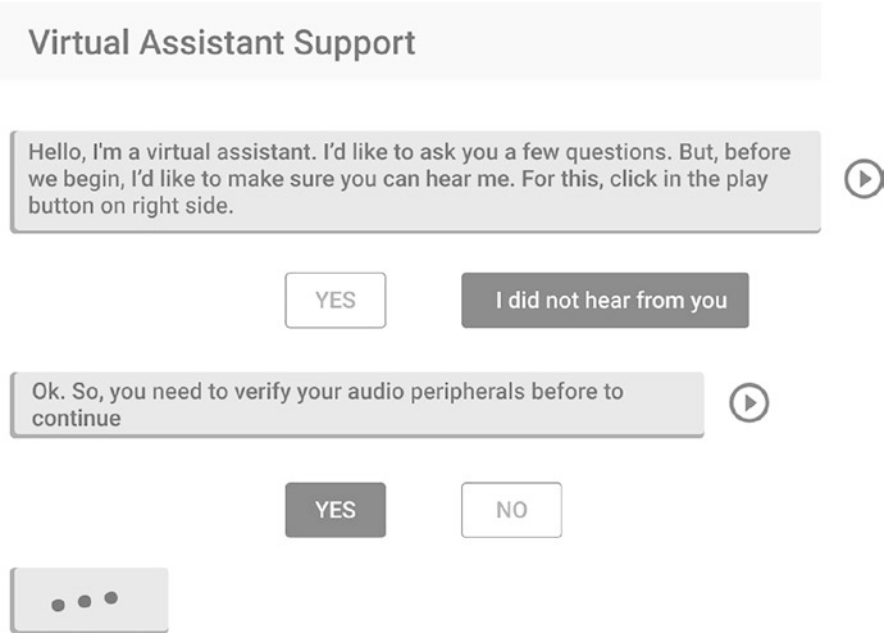

Fig. 18.2 An example of a chatbot initiating a dialogue with the user

most relevant information available, which can be a particular movie to the user to watch, for example. Some examples are answering chatbots, smart voice assistants (e.g., Apple Siri on iPhone), and device-embedded voice assistants, which can be found in Smart Speakers (e.g., Amazon Alexa on Amazon Echo dot). These assistants can use several modalities to communicate with their users. Figure 18.2 illustrates an example of a chatbot that uses text and voice to communicate with the user.

Figure 18.3 illustrates an example of an interface quite similar to the interface of Siri on Apple iPhones, which the assistant interacts with the user through voice activation. The user's second request was successful because there was a Netflix skill installed on the user phone, which was taken from the assistant's skill store.

Commonly, Voice User Interfaces (VUI) are voice command interfaces built into smartphones and assistant devices, also referred to as CA, IVA [8] or Intelligent Personal Assistant (IPA) or Personal Digital Assistants (PDA). In [9], the term used is Voice-based Conversational Agents (VCA), with voice as the main mode of interaction, but these are not limited to it. IPA variants can be thought of as an ECA [10].

Though there is no consensus on the nomenclature used, most existing voice assistants are often called conversational agents [11]. Such conversational systems present variations in speech patterns to communicate with the user and to process dialogue. For instance, the pattern "Question and Answer" simulates a limited speech and vocabulary dynamic of a voice assistant that is recognizable within a pre-established narrative context. In all cases, there are studies of several human,

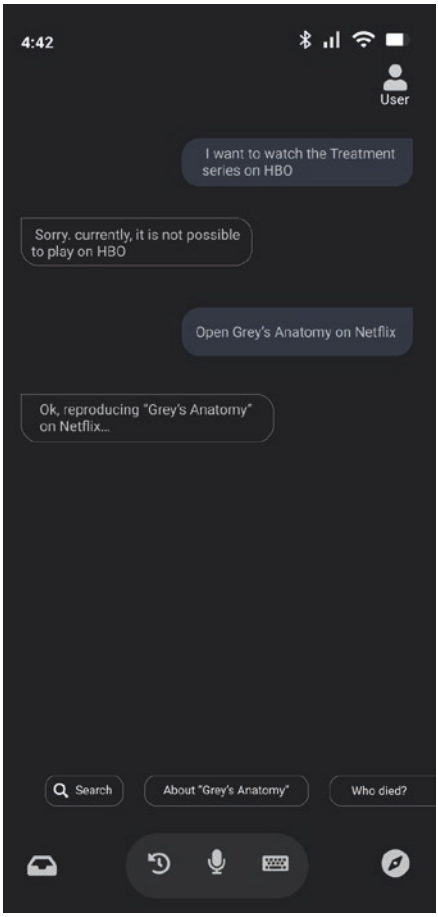

Fig. 18.3 The user voice activates the interaction using Siri smart assistant

contextual, linguistic, and technical aspects that affect the interaction between the human user and the agent/assistant.

Self-health management is defined as the degree to which a chronically ill patient is able and willing to control their daily life [12]. Effective self-health management is impacted when a person lives with more than one chronic health condition [12]. Hence the importance of existing technological resources for shared and collaborative health management [13]. In order to engage the patient with health management activities, Health CAs have different purposes: as a counseling agent [14], a goal-oriented health management agent [15], and a coaching agent [1]. Dialogs are designed, integrating other services (such as a telenursing service for family caregivers) [16], using feelings [17, 18], in order to motivate a user to have healthier behavior, for example, and are required to respect some dialog design guidelines [19]. Some examples are to explain medication instructions to patients with chronic disease using a frame message [20, 21], give healthy living advice including mood

[22], customize assistance messages by health status [23], etc. The Ada application is a Health CA for symptom assessment with good accuracy. The Ada app's accuracy of condition, suggestion, and urgency advice is higher than that of the best-performing symptom assessment app reported in a previous study (61%, 77%, and 52% for conditions suggested in the Top 1, Top 3, and exactly matching urgency advice, respectively). These results are relevant to the application of symptom assessment in primary and community health, where medical quality and safety should determine app choice [24].

Recently, the authors of [25] considered that a CA makes health care accessible, and effective in disseminating health information among a population with problems of Health Literacy. Inadequate or Limited Health Literacy (LHL) is associated with a personal attribute defined as the non-ability to access, understand, and use health-related information [26]. This means "the degree to which individuals can obtain, process, and understand the basic health information and services they need to make appropriate health decisions" [27]. Despite this, research in conversational user interfaces suggests different forms of interaction, adaptive to users, although voice assistants fail to attend to these types of users who are a minority [28, 29].

The fact is that although CA technologies still have their limitations, they can successfully automate many activities in healthcare delivery. Experiments with computational linguistics have shown that people will often prefer to rely on a discrete and non-judgmental artificial assistant over a human agent. Peng et al. [30] presented a study that reported patterns of perception of humanity in CAs with reliability components, such as competence, benevolence, and integrity. An interesting result is that a CA was perceived as a machine with a higher confidence level than a human CA, but they present great asymmetry between speech and understanding, that is, they express themselves very well with a "human" voice but have a low ability to understand and develop dialogs, undermining effective health communication.

The opportunity arises to combine certain conditions, these being the advantages of the machine's discretion and impersonality with the human character of those "behind" the technology. This would allow, in eventual circumstances, the human professional to replace the agent using the same channel, which tends to increase the level of adherence in treatments for chronic diseases, being that there is "accountability to a coach who is seen as trustworthy, benevolent, and having expertise" [31]. Thus, it is also possible to imagine the performance of the CA in conjunction with healthcare providers, who are known to the patients and assist them in face-to-face moments during treatment, which is known as the hybrid model.

In this sense, the guiding question is: which characteristics of the dialogue between a Health CA and an LHL user can lead to effective health communication? To answer this, we studied an integrated action plan to list the main requirements of a dialogue with a health management CA.

The objective of this chapter is to describe Health CAs and their applications in health and nephrology, presenting their architecture, characteristics, and the necessary requirements for an effective health communication with LHL patients, configuring the agent as another tool in promoting treatment adherence.

As a work methodology, we looked for studies on Health CAs and their applications in the health context (health management and continuous care), both in the areas of medical informatics and computer science. Then, we apply a process for specifying the requirements of a Health CA centered on the needs of the target users and on a guidelines plan, which integrates different stakeholders. Finally, we exemplify the use of a Health CA with an LHL patient with Chronic Kidney Disease (CKD) to manage their health. In [32], patients' motivation to engage with CKD management activity will be influenced by their understanding of the risk of disease and the relative benefits of different treatments. The process of managing diet, medications, and appointments will depend on adequate understanding of written and numerical instructions. A quarter of people with CKD are LHL [32], which has consequently reduced use of preventative medicine, demonstrated a poorer ability to manage medications and thus increased mortality.

The following results are achieved in this chapter: concepts and guidelines for assistive communication, based on a hybrid dialog model with patients, as well as presenting opportunities and risks in the use of existing solutions in the studied literature. Aspects related to the validation of these interaction scenarios motivate future work.

This chapter contributes to the goals outlined by the workgroup entitled "Health Communication and Health Information Technology" (HC/HIT) [33]. The HC/HIT goals were defined with a view to achieving effective health communication by 2030. The plan is part of the Healthy People 2030 initiative, which focuses on improving health communication so that people can easily understand and act on health information. We describe requirements and dialog forms for the design of a Health CA, which can guide CA developers.

18.2 Architecture of a Health CA

Figure 18.4 shows that the architecture of a Health CA is focused on continuous monitoring. The modular architecture allows for the development in interdependent layers, and thus, the advancement of the area can take place asynchronously [34]. A dialog session can be initiated by the system or by the user, via voice or text. In the first case, the Monitoring Decision System of the Health CA makes the dialog decision based on Health Knowledge and Data, going through the NLP Dialog Management layer, and finally the message is presented to the human user. It is important to emphasize the importance of the agent initiating the dialogue in the case of chronic patients. These individuals often find themselves in a situation of depression, where they probably would not initiate the dialog, making the technology innocuous. In the second case, the human user initiates the dialogue, and the

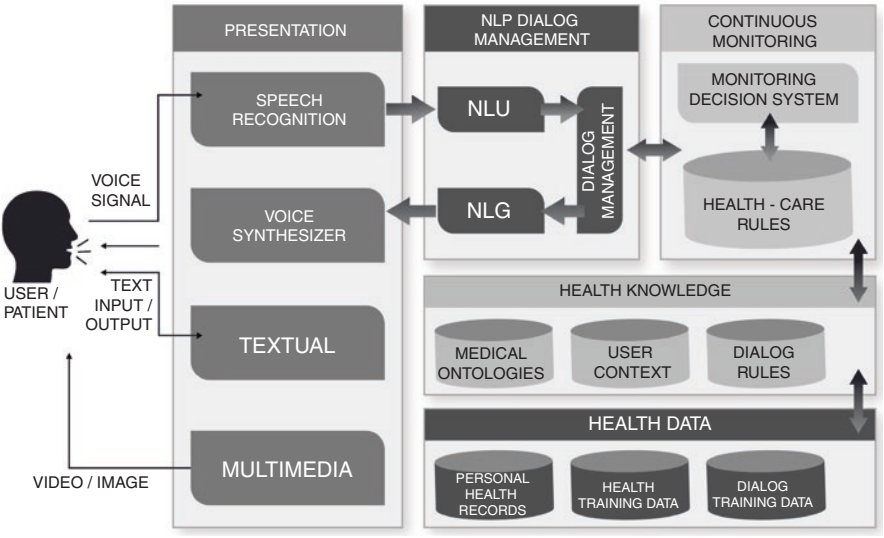

Fig. 18.4 Architecture of a Health CA

Health CA presentation layer sends the message to the NLU module (understanding language), which initiates the processing and understanding of the dialog.

Each layer of the Health CA architecture is explained below:

- Presentation—this layer is responsible for receiving the user's voice signal and transforming it into text, through the Speech Recognition module [34, 35], or receiving from the NLP Dialog Management layer the text to be presented to the user and transforming it into speech language through the Voice Synthesizer module [36]. Optionally, the interaction can take place through text or multimedia files (video or images).
- NLP Dialog Management - in this layer, the user's input text is submitted to the Natural Language Understanding (NLU) process in order to identify the user's intentions and recognize the entities [37]. On the other hand, the Dialog Management module creates conversation threads, following dialog rules predefined or learned from a machine learning model based on a dialog training dataset (data coming from the Health knowledge and Health Data layers), and the Natural Language Generation (NLG) module is responsible for generating the text into language that will be sent to the Presentation layer, for speech synthesis [38].
- Continuous Monitoring—Information generated in the Health knowledge layer is inputted to this layer, where automated healthcare decisions are made to monitor the patient's health [39].

- Health knowledge—health information retrieved or created from existing sources or from the Health CA data layer is stored here. This layer contains medical knowledge bases, i.e., ontologies, e.g., the MEsH [40], and a dynamic repository of patient-related information that is built and updated using automated data analysis and interpretation tools [39].
- Health Data—It contains the structured data sources (e.g., personal health records) and unstructured data used in training machine learning models (such as health training data and data from dialog threads).

18.3 Applications of a CA

Health CAs for monitoring a treatment can be used in a number of ways. We highlight solutions and characteristics regarding the context of use, the participation of users (patient, patient's family, caregivers, healthcare professionals) involved in the interaction and design of the solution.

18.3.1 Health CA and its Context of Use

Continuous patient monitoring can be done at home or at the hospital, using sensors integrated to the technological solution and a combined means of communication (email, telephone, WhatsApp, teleconference, etc.). Health CAs for intelligent health monitoring (Internet of Things - IoT, Internet-connected medical devices, etc.) make it easier, for example, for patients to transition from hospital to home care sooner, which saves costs and increases recovery rates [41]. A "virtual caregiver" platform equipped with a CA and connected devices have revolutionized elderly care, digital therapy, and continuous care, especially during the post-treatment recovery period for health checks [39, 42].

In the Sweet-Home project, Portet et al. [43] assessed the acceptability of voice interfaces by autonomous elderly people in a home context. In this work, the authors sought to test the four important aspects of Smart Home Care: voice control, communication with the outside world, human activity interrupting the system, and electronic agenda. The results of the study showed that the elderly prefer voice interfaces over other methods of interaction, such as typing or touching interfaces, but that they were concerned about their privacy. According to [44], sophisticated applications, for smartwatches for example, are still a long way from being a category for elderly users. The author emphasizes that CAs seem to have greater potential to reach this population, but issues such as discoverability (the elderly subject's ability to find the correct application in such a device) and poor dialogs are barriers to accessibility.

In [23], black women undergoing treatment for cardiovascular disease were monitored individually and in their own homes. Interventions were made for

adherence to a health protocol (e.g., blood pressure) varying the format of communication (e.g., text message and audio), communication time (e.g., daily, monthly), and the type of information capture (asking or collecting automatically). The outcomes were presented showing the relationship between each type of intervention and the patient's blood pressure (non)adherence.

Another type of context to consider is the patient's social context [45], where the voice communication with electronic devices is useful in increasing trust and, subsequently, strengthening social bonds. The opposite result was identified in [46], for some elderly participants who did not trust the health information provided by the CA. A similar proposal for the need for a co-located human presence user, through the interaction with a CA via chat is given in [47].

18.3.2 CA in the Integration of the Healthcare Team and Patients

The role of information technology in health care has long been an object of debate. Will it replace human healthcare professionals? Will assigning healthcare tasks to a machine do more harm than good to patients? Similarly, how should we view technology-propelled patient empowerment?

Smartwatch users can take an echocardiogram (ECG) test for free, anywhere and anytime, and share the results with their physician at their leisure [48]. This study [48] performed a review to determine "*whether the photoplethysmography (PPG) technology, employed in the wearables to monitor heart rate, is accurate enough to aid in the diagnosis of Atrial Fibrillation (AF) that may remain asymptomatic or paroxysmal.*" The authors reviewed trials involving *Apple Watch, Kardia,* and *Samsung*. The authors concluded that the strategy is useful, *"Intermittent short ECG recordings repeated over a longer-term period produced significantly better sensitivity for AF detection, with 4 times as many cases diagnosed compared with a single time-point measurement."* Besides taking the measurements, the app instructs its user on how to use these measurements and their limitations. Having easy access to this information may promote its frequent use and generate anxiety in the user. On the other hand, it might lower overall healthcare costs (private and public).

18.3.3 CA and Design Solutions for LHL Users

In this subsection we present system design solutions to help LHL users. Most of the examples identified in the CA literature are for elderly patients, who have problems similar to those of an individual with LHL [49]. The interaction of elderly people with technological solutions is affected by low visual acuity, memory loss, and decreased manual dexterity [50], which hinders their ability to use quantitative

information, and speak and listen effectively (oral literacy) 12]. The medical informatics area is still lacking in CA solutions for LHL users.

In a systematic review [51], 38 studies using different technologies, not including CA, presented solutions to help LHL users improve their understanding, their skills related to health and their behavioral intention toward health care. In this review, it was analyzed that there is a consensus on the importance of information design suggestions, e.g., presenting essential information by itself or first, presenting information so that the higher number is better, presenting numerical information in tables rather than text, adding icon arrays to numerical information, and adding video to verbal narrative. In [52], the authors identified 309 health and fitness Skills for Amazon Alexa and Google Assistant, even though they support monitoring activities, they are not focused on this audience. The authors of [53] suggest that natural language techniques help users to get what they want more, improving the mapping of their intentions, grouping similar tasks, and accepting grammatical variations. Other tips for online dialogue are given in [54], in which the authors evaluated the impact of providing patients with a literacy-appropriate diabetes education guide accompanied by a brief counseling designed for use in primary care.

In Cheng et al. [55] the authors intended to aid self-management for elderly patients with diabetes by proposing an application that uses the voice assistant Google Homepage and a web interface for data visualization. After a test carried out with ten elderly users, focusing on the voice component of Google Homepage, they perceived good general acceptance. Most people attributed its high usability to speaker functionality and the natural flow of conversations. Noh et al. [56] proposes a virtual interface that simplifies the interface of a smartphone for the elderly. The proposal displays menus according to the user's situation and makes requests through an intelligent agent. Voice recognition technology is applied to receive information from users together with gestures. A preliminary study to evaluate the proposed method was carried out with five elderly people. Four of the five approved the method, while one was not comfortable using the smartphone with the new interface.

In [57], the authors propose an educational conversational game dealing with the usefulness, reliability and security of health information, which is retrieved from the web and presented to learners. The objective of the game is to teach (young) players to find, understand, and select health information online and apply their knowledge to make health decisions. According to the game's narrative, the player interacts via chat with various characters (family, healthcare provider, etc.), who help them understand the content. Another example is given in [21], a conversational agent presents to elderly patients some educational content in message framing, with effects described either as gains of taking or losses of not taking the medication. This content frame design technique is used in several works aimed at understanding LHL patients [20].

In summary, despite the implementation of guidelines in the design of applications, users are still concerned about the privacy, ethics, and security aspects of health information. We believe in the joint use of guidelines in the design of applications with healthcare policies [58] to support the definition of reliability requirements [59], personalization, integration between data and between stakeholders, etc.

In the next section, we briefly describe an approach to developing a Health CA, using an integrated action plan for effective health communication, and following a design thinking process [60]. It is then applied in describing an illustrative example of a Health CA for CKD patients.

18.4 Development of a Health CA for Monitoring, and its Interventions in CKD Patient Communication

The main activities carried out in the development of the Health CA in question were:

(i) Definition and collection of health knowledge bases and data - medical and nephrology ontologies, patient personal data, and health dialog training data must be defined and retrieved in conjunction with the Health CA developer. Training data will be used in machine learning models. The result of this activity will be registered in the layers of Health Knowledge and Health Data of the architecture.
(ii) Definition of data and knowledge of users - for the idealization of likely CKD patients who will be Health CA users, scenarios of use should be defined, seeking to identify characteristics and context of use, education level, vocabulary spoken by user, etc. The result of this activity will be registered in the layers of Health Knowledge and Health Data of the architecture.
(iii) Definition of Health CA dialogs - this activity characterizes the possible dialogs between the user and the CA, as well as the functional requirements and the user experience. As a result, we have the conceptual definition of the Continuous Monitoring layer of the architecture.
(iv) Specification of the Health CA dialogs - based on the characterization of the dialogs resulting from the previous activity, at this moment, the entire dialog script must be specified - how the user accesses the CA, who starts it, how it develops, and how it ends. As a result, we have the definition of the Dialog Management module of the NLP layer of the architecture.

It is important to emphasize that the validation activity has not yet been carried out, as will be discussed later. The results of activities (ii) to (iv) are explained in the following subsections.

18.4.1 Characterization of Dialogues for Health Treatment Monitoring

In this context, assisting a person involves helping them to prevent or delay kidney failure and End-Stage Kidney Disease (ESKD). This action aims to increase the proportion of people on Medicare who get follow-up care 3 months after kidney injury—CKD-03 [61].

We highlight two dialogue Strategies (S1 and S2) for an example Health CA, one not depending on the other [62]:

- S1: Establishment of a healthcare plan; for the establishment of the general objective of the patient and the activities that he/she must carry out to reach the objective, within a specific time limit; and.
- S2: Monitoring of the established plan; for troubleshooting.

In the first S1 strategy, the establishment of a health goal can be provoked from the following reflective question to be asked by the healthcare provider to the patient: "Is there anything you can do next week to improve your health?" [62]. The interaction possibilities between a CA and the user can then be about what the user will be doing in the week and how the assistant can help them. In regard to the possibilities of interaction, the Health CA: asks the user questions about their health and can follow an established care plan, which is part of a treatment [47]; deliver educational materials to explain to the user the necessary behavioral changes [44] to adhere to the plan; and configure personalized help that the user needs to follow the plan (such as receiving reminders, communicating with humor [22]), etc. It is important that the patient has the confidence that they can achieve the objective with a plan. However, the uncertainties regarding the proper execution of the plan by the patients motivate the establishment of the second strategy.

In the S2 strategy, configured customized help must be provided by the Health CA. As the patient responds to interventions, reminders, motivational advice, a dialogue is maintained. During follow-up (with the support of technology and/or by the healthcare provider), problems that impede the plan's success must be resolved. These can be issues that go beyond lack of patient commitment, usability issues and/or issues with patient access to the service, etc. The purpose of this follow-up is to open up space for clarification and answer questions about the plan and related matters. This can be done by phone, email, social media programs (Telegram, Snack, WhatsApp), chatbot, and with web programs. Some metrics to assess the support provided to the user are generally [52, 63]: Engagement, which measures the user's involvement and interest; completeness of the plan as a function of the completion time; conversation quality, which measures how efficient the Health CA was in helping; and the type of communication established, to verify the user's behavior regarding the interventions received by the assistant.

Each of these strategies requires functionalities implemented in the Health CA, which are presented below.

18.4.2 Definition of the Health CA Requirements

The Health CA must have requirements to enable users' experiences with the dialogues defined for the S1 and/or S2 strategies, as well as to integrate with healthcare providers in order to support their organizational and social policies.

The requirements must be defined, based on the concepts described in this text, as well as the 19 objectives described by the workgroup in (HC/HIT) [33].

Table 18.1 contains the requirements proposed here, organized into the two dialogue strategies, in order to show how the example Health CA supports the effective health communication action. The third column illustrates the HC/HIT objectives, with their respective numbering, associated with each requirement.

Table 18.1 Monitoring and interventions for communication with people with LHL

Strategy and requirement	Requirements with example functionality and dialogs between a patient and a Health CA	Objectives to improve communication with LHL people
S1–1	Enable connectable dialogs with other skills. (example: Connection with a smart watch) ensuring the individual's self-perception during the activity (autonomy, competence, feeling of connection with other people)	Increase the proportion of adults offered online access to their medical records—HC/HIT-06
S1–2	Guide the importance and greater effectiveness of the plan so that the patient is confident that they will be able to follow the agreed plan. Example: The CA asks the user to choose what he/she needs to do, when and how often	Increase the proportion of adults whose healthcare providers involved them in decisions as much as they wanted—HC/HIT-03
S1–3	Check the user's understanding of something explained. Example: The CA asks the user to explain how they will reduce the amount of sodium	Increase the proportion of adults whose healthcare provider checked their understanding—HC/HIT-01
S1–4	Provide an explanation with a clear vocabulary, for the delivery of personalized content, which is reliable and with both sides covered (positive and negative) [20]. Example: When the patient says they are not willing to eat without salt, a story is given to them in another way, in order not to provoke resistance to the treatment	Increase the proportion of adults who report that their doctors or other health providers always explained things in a way that was easy to understand—HC/HIT-D11
S2–5	Present information in appropriate modalities (graphics, images, icons, arrows, hyperlinks)	Increase the proportion of people who say their online medical record is easy to understand—HC/HIT-D10
S2–6	Provide a means of communication for the user to have support from the agent together with the healthcare provider and/or family. Examples of a CA dialogue to report problems, could be: "Explain to me what did not work as planned"; [32] "would you like me to call someone to talk about this?"	Decrease the proportion of adults who report poor communication with their healthcare provider—HC/HIT-02
S2–7	Promote engagement (i.e., express empathy, accountability, and commitment) using different means of dialogue [64] and a personalized dialogue to remedy problems. Examples of CA's lines are [62]: Hello, would you like to try another plan?... Do you have a new idea?	Increase the proportion of messages in news stories that show empathy, accountability, and commitment—HC/HIT-D04

18.4.3 Modeling of the Dialogue with the Health CA in Development

The clinical pharmacist works with the multidisciplinary team, preventing, detecting, and solving problems related to therapy, both during hospitalization and discharge from the hospital, and prepares the Pharmaceutical Guidance Table (PGT) [65] and a care plan [66].

In this case, the purpose of using Health CA is educational, and the patient and their caregiver need relevant guidance and instructions to delay kidney failure and end-stage kidney disease for this individual.

The first strategy is a dialog model to configure the Health CA according to the PGT of a patient, which contains the patients' medication plan and the contact of the healthcare provider [65]. In the second strategy, the CA presents intervention scenarios, reminders for the patient to take their medications, motivational advice, and explanations, and if necessary, it enables communication with the professional.

The Health CA approaches educational topics such as correct mode of use, storage of medicines, hand hygiene, etc.

The dialog model for a certain topic complies with the requirement S1–4 in Table 18.1, that is, the concept of hyper stories structured the dialogue [67], providing contextualized explanations in relation to the user's knowledge. Depending on the answers, the dialogue can lead the user (patient) to one or the other path. The hyper story on a topic with questions/answers is triggered by to some event or stage of treatment on the seventh day after the patient has started the care plan and is repeated every 2 days (see Fig. 18.5).

The advantage of this way of organizing the care plan by hyper stories is the simplicity of communication. It is possible to use different communication modalities to help LHL users. In this case, a video was projected, but electronic or even printed notebooks can be distributed to the patient. This material must be made by the various members of the healthcare team.

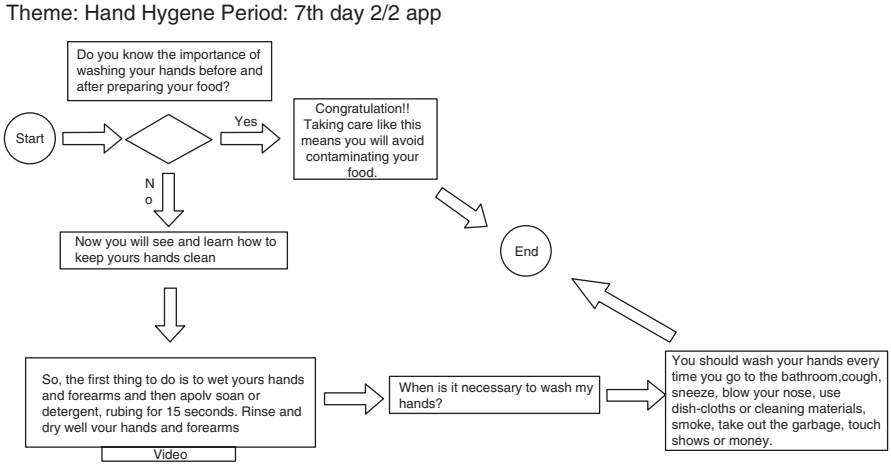

Fig. 18.5 Interaction dialog flowchart

The CA also maintains dialogues to intervene and remedy problems in a personalized way that complied with requirements S1–3, S2–6, and S2–7. As an example (see Fig. 18.6), we defined a scenario "drug-related problem" where: (a) the CA

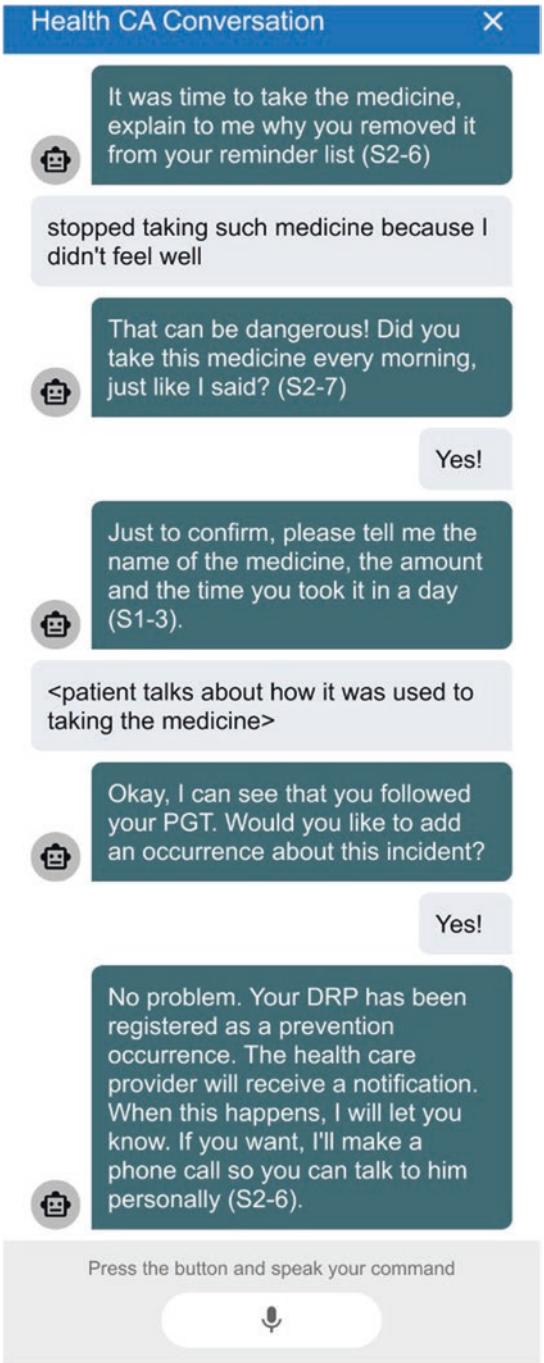

Fig. 18.6 Dialog description between a patient and the Health CA in development

adjusts to the patient's responses; (b) the CA acts to reinforce that the patient should take their medications properly; (c) the CA calls for the healthcare provider.

In an interaction with questions/answers, breakdowns in conversation are common, leading to negative user experience [68]. Repair and pattern strategies (confirmations, providing options, explaining) must be applied. Thus, it is important to define the phrases that the assistant should speak to the user. Each sentence corresponds to a command spoken by the assistant. If a user's possible responses are already part of a command, the user must choose a response option. But when users' responses are open, there is no predictability of what a user can say. For this, a thesaurus is created, containing alternatives for user input, which are the possible responses of users throughout the interaction.

18.5 Opportunities and Risks

Opportunities and risks are presented in the form of the topics given below.

18.5.1 Implementation of a CA and Risks of Interaction Bias

When implementing a CA, there are risks of interaction bias caused by factors that affect the voice (such as education level and culture) [68, 69].

There are practices and laws for medical ethics in clinical research which have different levels of acceptance depending on the country. This fact can be called ethical dumping, which can be described as the export of sensitive ethical practices from more developed countries to less developed countries, which have requirements below the average standards, and would be inadmissible in developed countries [70]. Similarly, there are also studies on the lack of ethics in conversational systems. An example is a CA being less efficient in understanding the speech of a user who lives in a less favored region in relation to another one who lives in a "better" region [29]. To address this problem, the Speech Recognition module (Presentation layer of the Health CA architecture) must be trained in voice datasets that address the diversity of accents, dialects, regionalisms, and common expressions. Here again recruitment of individuals from less developed countries will be needed, and the CAs should develop requirements based on regulations and transparency. Otherwise, CAs will not be a viable solution to reduce disparities in healthcare service use and/or health outcomes among different racial, ethnic, cultural, or age groups.

18.5.2 Content Validation and Curation Opportunities

As previously mentioned, the validation activity has not yet been performed. However, a method applied in the evaluation of educational artifacts in the health area is presented and discussed here, but which does not yet have a use in CA [71].

The validation method is the inspection of the material produced, in which experts check several items of each of the evaluation criteria [72]. Specialists are usually researchers and professionals working in the areas of pathology, whose treatment technology seeks to support, as well as design the information. The criteria with their associated items are given below. In the Objective criterion, the following is evaluated: if the content is compatible with the target audience; whether the information/content can guide patients about the importance of self-care; if the content is motivating and encourages the individual to continue interacting with the technology; and whether the content can circulate in the scientific community in the field of public health. In the Structure and Presentation criterion, the following issues are addressed: attractiveness, correctness, organization, clarity, and simplicity. Finally, in the Relevance criterion, the following are evaluated: whether the tool allows the patient to acquire information and clarify doubts; whether the content encompasses essential points the patient needs to know; and whether the material is suitable for use.

The criteria go beyond validating content, they are about patient interactions with these contents, as well as the involvement of the health team and family care.

For the method to be adapted for validation of content and interaction scenarios to be delivered to patients, as described throughout Section 4 of this chapter, it is suggested that Table 18.1 is analyzed, as described in Section 4.3. Specifically, check whether the criteria being proposed in this method validate the defined requirements. For example, we see the possibility of using the Structure and Presentation criterion to validate requirement S1–4 on the importance of reliability in health information. That is, this criterion affects the way the user perceives and judges the information obtained from a CA, thus helping the user to discern between sources of health information, which is a key component for digital health literacy [73].

Other kind of validation need to be applied, studying whether virtual counselors become emotionally, socially, and/or culturally competent, they will maintain, decrease, or increase people's openness and self-disclosure with computer-based health interventions [74]. Designers and AI engineers must collaborate with health professionals in new ways to create both the material and its application experience [75].

18.6 Conclusion

In this chapter we describe an approach to developing a Health CA presenting its architecture, characteristics for designing natural language dialogs, and the requirements for effective health communication with LHL patients. It was applied to illustrate an educational interaction scenario with a CKD patient as a user. It is still necessary to implement a health CA developed with this approach in a real context, to validate the effectiveness in getting LHL users to monitor themselves, with the necessary automatic interventions and human integrations. This study will also evolve to include other dialogue strategies, such as diagnosis, as well as considering other guidelines and organizational plans, which involve experts and society.

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Chapter 19
Mobile Health in Nephrology

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19.1 Introduction

Mobile health (mHealth) has been defined by the World Health Organization as a medical and public health practice supported by mobile devices (e.g., mobile phones, patient monitoring devices, personal digital assistants, and other wireless devices) [1]. In intervention studies, mHealth has improved patient-reported results, reduced the use of health resources, and saved health service funds [2].

Mobile technology has unique characteristics, such as quick and easy access, and has become part of the routine for several people around the world. Mobile applications (apps) are well accepted by patients and have more effective therapeutic results than using flyers and pamphlets for health education [3]. In nephrology, mHealth

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has been used to help patients with kidney diseases by managing their lifestyle and monitoring their diet, treatment, and symptoms, especially in the context of chronic kidney disease (CKD) and kidney transplant [4].

Most mobile apps available provide information on the complex treatment of kidney diseases, possible drug and/or food interactions, adverse reactions, and contraindications. Hence, mHealth technology is an innovative strategy that helps patients have correct and safe therapy, adhere to medication use, manage diseases, and control vital signs. It is a growing industry, with a great potential to improve self-management in people with CKD [5].

Although it is a promising technology, it is not clear whether people with CKD are ready to use mHealth in their everyday health care. This is due in part to the many apps available and, in many cases, the little information provided by developers regarding their function, quality, and usability so patients can choose the most adequate one. Moreover, health apps are not currently required to be scientifically based, certified, or regulated, so consumers are at risk of choosing clinically inadequate and potentially harmful ones [6].

19.2 mHealth Apps

Various classifications are used to describe mHealth apps. A simple method categorizes them as either passive or active. Passive mHealth apps show pages with statistical health information or allow users to enter health information with manual keys. In contrast, active mHealth apps—the group to which sensor-based mHealth belongs—generate health data [7]. Billions of mobile device users worldwide can easily access smartphone integrated sensors. Environment and position sensors have been integrated into ever-advancing devices to increase and improve their functioning—microphones detect sound, cameras register the visual world, and touchscreens make it easier to access their flexible displays and initiate commands [8]. For instance, sensor-based apps available in the main app stores are used for imaging diagnosis (using the camera to verify eye and skin condition), photoplethysmographic monitoring (estimating the pulse and arterial pressure), and sound diagnosis (using the microphone to verify sleep apnea) [9–11].

Apps stores, such as the ones by Apple and Google, return literally millions of search results on health, giving options to users, though making it harder for them to choose the best app. User satisfaction, convenience, efficiency, effectiveness, and learning capacity are some of the most considered attributes when defining the usability of an app. Information technology scholars believe that usability tests should focus exclusively on the target audience of the app. This would help the software have a friendly interface, and end-users would be able to effectively benefit from it [12]. A good app must be fast and dynamic, provide the user a pleasant experience, and have functions and characteristics that ensure its adequate use.

Information on app function, aesthetics, and usability can be obtained while using it. However, the lack of adequate quality indicators makes it less usable by patients and professionals. These products usually lack details on information precision, personal data privacy, safety, and clinical effectiveness. Moreover, there is no consolidated service where overall and specific app conformity and clear indication for use can be verified [13].

Online platforms that certify the operationality of mHealth apps may play an important role in raising awareness of the quality of mHealth material distributed to patients and health professionals. Happtique is one of such platforms, providing mHealth solutions to all fields of health, with the mission of integrating mHealth into patients' care and daily life. It uses mobile platforms to help health professionals prescribe mHealth apps and engage patients in actively monitoring and managing their health [14].

19.3 Apps for Kidney Healthcare

Apps are being increasingly used in nephrology. Hence, studies aim to provide information on apps with good usability and content, based on guidelines and specialized scientific literature. In 2014, Diamantidis and Becker reviewed the use of mobile technology to treat CKD patients. They revealed that, from the first instant messages and early Internet connection to current mobile apps, device accessibility, app usability, and their novelty were the main factors for users' willingness to use such technology. They pointed out that most CKD patients expected to have greater contact with their physicians by using an interactive system. They also concluded that mHealth use is an advancement in the treatment of kidney diseases, as it provides innovative solutions to inform and engage patients and improve patient–physician communication [15].

Many aspects can be addressed in CKD self-management, such as specific knowledge of the disease, self-care behavior, self-management of symptoms and problems, interdialytic weight gain, and management of drugs. It can also help obtain and maintain social support, maintain social and occupational roles, change lifestyles, develop and maintain a positive attitude and care with mental and physical well-being, and manage the time until the beginning of renal replacement therapy (RRT) and survival after beginning it [16].

However, current CKD apps lack functionalities to change the lifestyle, build and maintain an effective relationship with health professionals, manage drugs, obtain and maintain social support, and maintain social and occupational roles. This last item includes continuing to work, having a hobby, and maintaining domestic relationships and roles. Therefore, when developing self-management apps for CKD patients, these functionalities should be improved to meet their needs and provide patient-centered CKD care [17].

In a systematic review of the literature, Siddique et al. [18] assessed mobile apps that would be possibly used by CKD patients, helping them adhere to medical treatment and nutritional therapy. The authors searched the two largest mobile app platforms (Google Play Store and Apple App Store), using 13 related search terms, and retrieved 431 apps. Of these, 235 were duplicates, and 162 were removed for not segmenting CKD patients. Of the remaining 34 apps, 18 were excluded after the final assessment because of their limited functions, and four were excluded because their language was inadequate. The definitive analysis had 12 apps. They were assessed with the Mobile App Rating Scale (MARS), which has four broad categories of objective quality criteria, namely: app engagement, functionality, aesthetics, and information. Overall, 11 (91.7%) apps reached the minimum acceptable score (3.0) in the MARS classification. Five of them were developed in collaboration with respectable specialized organizations – e.g., the National Kidney Foundation (NKF), in the United States, the National Kidney Foundation Malaysia, and the National Health and Medical Research Council (NHMRC), in Australia. The high information scores obtained by these apps may be ascribed in part to these organizations' involvement, as credible and legitimate sources of information are part of MARS assessment sub-criteria [18].

Another study assessed apps for CKD patients, available in Apple App Store (in the United States) and Google Play Store. It found that most of the 28 apps they assessed were free to download, aimed at adult CKD patients that were not on dialysis and their caregivers, and focused on patients' education. Assessments were made by patients and nephrologists; however, patients, nephrologists, and consumers usually disagree about the quality of an app, which potentially results in diverging perspectives on what makes an app useful. Only two out of the seven apps with tracking functions warned users when they entered extremely abnormal values, raising concerns about the safety of these apps [19].

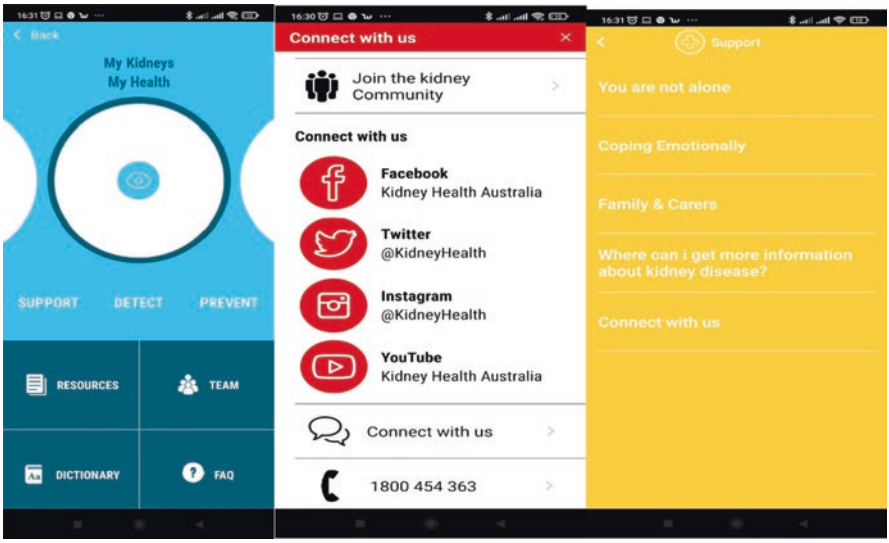
Apps used for self-management of CKD are presented below. Some are intended to prevent CKD progression, others are used to manage some specific elements involved in the complexity of the disease.

19.4 My Kidneys, My Health Handbook

This app was developed by Kidney Health Australia and is freely available in English at the Apple and Google stores. Its purpose is to educate and support people with CKD from its initial stages. The main resource is educational information on how to detect kidney diseases and self-manage them. It is interactive, assessing CKD risk and providing self-management tools. It reminds them of appointments

and medicines and calculates and monitors their water intake, weight, and phosphorus [18].

The app also gives support information about the Kidney Health Australia community, with direct links to this and other resources on kidney diseases and the story of different people with kidney diseases. The following functionalities are the most assessed by both professionals and patients: education on CKD, education on hypertension, education on diabetes and glucose control, education on cardiac diseases, and information exchange via text messages, e-mail, or social media [19].

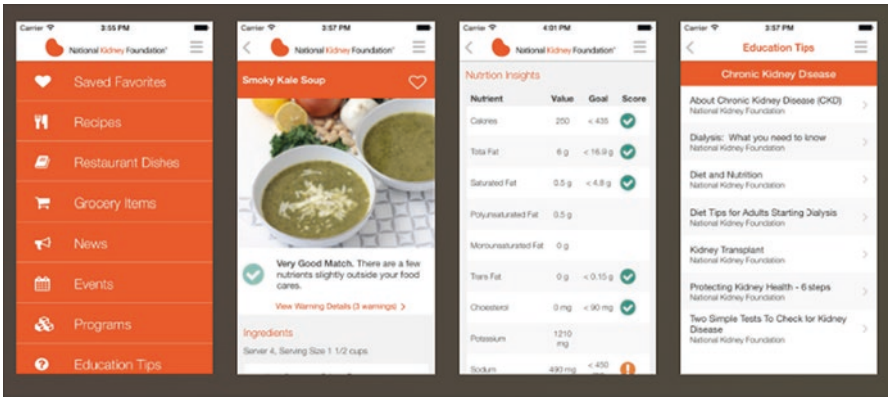


Source: Google Play Store.

19.5 My Food Coach App

This diet-focused app was developed by the National Kidney Foundation, USA, and is available for free in English in the official Apple and Google app stores. A restricted diet is one of the pillars of CKD treatment. Therefore, patients must adhere to adequate nutrition and manage it. However, they may feel overwhelmed by the complexity of the diet and feel strongly discouraged to follow it. This app was designed as a portal, where users can get personalized information, support, and education on nutritional needs and changes that may slow the progression of

kidney diseases. The user profile helps find recipes and ready meals in supermarkets, control weight, and create a library with favorite foods/recipes. The information in the app is based on American instructions, which may not apply to other countries whose cultures and lifestyles are different [18, 20].



Source: https://www.healthnavigator.org.nz/apps/rn/my-food-coach-app

The app stands out for its engagement, especially because of GPS, which finds restaurants and their menus. Moreover, it is highly interactive, supporting access to a registered nutritionist [18].

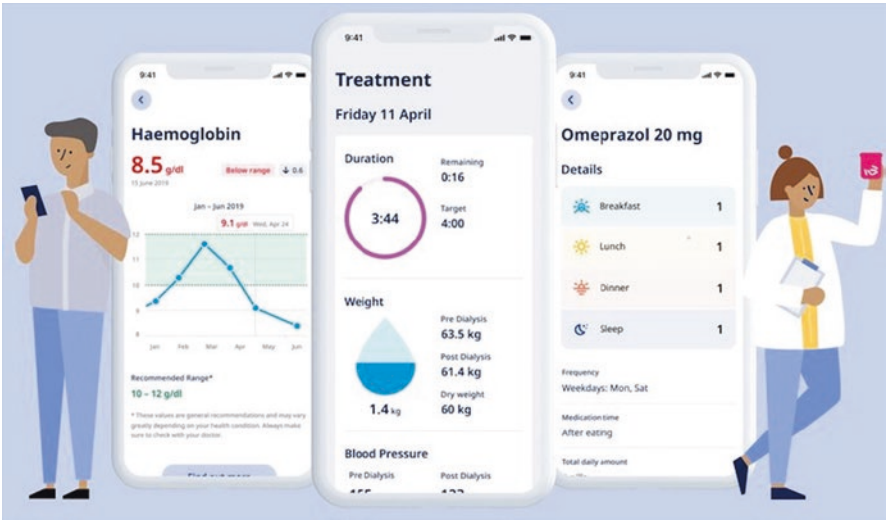
19.6 H_2O Overload

H_2O Overload is an app developed by the National Kidney Foundation, USA, to control the patients' water intake. It is freely available on IOS and Google platforms. Although it does not have many features, its functionality is rather important because limited water intake is one of the main obstacles reported by CKD patients. Its resources track weight, liquid intake, and arterial pressure, inform about kidney diseases, remind of appointments, enter data on drugs, take notes on questions to make to physicians, and send them e-mails with graphs of the patient's progression (arterial pressure, weight, and water intake) [21].

19.7 Fresenius myCompanion

This app is free and available in the two main mobile app stores (Apple Store and Google Play Store). However, it is only for patients registered at the Fresenius Medical Care dialysis clinic—a multinational German company that provides

products and services for dialysis. The app allows patients to see all data on their treatment (e.g., prescribed drugs and laboratory examinations) and have a more active role in their therapeutic plan. Furthermore, information available on the mobile phone can be easily shared with friends and family. Fresenius Medical Care highlights that patients need an access code (generated by the company's EuCliD system) to set up their account [22].



Source: https://fmcna.com/insights/amr/2021/connected-health-fresenius-medical-care/

19.8 MiKidney

MiKidney is an app developed in Ireland in 2016 to record detailed personal data, medical history, blood data, weight, and current list of drugs. It provides information on CKD, drugs for CKD, renal diet, RRT options, symptom management, and health maintenance. The app also tracks exercises, records daily physical activities, reminds patients of their commitments, and saves questions they need to discuss with the multidisciplinary team. Moreover, the app has a traffic-light score system – My Renal Rating—that gives feedback to users, including motivational messages [23].

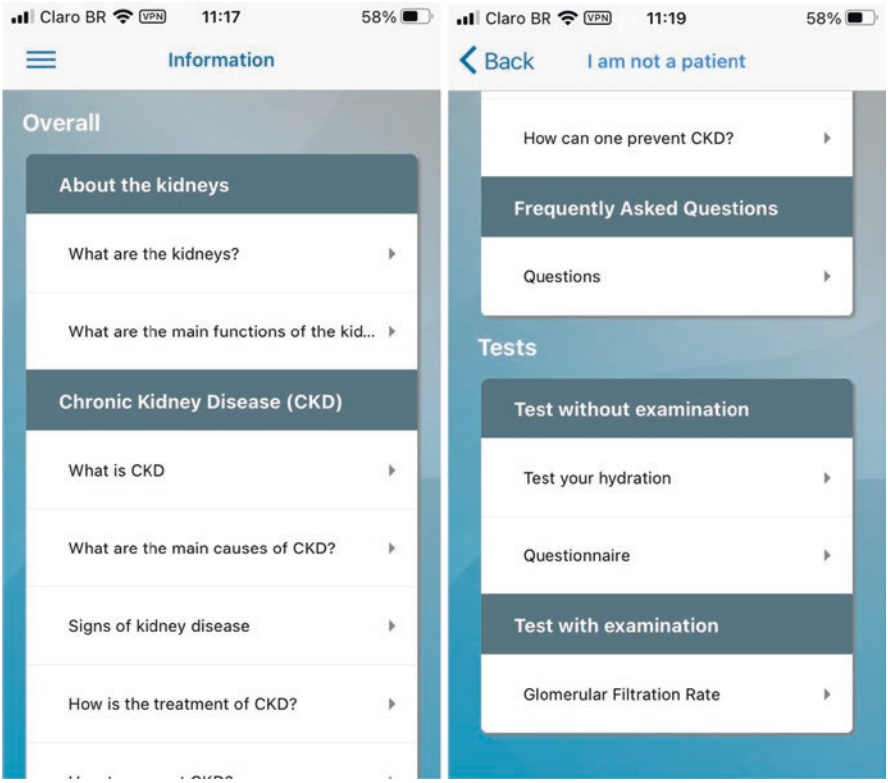
Patients get positive feedback on drug and diet adherence, improving their symptoms and renal function parameters in real time. MiKidney has the potential to get patients actively involved in consultations with health professionals, providing necessary real-time information to both parts in a readily accessible platform [23].

19.9 Renal Health

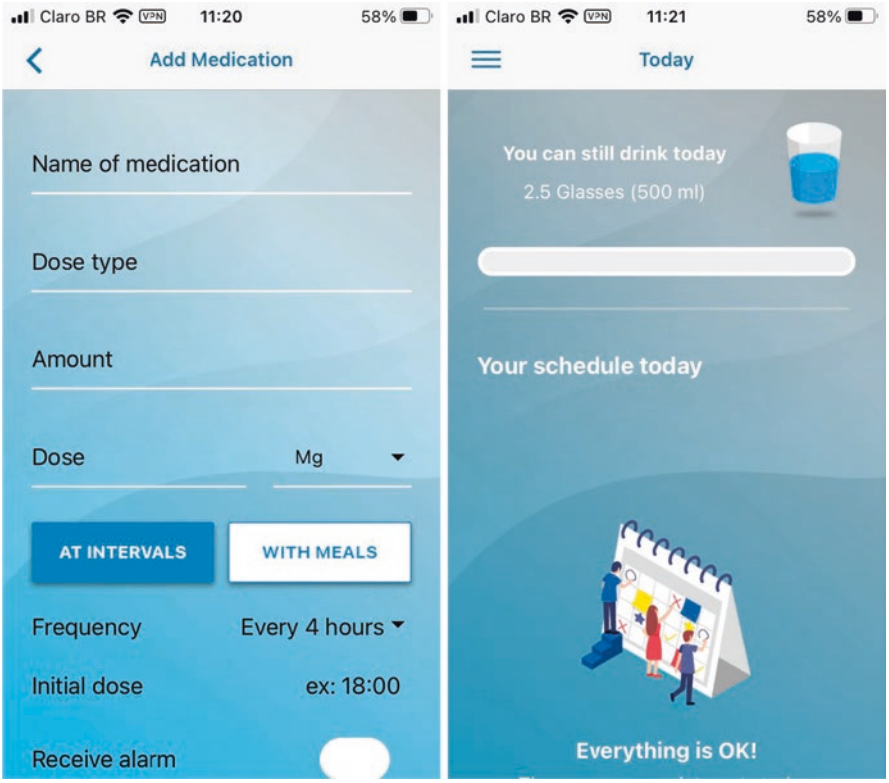
Renal Health is a free mobile app developed in Brazil, available in Portuguese, Spanish and English, at the Google Play and Apple stores. It has functionalities for the population without a kidney disease, with information about CKD diagnosis and screening, as well as for CKD patients undergoing RRT—whether hemodialysis, peritoneal dialysis, or kidney transplant, focused on self-management strategies.

Those not diagnosed with CKD find information on disease definition, signs, and symptoms, preventive measures, causes, treatment, and frequently asked questions. The app also stratifies their risk of CKD and calculates their glomerular filtration rate based on a serum creatinine test [24].

As for patients undergoing RRT, the app helps self-manage the disease with water and food intake control, drug reminder, and examination follow-up with graphs and the option to indicate where they get treatment [21].



Source: Apple Store.

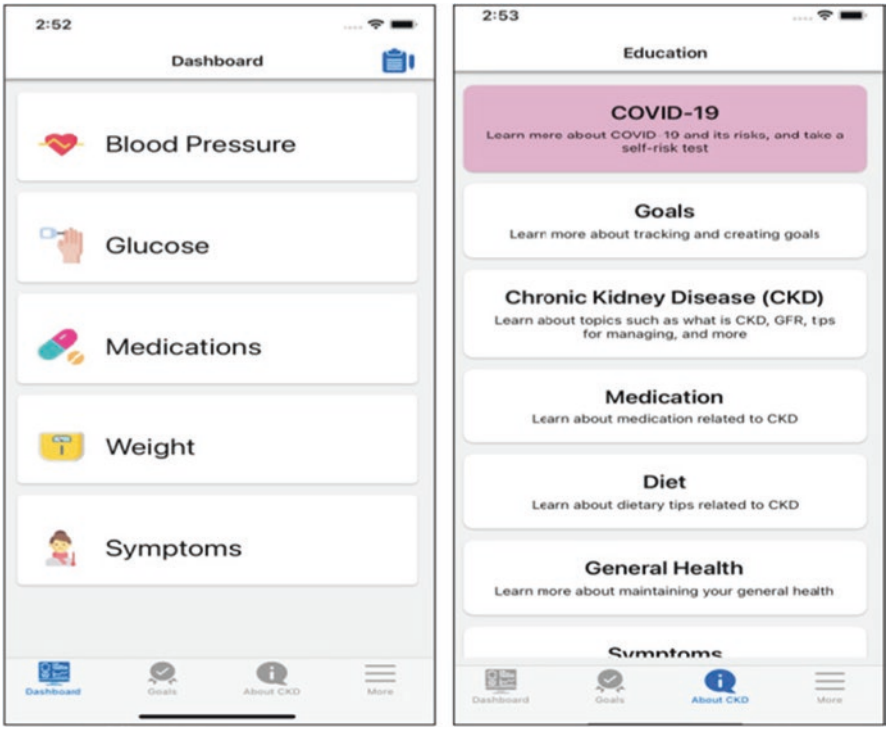


Source: Apple Store.

19.10 CKD App

More recently, researchers at Loyola University Chicago, USA, developed the CKD App, intended for CKD patients not undergoing RRT. It is available in Apple Store and its purpose is to improve CKD self-management with a holistic approach by patients who are not dependent on dialysis. Users can enter health data, including arterial pressure, weight, blood glucose levels, symptoms, and severity. The app also sets personalized goals (using colors to interact—green for goals they have achieved and red for goals they have not achieved yet), records drugs they are currently taking, and reminds users to take them. Patients can also use it to join recurrent synchronous meetings with support groups focused on CKD education and mediated by a health professional. Due to the COVID-19 pandemic, the app has an interface informing signs and symptoms of the disease, tracking symptoms of

COVID-19, informing when they should contact the physician, and tracking influenza and COVID-19 vaccinations [25].



Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8717130/#box1

19.11 MultCare

MultCare app is adaptable to multiple technologies (such as mobile phones, tablets, and computers). Apps like this one, whose interface runs on any type of browser, can be more broadly used. It was developed by Brazilian researchers, and its public audience is people without TCKD. Its main purposes are to monitor risk factors and follow up and slow down CKD progression. Hence, they considered the two main risk factors (diabetes and hypertension), which are also the most relevant aspects in monitoring. Users can also enter and maintain data on prescribed drugs, allergies,

and examination results – which helps identify the risk of the disease. Thus, patients in its earlier stages can be referred to a nephrologist and prevent future clinical complications due to late diagnosis. The app developers followed the KDIGO, KDOQI, European Society of Hypertension, and European Society of Cardiology guidelines for the management of arterial hypertension [26].

19.11.1 App Characteristics for Good Effectiveness

Apps are known to positively influence kidney disease self-management, both in its early stage or in RRT. Therefore, it is a great aid to health professionals. Technological advancements and the diversity of apps available in platforms help increase their use. However, the effectiveness of this tool depends on some factors.

19.11.2 Reaching People of Different Ages and Social Classes

Considering that most CKD patients are older, the interface must have large and clear texts with simple and objective language. Free access in a variety of platforms is also important to increase accessibility [27].

19.11.3 Having Resources to Help Manage Various CKD Problems

Many apps only give basic information on the disease or a few treatment-related items, such as their diet, drug use, or water intake. The more comprehensive the app—encompassing CKD progression, diet treatment options, lifestyle problems, physical activity, drugs—the more attractive it is to patients [27]. Unlike other chronic diseases, CKD patients usually have comorbidities, such as diabetes, hypertension, obesity, and cardiac diseases. Therefore, a CKD app must address these multiple facets of disease self-management.

19.11.4 Interactive Resources

It is greatly important to give users the option to enter data on their health status and contact the health team when necessary. Most CKD apps do not address clinical care nor promote health behaviors with motivational feedback, goals, or interaction with professionals [25].

19.11.5 Data Security and Privacy

App security is essential to keep hackers from accessing information or using cryptography to introduce malware into the software, thus intercepting or stealing confidential health data [27].

19.12 Conclusion

Mobile devices are part of the population's daily life, bringing about significant changes in people's lifestyles. Their impressive growth has led to the appearance of countless apps developed to help common users and health professionals. The use of mHealth technology promotes health care and monitoring. Its practicality and ease of access have been forwarding its great expansion. Many apps for kidney diseases are available on the main platforms. They have useful tools, as they allow patients with kidney diseases to self-manage various aspects of their health. Moreover, family, caregivers, and other professionals related to the treatment benefit from these apps. As a result, costs are reduced, and patients have a positive impact on their quality of life. Nevertheless, despite these benefits, further studies are needed because many available mobile apps are not regulated or scientifically based and did not have its impact on patients' clinical outcomes measured. These tools must be universalized, creating apps that can be used by older people and with lower educational attainment. Also, health professionals need to be made aware of these apps, to include them in their routine care for this population.

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Chapter 20
Telenephrology: A Resource for Universalizing Access to Kidney Care, Perspectives from Latin America

Carlos Zúñiga-San Martín

20.1 Introduction

In the last 20 years, the use of telehealth and telemedicine has had an explosive growth worldwide [1–6]. Through these digital modalities, care and/or actions are carried out that allow evaluating, diagnosing, treating, controlling, and educating patients, family members, and health teams. The growing number of publications related to telemedicine, and in particular telenephrology, reflects the growing interest in incorporating information and communication technologies (ICTs) in the health area [7–13].

In the nephrology community, it becomes of special interest to know the benefits that telenephrology could provide in the prevention and comprehensive approach to chronic kidney disease (CKD), one of the main health challenges that impacts public health worldwide [14–19]. The growing demand for care and treatment of people with CKD, associated with the shortage of nephrologists, requires the search for and implementation of innovative models that allow a timely response to these demands [14–21].

In this chapter, a proposal is presented in order to use telenephrology as an articulating instrument in a systemic strategy to address CKD in a health network, supporting the integration and coordination between primary health centers (PHCs) and the most specialized levels.

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It is expected that digital modalities favor early diagnosis of the disease, timely treatment, and quick access to specialized care for people with CKD.

The proposal is based on the premise of person-centered care and telenephrology (TN) experiences reported in publications of the specialty and in Latin American countries, some already consolidated and others under evaluation as proposals for development and public policies in renal health [7, 18–21].

Likewise, some telematic continuing education experiences carried out by regional scientific societies and the use of ICTs during the COVID-19 pandemic are reported, a period in which a more frequent and widespread use of telemedicine has been observed.

20.2 Chronic Kidney Disease: A Silent Epidemic

CKD is a public health problem with an alarming increase in its incidence worldwide, which is associated with the sharp prevalence of the two highest-risk diseases: high blood pressure and diabetes mellitus, and the low levels of control of these pathologies at a world range [14–18]. In this regard, health organizations and international scientific societies have pointed out the urgent need to implement public prevention policies [14, 16, 18, 22–27].

There is evidence and consensus that early diagnosis and timely treatment of CKD are especially important in the evolution of the disease, since both actions can slow down or stop progression to advanced stages, along with preventing complications, reducing associated cardiovascular events, and avoiding the need for renal replacement therapy (RRT) [14, 22–27].

According to the Kidney Disease Improving Global Outcomes (KDIGO) classification, most guidelines recommend that patients with CKD stages 1 to 3a should be monitored in PHCs and those in advanced stages 3b to 5 should be referred to a nephrologist for more specialized study and treatment [14–19, 24–27].

Compliance with this last recommendation is limited in many countries, due to the lack of specialists reported worldwide, being more critical in low- and middle-income countries [16, 18, 28–30]. The shortage of specialists makes it necessary to prioritize in-hospital nephrology care and postpone the evaluation of patients referred from primary care, with the consequent increase in waiting lists and delay in the timely diagnosis and treatment of the disease.

In Latin America, CKD is also one of the main health challenges affecting the quality and life expectancy of people and the management of health systems in the region, ranking second as a cause of quality-adjusted years lost [18].

Unfortunately, only isolated epidemiological data are available on the prevalence of CKD in sporadic population surveys in some Latin American countries. Available information comes from data obtained from the ERC and/or RRT records. Since 1991, the Latin American Registry of Kidney Dialysis and Transplantation

(RLDTR) has collected data on patients undergoing dialysis or transplant therapy in countries affiliated with the Latin American Society of Nephrology and Hypertension (SLANH) [31]. Its latest report, in 2019, describes a sustained increase in prevalence, with currently more than half a million patients receiving RRT in Latin America, of which 150,000 people were admitted in the last 10 years [18, 30–32]. This access to RRTs is not homogeneous, and there are great inequities among the different countries, but also within countries [18]. Access to RRT in Latin America is strongly linked to the income level of the countries: in those with high income, more than 75% of patients have access to RRT, while in those countries with medium or low income, the percentage of patients that have universal access to RRT is less than 20% [18].

Regarding the diseases with the highest risk of developing CKD, diabetes mellitus (DM), and arterial hypertension (HBP), both show an alarming growth in Latin America in the last 20 years. Diabetes mellitus (DM) is one of the main health problems in Latin America and is the fourth cause of loss of healthy life, reaching the first place in several countries of the region [33]. The number of people with DM has tripled in the region since 1980, and it is estimated that of the 33 million who currently suffer from it (excluding Mexico), the prevalence will reach 49 million in 2045. The increase in prevalence has been greater and faster in low- and middle-income countries than in higher-income countries (https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf).

In turn, between 20% and 40% of the adult population in the region suffers from hypertension (in some countries this percentage reaches 48%). This figure implies that around 250 million people suffer from high blood pressure (https://www.paho.org/es/temas/hipertension).

Along with the above, the lack of specialists reported worldwide reaches critical levels in some Latin American countries. In the last report presented at the SLANH 2019 Congress in Lima, Peru [31], the average number of nephrologists in Latin America was 16 pmp (range 4–51), a rate lower than the 20 pmp agreed by SLANH and PAHO as a reasonable objective [18, 31, 32]. Likewise, it is presumed that the number of renal professionals in nursing, nutrition, social service, and psychologists is also insufficient to address the demand for face-to-face care in each of the stages of CKD.

The complex epidemiological context and its impact on people's health urgently require policies to promote kidney health in the population and implement strategies focused on prevention that facilitate early diagnosis, stop or slow progression, reduce cardiovascular morbidity and mortality, and allow timely and informed admission to dialysis/transplantation or non-dialytic conservative treatment in the final stage [14, 16, 18, 22, 34]. Likewise, since diabetes mellitus and arterial hypertension are predisposing diseases, their timely diagnosis and treatment should be incorporated into CKD prevention programs at the primary care level [14, 16, 18, 22, 34].

20.3 Role of Prevention in a Renal Health Strategy

As an objective, prevention considers a wide variety of interventions aimed at reducing risks or threats to health [22–27, 35, 36]. A preventive model in CKD should be based on the three classic categories of prevention: primary, secondary, and tertiary [35, 36] (Fig. 20.1).

In primary prevention, interventions are aimed at avoiding the disease in the population at risk. Education and promotion of healthy lifestyles, as well as adequate and timely screening and control of people with risk factors or those susceptible to developing CKD, such as diabetes mellitus, high blood pressure, obesity, cardiovascular disease, among others. Secondary prevention addresses the therapeutic management of CKD already diagnosed, and its interventions aim at investigating, stopping, or slowing down the progression of the disease and preventing complications.

Finally, in the advanced stages of the disease, tertiary prevention, along with preventing, screening for, and treating associated complications (especially cardiovascular, anemia, and bone/metabolic complications), is aimed at promoting informed and timely admission to any of the four treatment options: hemo/peritoneal dialysis, kidney transplant, or conservative/palliative treatment. These interventions focus on preserving the person's functional capacity and residual renal function and improving their life expectancy and quality of life [35, 36].

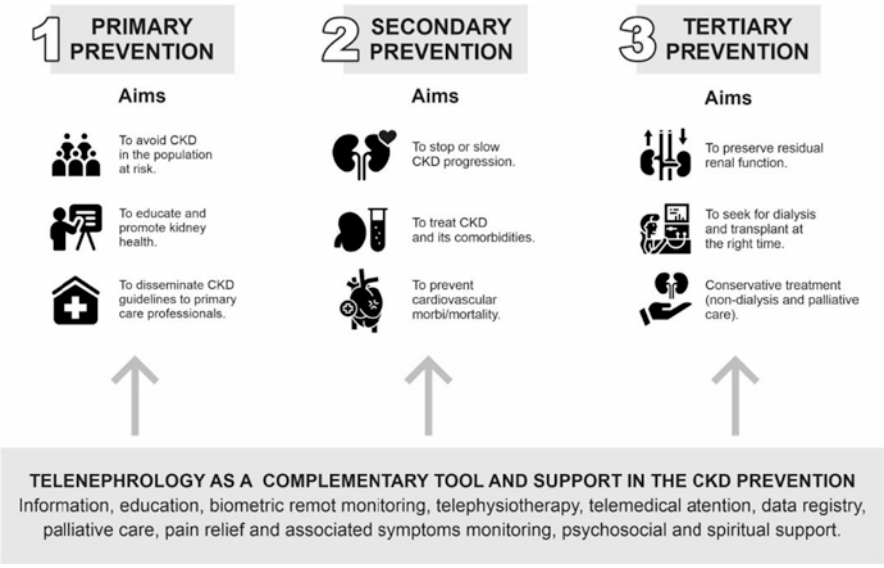

Fig. 20.1 Objectives of CKD prevention. Complementary role of digital nephrology

20.4 Computerization and Prevention of CKD

Considering the need to facilitate decentralized and agile management in a preventive strategy, it has been proposed to develop models that consider the use of ICTs in renal health programs, including the telemedical care modality (synchronous or asynchronous), monitoring and control distance, data recording/analysis, and education of people with CKD [7–13, 18, 37–47].

The scope of application of ICT in healthcare is very changeable due to constant technological advances. In practice, the different concepts used can be confusing, and, in many cases, there are no complete and definitive definitions. In this chapter, the terms telehealth and telenephrology refer to the following definitions:

(a) Telehealth is an umbrella term that covers a wide range of health and care services delivered through information and communication technologies (ICTs). It encompasses a growing variety of applications and services using two-way video, the Internet, email, smart phones, wireless sensors, and other forms of telecommunication technology. These applications support long-distance clinical healthcare, videoconferencing, transmission of still images, remote monitoring of vital signs, patient and professional health-related education, public health, and health administration [39].
(b) Telenephrology (TN) is a term that refers to the application of telehealth in kidney care. TN facilitates direct communication between providers (PCPs and kidney specialists) or between patients and providers to exchange information for care delivery [39].

The use of ICTs in the three categories of prevention described above is a valuable support tool that facilitates continuity of care, articulation, and coordination within and between the different levels of care in a health network, especially when there is difficulty of access to professionals for a face-to-face consultation. Likewise, it facilitates expedited communication with the patient and better control of risk factors, particularly in people with a higher risk of complications, allowing continuous monitoring of health data. Figure 20.1 shows the telematic benefits that support CKD prevention, from kidney health promotion to RRT and palliative care at the end of life.

Computerization in nephrology, along with supporting access and care for patients with CKD, generates a large amount of clinical and socio-demographic data (big data), which, when analyzed by artificial intelligence, will provide valuable information and knowledge for decision-making in health teams with more precision, as well as for the development of clinical guidelines and public health policies [45]. In addition, it will allow optimization of human and material resources, integration of both clinical and administrative processes, information registration, and reduction of health expenses. Figure 20.2 summarizes the benefits and services that digital nephrology would offer people with CKD and their families to strengthen renal prevention interventions.

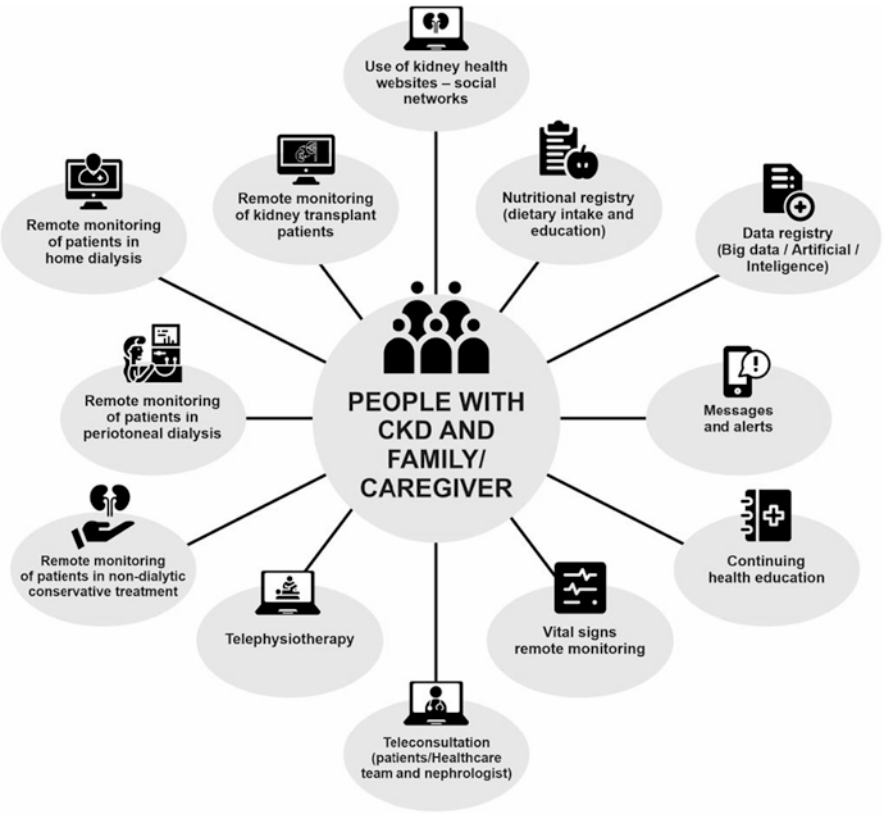

Fig. 20.2 Health assistance and services provided by digital nephrology to CKD patients. Modified from Stauss M et al. [47]

20.5 Telehealth in a Systemic Strategy of Network Renal Health: A Proposal Under Evaluation

As part of a health network, theoretically, patients with CKD move between the different levels of care according to the stage of the disease and clinical requirements. However, these levels of care are usually not integrated or coordinated with each other, which affects the continuity of care and timely care of the patient.

The proposal to incorporate digital technology into a systemic approach strategy for CKD considers the use of ICTs as articulators for the integration and coordination of promotion, prevention, and care work in a health network, from primary care to the most specialized levels (Fig. 20.3). In turn, the use of ICTs allows the collection and sharing of relevant clinical and epidemiological information for health management in the respective geographical area, as well as making diagnostic and therapeutic decisions in a timely, expeditious manner and efficient.

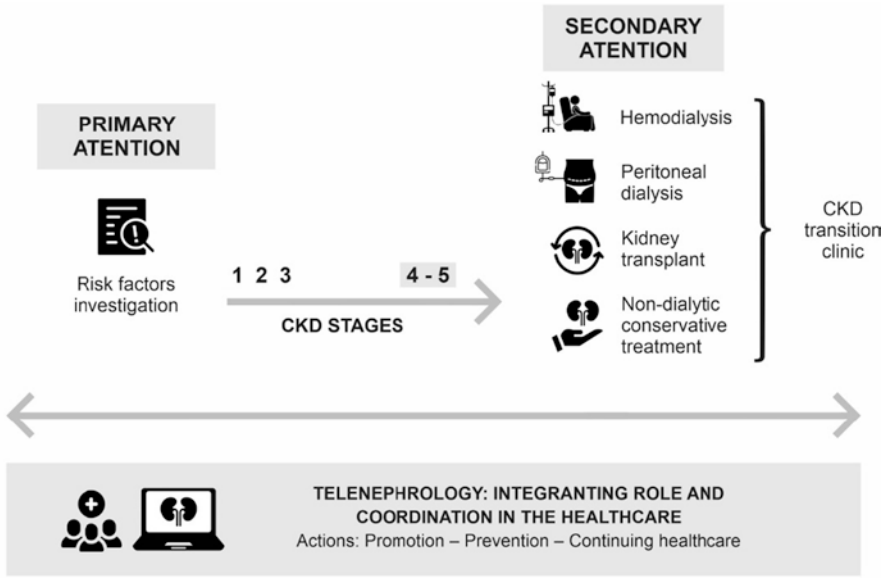

Fig. 20.3 Telenephrology: role in a renal healthcare network

The proposed modality establishes that once the person with CKD has been detected in a health network that requires specialized evaluation, the primary care physician can send relevant clinical and laboratory information through a digital platform designed for asynchronous evaluation to a teleconsultant nephrologist, who responds to the PHC doctor via the same platform. Once the clinical information is analyzed, the nephrologist has two response options (triage): (1) to counter-refer the patient back to the referring establishment to either obtain new information or undergo exams or provide suggestions or recommendations for continued treatment; (2) to refer the patient to a more complex center in the network for a face-to-face evaluation and further specialized studies or treatments [7, 47, 48] (Fig. 20.4).

For the objectives of this proposal, the asynchronous TN model is considered more effective than the synchronous TN model (video conference between the specialist and the PHC doctor) because the asynchronous model does not require both professionals to be available at the same time, uses less technology, and reduces the time per nephrologist consult [7].

This strengthens the continuity of care centered on people from the early stages of the disease until admission to renal replacement therapy (dialysis/transplantation) or non-dialytic conservative treatment, avoiding unnecessary travel and optimizing the scarce resource of specialists [7, 37, 39, 45, 46].

Expedited access to medical specialists via telematics also has a training and continuing education role for primary care professionals, especially in the implementation and application of CKD clinical guidelines, as well as support in management and diagnostic decision-making and/or therapeutic.

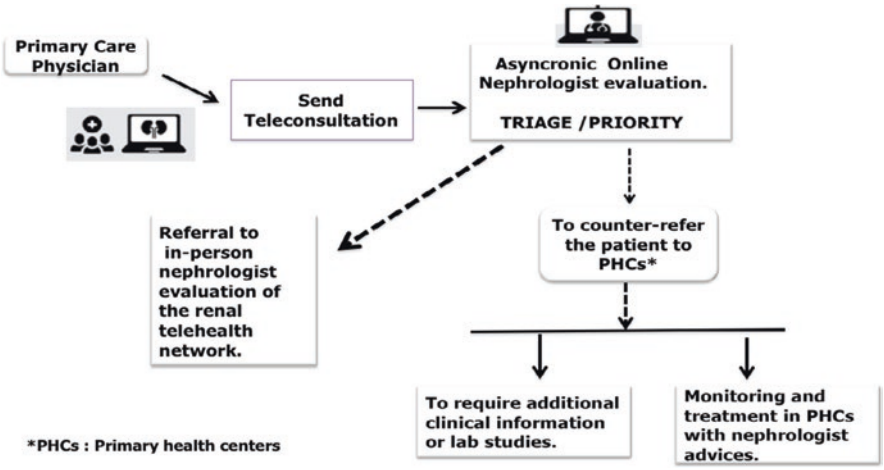

Fig. 20.4 Workflow of asynchronous telematic care by nephrology in a health network. Modified from Zúñiga C, et al. [7]

In practice, teleconsultation results in a triage, which allows selecting and classifying the clinical risk, severity, and complexity of a given health problem [46–48]. For example, it prioritizes whether the care is pertinent, urgent and whether it requires a referral for a face-to-face evaluation or can continue to be monitored electronically. The latter makes it possible to decongest medical centers and emergency services, which have been especially relevant during the current COVID-19 pandemic [11, 49]. However, the telematic modality complements but does not replace the face-to-face clinical evaluation when it is indicated.

Table 20.1 summarizes the advantages that telematic care would have in a kidney health network strategy [46].

In cases of natural disasters (earthquakes, floods) or health emergencies such as the COVID-19 pandemic, telematic care would allow continuity of care, monitoring and control of chronic patients at home, especially for inhabitants of geographically remote or difficult-to-access areas, avoiding unnecessary travel and mitigating the risk of contagion [7, 11, 46].

The disadvantages that telematic care would have in a kidney health network strategy are summarized in Table 20.2. Telenephrology initiatives are being widely accepted by practitioners and patients, but comparison with the conventional system and the impact of provider and patients' experience of care, population health, and costs is still unclear [9, 10, 39]. There are significant barriers to adoption, some common to all aspects of telemedicine and some specific to nephrology. These limitations can be categorized into four categories of issues: (1) reimbursement, (2) clinical, (3) legal, and (4) societal [9, 10, 39].

Table 20.1 Advantages of telenephrological care in a health network

Improves timely access to the nephrologist • Solves the waiting lists • Prioritizes specialized care according to the severity of the patient • Allows more decisive consultations by having exams previously requested
Improves quality of care • Defines clinical risk, gravity, relevance, care modality, triage • Provides prompt and timely care
Stimulates work in the care network • Decongests secondary level medical centers, especially in catastrophes and health emergencies • Frees face-to-face consultations by a nephrologist and optimizes the scarce resource of specialists in nephrology • Strengthens resolution at the first level of care
Strengthens people-centered care • Facilitates access to a nephrologist from remote areas or disaster situations • Equity and democratization of car • Allows continuity of care (monitoring consultations—chronic control) • Attenuates the anxiety of the person waiting to access a consultation • Avoids irrelevant face-to-face consultation
Indicator monitoring • Compiles and records clinical history and advances request for examinations • Allows monitoring management and clinical indicators • Records clinical and epidemiological data (big data), to plan interventions and public policies in renal health continuing education • Promotes continuing education, support in decision-making for clinical management and the empowerment of professionals at the first level of care
Environment and patient costs • Contributes to reducing environmental pollution (carbon footprint) by retail displacement number and face-to-face evaluations • Reduces out-of-pocket expenses for transportation

Table 20.2 Disadvantages of telenephrological care in a health network [10, 40]

1. Lack of long-term outcomes data comparing telenephrology with conventional systems.
2. Limited ability for physical exam.
3. Potential detrimental impact on doctor-–patient relationship. (tele-based vs. in-person).
4. Unproven reliability of smartphone apps.
5. Costs and lack of availability of equipment.
6. Lack of expertise in handling equipment.
7. Issues with reimbursement of providers and facilities.
8. Barriers due to medical legislation.
9. Uncertain malpractice and legal frameworks
10. Challenging for patients with hearing or visual impairments.
11. Costs and time required to acquire and train on equipment

20.5.1 Administrative, Technical, Legal, and Ethical Considerations on the Use of Telehealth in a Renal Health Network Strategy

The implementation of the proposed strategy requires changes in clinical/administrative management and a reorientation of the work of the teams in the health network at each level of care. To achieve this goal, it is essential to have the commitment, motivation, and cooperation of health planners, administrators and managers, service providers, primary care health teams, and participating groups of specialists. It is necessary to educate and raise awareness at the different levels of the network about the health impact, the high burden and cost of CKD, and to provide information on proven, feasible, and cost-effective interventions that can reduce morbidity and mortality and the cost associated with CKD [1–10, 15–19, 22–27, 35, 36, 45]. Likewise, it is necessary to promote the implementation of ICTs as a complementary technological resource to improve clinical/administrative management, data recording, continuity of care and quality of care throughout the health network for the direct benefit of people (Table 20.3) [1–10, 40, 45–47].

The essential technical requirements to develop digital health activities deal with having the necessary devices, information systems, platforms, and easy access to the internet. Likewise, it is required that the professionals who provide telematic care, as well as the patients who receive it, have the knowledge, skills, and adequate digital literacy (Table 20.2) [18–23, 46].

As in any health service, the minimum standards of safety, efficacy, and quality of care must always be protocolized and guaranteed, as well as ensuring the rights of privacy and digital confidentiality [50–52]. It is therefore imperative to have validated platforms and databases that ensure the protection of data, especially those sensitive to people. It is also necessary to have a legal framework that establishes rights, duties, and regulations for patients and those who perform telematic health care [46, 50–54].

The ethical requirements in telematic care are not different from face-to-face care, and they are based on a pact of trust between the patient and his medical team [46, 50–53]. Patients must be able to trust that their treating team will put their well-being above other interests, provide competent care, transparent and complete

Table 20.3 Telehealth implementation requirements in a health network

• Reorientation of clinical/administrative management in the health network
• Commitment, motivation and cooperation of directors and chief executives
• Devices, software, information systems and internet connection quality
• Digital literacy for the use of online networks (health teams and patients)
• Validated platforms that ensure confidentiality and protection of data
• Informed consent—Professional identification—Secure data registration
• Legislation/legal framework and care protocols that guarantee the lex artis and safeguard the principles of medical ethics
• Expedited coordination with service centers for eventual referral and ICT technical contingency protocols

information for decision-making, respect the person's privacy and confidentiality, and take the necessary actions to guarantee the continuity of care [46, 50–54].

Finally, to ensure the continuity of health care, it is mandatory that care services have protocols and referral channels so that the person attended in the telematic modality (especially the first care) can be referred, if required, to a face-to-face assessment as fast as possible. Likewise, it is required to have contingency plans for technical problems of ICTs that could interfere with adequate care.

20.5.2 Report of Telenephrology in a Renal Health Network Strategy. Chilean Experience

The first report in Latin America on the use of telenephrology in a Networked Renal Health Strategy was published by Zúñiga et al. [7]. The strategy was implemented in 2012 in two public health services in the cities of Concepción and Talcahuano in southern Chile.

As reported, in 6 months, it was possible to reduce the waiting time for specialist care from an average of 225 days to 2.5 days for telematic care and 30 days for face-to-face care (Fig. 20.5). Of the 4668 patients evaluated, 57.3% did not require face-to-face evaluation by a nephrologist and were referred to primary care with therapeutic recommendations from the specialist.

The investigation and timely telematic referral of patients with CKD in advanced stages 4–5 made it possible to streamline and prioritize face-to-face care by a nephrologist and educate, without urgent urgency, about treatment options: hemo- or peritoneal dialysis, kidney transplant or treatment non-dialytic conservative. The

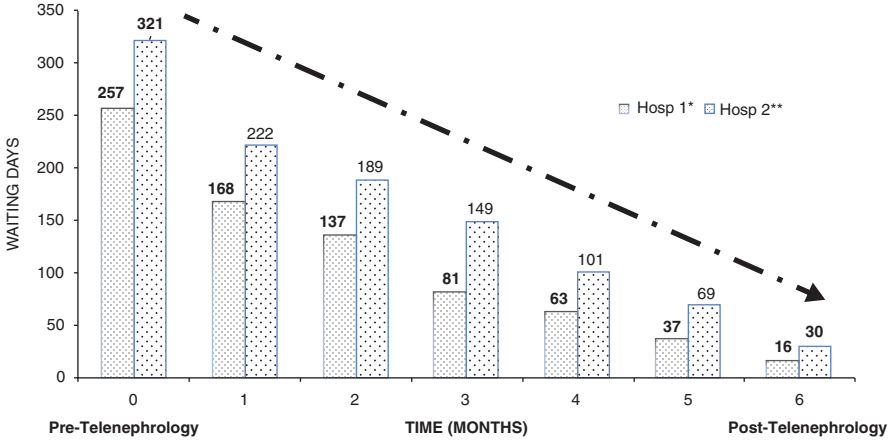

Fig. 20.5 Changes in the wait time for face-to-face evaluations at both the hospitals during the first 6 months of telenephrology (TN). * Hospital 1: Hospital Las Higueras—Talcahuano—Chile. ** Hospital 2: Hospital Regional—Concepción—Chile. (Modified from Zúñiga C, et al. [7])

choice of peritoneal dialysis increased from 5% to 16.3%, admission to hemodialysis with arteriovenous fistula increased from 28.3 to 60.3%, and admission to emergency dialysis without prior evaluation was minimized to 0.9%.

The satisfaction evaluation of the use of telenephrology by primary care physicians was 86.7%, highlighting the continuing education and prompt access to the specialist.

In 2018, the Chilean Ministry of Health implemented the Digital Hospital, a public policy which aimed at promoting the use of telehealth in all specialties of the healthcare network (https://www.hospitaldigital.gob.cl/#). Considering the auspicious results previously reported [7], telenephrology was one of the first specialties to be incorporated into this national telemedical plan. Since its implementation, 15,685 asynchronous teleconsultations have been carried out in nephrology (2019: 50.8%; 2020: 31.9%; 2021: 17.3%). Most of the patients evaluated (61.7%) were referred to continue their treatment at the primary care level with specialist recommendations, thus optimizing the limited availability of face-to-face care aggravated by the shortage of nephrologists in the country. During the COVID-19 pandemic period (2020–2021), the use of telenephrology was restricted, but it allowed the continuity of control of CKD patients and decongested primary care and emergency centers.

Recently, the Chilean Ministry of Health has established the Network Renal Health Strategy as a public policy, based on the previously reported experience, where the use of digital technologies plays a substantial role as an articulator of the administrative and clinical management of the comprehensive approach of CKD [19].

Our first results are promising, but it will be necessary to compare the application of this strategy in other countries with similar epidemiological and socioeconomic realities.

Likewise, in future studies, the impact of this telemedical strategy in the follow-up of patients with CKD, the decrease in CO_2 emissions related to the reduction of displacement, and the level of satisfaction of patients and PHC teams should be evaluated, as well as the effective cost evaluation of this innovative assistance modality.

20.6 Telehealth and Telenephrology in Latin America

The use of telenephrology in Latin America has had a progressive but uneven development between and even within countries. In this regard, the SLANH Renal Health Committee conducted a survey in the second semester of 2021 to find out the level of use of telemedicine in nephrology in the countries that make up the Latin American Society of Nephrology and Hypertension (SLANH), as a preliminary instance to obtain information and design proposals to promote telenephrology [21]. In addition, 15 of the 22 Societies participating in SLANH responded. Only four

countries reported using telenephrology for the care of patients with CKD in the public system and primary care. Two of them with national coverage and two with regional coverage.

In five countries, the use of telenephrology was reported for the control of patients undergoing RRT renal replacement therapy (three in peritoneal dialysis and two in kidney transplant). Only one country reported having educational content on telehealth/telenephrology in its curricular programs for nephrologists in training [21].

Although there are Latin American experiences regarding the use of ICTs in nephrology, published or reported in specialized conferences or congresses, their use is not yet incorporated into public health policies or strategies in all countries. The referred survey evidenced a digital gap that urges the development and implementation of national and regional strategies that promote the use of telehealth/telenephrology. These actions should include access to and use of technology in remote care and monitoring of kidney patients, continuing education and training of patients, care teams, and professionals in training.

Until now, in Latin American countries, the most developed telemedical activity has been continuing education through online courses in synchronous and asynchronous modalities.

20.6.1 Use of Continuing Tele Education in Nephrology and Prevention of CKD in Latin America

In 2013, the SLANH organized a telematic continuing medical education program for Latin American nephrologists and kidney professionals. Logistical, IT, and educational support was provided by Evimed, a Uruguayan educational company specialized in telematic continuing education in the field of medicine. Since then, multiple courses have been held with the participation of professionals from the region who work in different areas of nephrology, some of them residing in remote areas of Latin America [20].

The modality of these distance education courses are telematic, asynchronous and/or synchronous, and bilingual (Spanish and Portuguese). Courses begin with a face-to-face online plenary launch conference, followed by lectures carried out by regional and global experts. Everything is complemented by multiple educational strategies, such as readings of selected articles, educational videos, evaluation of clinical cases (e-rounds), and pre and post course evaluations. It ends with a closing conference and evaluation of the course by the participants [20].

Along with contributing to the education of health teams, the program facilitates the access of health professionals from different Latin American countries who, for reasons of geographical distance, labor restrictions or economic resources, cannot regularly participate in courses, conferences, or face-to-face congresses of the specialty.

20.6.2 PAHO and SLANH Alliance for Telematic Continuing Education in Prevention and Management of Kidney Diseases

In June 2015, the Pan American Health Organization (PAHO) recognized SLANH as a non-governmental organization that supports institutional work priorities [55].

This strategic alliance between both regional organizations aims to contribute to the education of health teams and reduce the gap that separates patients with kidney diseases from access to specialized, timely, and quality care.

20.6.2.1 SLANH Telematic Course on Prevention and Management of Acute Kidney Injury (AKI)

In accordance with the Oby25 ISN initiative for the education and prevention of acute kidney injury (AKI) [56], the SLANH AKI committee implemented in 2015 two free courses, asynchronous online modality, one course for nephrologists and the other for primary care physicians. The Pan American Health Organization (PAHO)/World Health Organization (WHO) collaborated in disseminating the course among Latin American primary care physicians. The evaluation of the organizers reported that the telematic education modality was effective for learning about the prevention and management of AKI [57].

20.6.2.2 Telematic Modality Course of Prevention and Management of CKD for the First Level of Care in Latin America: PAHO/SLANH

In the context of the health challenge that chronic kidney disease (CKD) represents in Latin America, the education of health teams in primary care acquires greater relevance, considering that they are the first level of investigation, diagnosis, and treatment of the disease [14, 18, 22–27]. Under this premise, in July 2016, the Pan American Health Organization (PAHO) in conjunction with SLANH, organized the First Online Course on Prevention and Management of CKD for the First Level of Care in Latin America [58].

The course is free, with unlimited seats, and is especially aimed at Latin American primary care health teams, who are in charge of screening and treating patients with CKD in its early stages. Its modality is telematic, asynchronous, and self-learning, programmed to be completed by the students in approximately 50 h of dedication. Each learning module ends with an evaluation test, which only once passed allows you to move on to the following modules. The presentations are given by leading SLANH nephrologists, and the main topics addressed are: the epidemiology and

Table 20.4 Analysis of the survey of participants in the course for the first level of health care: first level of health care—PAHO/SLANH

	%
Considered the topics relevant to their professional work	98
Evaluated the educational resources favorably	96
Did not need to consult with teaching coordinators	93
Used a mobile device with internet access	86
Accessed the course from home	71
Referred as the greatest advantage the autonomy and freedom of timetable	65
Considered the time required for the course to be the greatest difficulty	47
Reported internet access problems	17

prevention of CKD, diagnosis and treatment in its five stages, strategies to prevent progression, diabetic and Mesoamerican nephropathy, management of comorbidities, and timely referral to the nephrologist [55].

From the beginning of the course until July 2021, 34,918 professionals have registered. The course satisfaction survey revealed a positive general evaluation by the course participants, highlighting free of charge, self-administration, attention by the tutor, practical applicability, and discussion of clinical cases (see Table 20.4) [59].

Among the pending tasks of the course one may mention evaluating the impact on the long-term results of the course, both in the knowledge acquired and its application in daily practice, and updating the contents and use a more modern, friendly, and easily accessible platform.

20.6.3 Initiatives Under Development at SLANH to Promote Telehealth in Nephrology Practice

20.6.3.1 Contents of Digital Nephrology in the Training of Nephrologists in Latin America

In 2015, the SLANH published some recommendations for the training of nephrologists in Latin America [60]. In its proposal, the "use of telemedicine to provide care services or health education, and/or develop continuous medical training activities" is promoted, considering the significant contribution of telehealth/telenephrology and its potential growth in the near future.

By this recommendation, it is intended that education on the use of ICTs in nephrology, its indications, advantages, limitations, and ethical and legal considerations are included in the contents of training programs. However, the aforementioned survey on the use of digital nephrology in the region, revealed that unfortunately this proposal has not yet been implemented in most of the Societies belonging to SLANH and is one of the challenges considered in the new institutional strategic plan [18, 21].

20.6.3.2 Digital Platform for the Management of CKD in Primary Care

The SLANH, through its Renal Health Committee, has proposed to promote the use of digital technology to address CKD in the Region [18]. In addition to continuing with telematic education on CKD for primary care teams, it plans to develop a digital platform that will facilitate quick and timely access of CKD patients to the nephrologist, referred by the primary care teams dependent on the public health systems. The SLANH would make this platform available to Latin American countries that require it, to carry out asynchronous teleconsultations carried out by nephrologists who participate in this initiative. In those regions of Latin America where there was a lack of specialists, SLANH would coordinate with the respective health authority and the local Society of Nephrology and would request support from national nephrologists or from other countries in the region, so that they could voluntarily respond to teleconsultations. This initiative is part of the social commitment and community involvement that inspires and sustains SLANH.

To achieve the proposed objectives, it is expected to establish a strategic alliance with PAHO and thus promote and coordinate the use of the platform in those Latin American countries that require it, adapting to health standards and technical, administrative, legal, and public policy conditions of each country.

20.7 Telenephrology in Latin America During the COVID-19 Pandemic

During the COVID-19 pandemic, the use of telemedicine has intensified in different parts of the world [11, 49, 61]. In the context of the health emergency, the SLANH and its affiliated Societies used social networks and ICTs (especially Twitter, Facebook, Instagram, and Webinars) to collect and record data from patients infected with RRT, deliver recommendations for prevention of contagion, educational content for confinement, instructions on vaccines, and management of acute kidney injury associated with COVID-19.

In Brazil, during the pandemic, various telemedicine services, including telenephrology (mostly concentrated in the private sector), have been providing assistance to the Brazilian population [40].

In Chile, the “Fundación Educacion Renal” and “Fundación Pro Salud Renal,” belonging to the Chilean Society of Nephrology, implemented a web platform for the containment, orientation, and education of patients with CKD who during the pandemic could not access their outpatient check-ups with their renal team (http://educacionrenal.cl/).

Through this platform, people made consultations to specialists through messaging or videoconferencing consultations, participated in forums open to the public and educational talks on different topics. Online support workshops were held

for patients and their relatives with CKD by a multidisciplinary team, who voluntarily supported the continuity of online care for patients with CKD during the pandemic.

20.7.1 Telenephrology in Patients with Advanced CKD Who Were Not on Dialysis During the COVID-19 Pandemic: Preliminary Report

In a report from the Renal Transition Clinic of "Las Higueras" Hospital in Talcahuano, Chile, which cares for patients with advanced CKD in stages 4 and 5 not on dialysis, the televideo consultation modality allowed avoiding unnecessary transfers, favoring confinement with nephrological control remotely and have a greater number of places for face-to-face hospital care at the most critical moments of the COVID-19 pandemic. In the group treated by telenephrology, the incidence of COVID-19 infection was similar to the general population and much lower than the population on hemodialysis. Contrary to expectations, the group controlled by telenephrology had lower lethality and admission to dialysis than the pre-pandemic year.

Regarding the level of satisfaction with the telematic modality, 88% of the patients reported being very satisfied with the care and 77.3% approved of this modality to continue their medical check-ups.

Considering the described results and the good acceptance by patients, the mixed use of telenephrology alternated with face-to-face evaluations post-pandemic should be evaluated as a new option for the outpatient control of people with advanced CKD, especially in older people or people with difficult geographical access [62].

In summary, the computerization of nephrology plays an important articulating and supporting role in a Renal Health Network strategy. It facilitates expeditious access, evaluation, and timely treatment of patients with CKD from the urban/rural primary care level and prioritize face-to-face care by a nephrologist for those with higher risk or severity. Likewise, telematic education for first-level health teams and local nephrology teams would strengthen CKD promotion, prevention, and care actions in a care network. It is relevant to point out that based on what was confirmed during the COVID-19 pandemic, telemedical care would allow continuity of care, follow-up, and control of patients with chronic CKD, especially in geographically remote areas and in situations of natural disasters or health emergencies. The results of the survey on the use of telenephrology in the region revealed a digital divide that calls for the development and implementation of national and regional strategies that promote the use of telehealth/telenephrology.

Finally, the clinical and epidemiological data collected can be later analyzed by artificial intelligence and will provide evidence for the development of public policies and territorial preventive plans coordinated between the different levels of a Health Network.

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Chapter 21
Innovations in Intensive Care Nephrology

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Acute kidney injury (AKI) is a syndrome with a variety of etiologies defined by a decline in kidney function. Continuous care is required for AKI patients, beginning with utilizing real-time renal function monitoring and biomarkers for early diagnosis. E-alert systems have been recently developed that can immediately notify the clinician of a kidney issue and allow them to deliver appropriate management promptly. Novel technologies like hemoadsorption can also mitigate the inflammatory process during severe sepsis by reduction cytokines and endotoxin levels. Post-AKI care is a new concept in management that has been shown to improve patient outcomes.

21.1 Current Criteria for the Diagnosis of Acute Kidney Injury (AKI)

Historically, the definition of AKI has been inconsistent. Numerous reports and definitions have attempted to improve articulation of the condition. The RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) criteria introduced in 2004 by the Acute Dialysis Quality Initiative (ADQI) workgroup proposed a unified standard for the diagnosis of acute renal failure. The diagnostic criteria is composed of the glomerular filtration rate (GFR) and urine volume. However, these criteria were found to lack diagnostic sensitivity [1]. In 2007, an expert committee known as the Acute Kidney Injury Network (AKIN) revised the criteria for diagnosing AKI, adding a threshold for changes in serum creatinine of 0.3 mg/dL within 48 h [2].

In 2012, the global non-profit organization Kidney Disease Improving Global Outcomes (KDIGO) released a combined RIFLE and AKIN criteria for the diagnosis of kidney injury [3]. The KDIGO definition established a standardized system for defining AKI in research and clinical practice. A consistent approach to applying KDIGO criteria may reduce the range of AKI definitions and enhance the comparability of clinical trials. The full KDIGO definition criteria can be found in Table 21.1 [4].

Table 21.1 Criteria for acute and chronic kidney disease [4]

	AKD			
	AKI	AKD without AKI	CKD	NKD
Duration	Within 7 days	≤ 3 months	> 3 months	
Functional criteria	**Stage 1:** Increase of SCr level by ≥0.3 mg/dL (26.5 μmol/L) for ≤48 h or ≥150% for ≤7 days or urine output <0.5 mL/kg/h for >6 h **Stage 2:** Increase of SCr level by >200% or urine output <0.5 mL/kg/h for >12 h **Stage 3:** Increase of SCr level by >300% or ≥4.0 mg/dL (≥353.6 μmol/L) with an acute increase of ≥0.5 mg/dL or urine output <0.5 mL/kg/h for >24 h or anuria for >12 h or acute RRT	GFR < 60 mL/min/1.73 m^2 or Decrease in GFR ≥ 35% or Increase in SCr by >150%	GFR < 60 mL/min/1.73 m^2	GFR > 60 mL/min/1.73 m^2
And/or		And/or	And/or	And
Structural/Damage criteria	Not defined	Marker of kidney damage (Albuminuria, hematuria, or pyuria are most common)	Albuminuria	No kidney damage

NKD no kidney disease, *AKD* acute kidney disease, *AKI* acute kidney injury, *CKD* chronic kidney disease, *GFR* glomerular filtration rate, *SCr* serum creatinine

21.2 Innovations in Diagnosing AKI

21.2.1 Continuous GFR Monitoring [5]

Continuous radio-frequency transmission monitoring is a non-radioactive method that employs a fluorescent GFR marker. It is non-invasive using substance elimination kinetics that can facilitate early diagnosis of AKI. The fluorimeter emits a specified frequency of light waves via an LED that is reflected by a photodiode. This approach enables remote monitoring and analysis in real-time.

Sinistrin is a polysaccharide, which is a member of the fructan family but has greater solubility than insulin in water. The FITC-sinistrin transdermal device has been extensively utilized to evaluate kidney function in biomedical research [6].

Relmapirazin (MB-102) is a novel fluorescent tracer agent that demonstrates comparable results to iohexol in preclinical research and human clinical trials with minimal adverse effects [7].

A transdermal GFR monitoring device is equipped with a screen and monitor cable which is a sensor toolbar attached to the sternum or manubrium, forehead, arm, and trunk [8]. The fluorescent tracer agent is administered intravenously. Measurement of GFR levels begins when fluorescence reaches its peak concentration. This GFR monitoring via transdermal patch will continue until the tracer agent no longer detects it. These kinetic clearance techniques also have potential use for patients with chronic kidney disease (CKD).

21.2.2 Continuous Urine Output Monitoring

Urine output is a measure that reflects the presence and the degree of AKI severity. Jin et al. [9] found that intensive urine output monitoring improved detection of AKI. The URINFO® system (FlowSense, Haifa, Israel) [10] is a commercial urine flow measurement tool. The disposable foley catheter is attached to the cradle, and the urine output is converted to droplets. Using optical technology combined with an algorithm, the flow and volume can be visualized.

Clarity RMS Sensor Kit® (RenalSense, Jerusalem, Israel) [11] is an electronic sensor using the principles of thermal transfer. This electronic device has been shown to provide more accurate information on urine output and fluid status than routine practice [12].

21.2.3 Clinical Applications

Continuous renal function monitoring can be performed as a screening tool in patients at high risk of AKI, especially hospital-acquired AKI. In critically ill patients, continuous GFR monitoring can determine the response to treatment during resuscitation with fluid or vasopressor administration.

21.3 Innovation in AKI Biomarkers

Over the past 10 years, researchers have searched for an ideal biomarker to diagnose AKI similar to the way troponin is used as a biomarker for myocardial infarction. In contrast to myocardial infarction where ischemia is the primary cause, AKI can be caused by multiple mechanisms such as ischemia, sepsis, and nephrotoxins. Due to its complexity, several novel biomarkers have been developed to improve the diagnosis, prevention, and management of AKI. To date, AKI biomarkers can be classified into three types of markers: stress, damage, and functional (Fig. 21.1) [13]. Although hundreds of biomarkers have been developed, only a few have been commercially launched and incorporated into clinical practice. The following four biomarkers have been widely studied in the clinical practice (Table 21.2).

Table 21.2 Summary of clinical applications of novel acute kidney injury biomarkers

	Type of marker	Predict acute kidney injury	Predict persistent acute kidney injury	Predict renal replacement therapy	Predict short and long-term outcomes
Urinary NGAL	Damage	++	+	+	+
Plasma NGAL	Damage	++	+	+	+
Urinary KIM-1	Damage	+	NA	NA	+
Urinary L-FABP	Damage	+	+	+	+
Urinary [TIMP-2]*[IGFBP7]	Stress	+++	+	+	+
Urinary DKK-3	Stress	+++	+	NA	+
Plasma Proenkephalin	Functional	NA	++	+	++

NA not applicable, + fair prediction, ++ good prediction, +++ excellent prediction

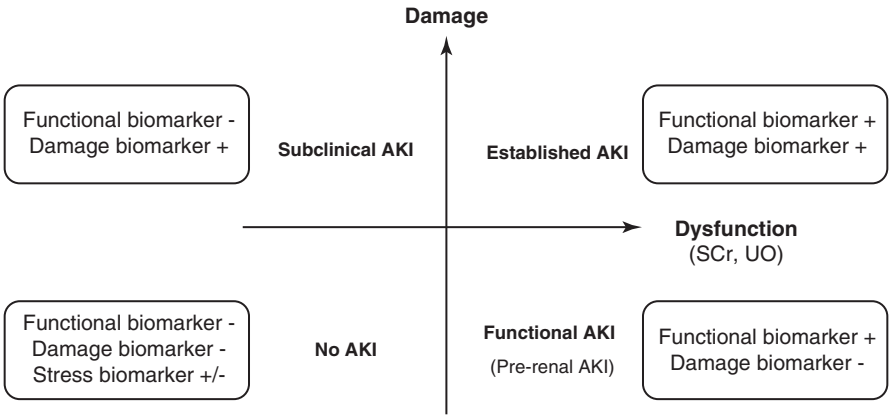

Fig. 21.1 AKI biomarkers can be classified into three types of markers: stress, damage, and functional

21.3.1 Neutrophil Gelatinase-Associated Lipocalin

Human neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDA glycoprotein of the lipocalin superfamily. NGAL is produced in the kidney mainly at the thick ascending limb and the intercalated cells of the collecting duct. Under normal conditions, the urinary NGAL level is low because filtered NGAL is almost completely reabsorbed. After renal tubular injury, NGAL increases rapidly within 2–3 h in both serum and urine. Urinary NGAL can increase almost 100-fold and serum NGAL can increase 20-fold before AKI can be detected by serum creatinine. Inflammation, particularly sepsis, is considered a confounding factor because NGAL increases in bacterial infection, systemic infection, sepsis, thrombocytopenia, cancer, and other systemic diseases [14]. At present, NGAL is the most widely studied AKI biomarker in clinical practice.

Many studies have demonstrated the role of NGAL as a predictor for AKI in critically ill patients, kidney transplant patients, and cardiac and non-cardiac major surgery patients [15]. A meta-analysis by Ho et al. [16] demonstrated the predictive ability of NGAL for AKI in cardiac surgery and found an AUC for uNGAL and pNGAL of 0.72 and 0.71, respectively. A recent meta-analysis also showed the capability of serum NGAL and urine NGAL to predict septic AKI with an AUC of 0.86 and 0.80, respectively [17].

For the diagnosis of AKI, NGAL helps distinguish acute tubular necrosis (ATN) from non-ATN or pre-renal AKI. uNGAL levels effectively distinguish between ATN and pre-renal AKI (AUC of 0.87) with a level >104 ng/mL indicating ATN and a level <47 ng/mL indicating pre-renal AKI. The high level of NGAL also predicted

the severity of AKI. Urinary NGAL also helps distinguish ATN and non-ATN among cirrhosis patients [18].

NGAL also helps in predicting persistent AKI and RRT [19]. uNGAL and pNGAL showed a fair prediction of RRT among critically ill patients with AUC of 0.72 and 0.76, respectively [20]. Srisawat et al. [21] recently published a feasibility study examining the effect of early RRT guided by a pNGAL >400 ng/mL. Although the early RRT did not significantly reduce the primary outcome of 28-day mortality compared to the standard RRT, the median number of days free from mechanical ventilation were significantly higher in the early RRT group.

21.3.2 Kidney Injury Molecule-1

Kidney injury molecule-1 (KIM-1) is a 38.7 kDa type 1 transmembrane glycoprotein that functions as an adhesive molecule and serves as a receptor to facilitate the clearance of the apoptotic debris for limiting the proinflammatory response. Under normal conditions, KIM-1 is expressed at a low level in the kidneys. However, in response to ischemic-reperfusion injury, KIM-1 is markedly upregulated in the injured proximal tubular epithelial cells and can be detected in urine for 24–48 h after tubular injury [22].

A meta-analysis [23] showed that urinary KIM-1 had a sensitivity of 74% (95% CI 61–81%), a specificity of 86% (95% CI 74%–93%), and AUC of 0.86 (0.83–0.89) for the diagnosis of AKI. The subgroup analysis also found urinary KIM-1 had better diagnostic accuracy in infants and children than adults, especially in patients undergoing cardiac surgery after cardiopulmonary bypass. In a cohort study of 201 hospitalized patients with AKI [24], urinary KIM-1 was reported to be significantly associated with dialysis requirements and hospital mortality. The TRIBE-AKI study [25] also showed that high concentrations of urinary KIM-1 predicted mortality at 3 years in patients with AKI. Recently, KIM-1 has been approved by the US Food and Drug Administration (FDA) as an AKI biomarker.

21.3.3 L-Type Fatty Acid Binding Protein

Liver-type fatty acid-binding protein (L-FABP or FABP1) is a 14 kDa soluble protein member of the lipid-binding protein superfamily. L-FABP is found predominantly in the cytoplasm of hepatocytes. FABP itself is present in many other tissues such as enterocytes, renal proximal tubular cells, and alveolar epithelium lung cells. L-FABP binds fatty acid uptake and facilitates the transfer of fatty acids between extracellular and intracellular membranes. L-FABP can be unregulated and detected

in urine after tubular damage. Currently, there is no standard cutoff point of L-FABP for diagnosis of AKI [26].

A meta-analysis of 28 studies showed L-FABP had an AUC of 0.72 (0.60–0.85) in predicting AKI among cardiac surgery patients [16]. In critically ill patients, Noiri et al. [27] provided evidence (AUC = 0.75) for a greater efficacy of L-FABP in predicting AKI compared to other biomarkers such as NGAL, IL-18, NAG, and albumin. L-FABP can also predicted short-term outcomes including AKI progression, dialysis, and 7-day hospital mortality in ICU setting [28].

21.3.4 Tissue Inhibitor of Metalloproteinase-2 and Insulin-Like Growth Factor-Binding Protein 7

Tissue inhibitor of metalloproteinase-2 (TIMP-2) is a 21 kDa multifunctional protein abundantly expressed in many normal tissues and first identified as a natural inhibitor of MMP, the enzyme that degrades the extracellular matrix. Insulin-like growth factor-binding protein 7 (IGFBP7) is a 29 kDa multifunctional protein that regulates the tissue's insulin-like growth factor and stimulates cell adhesion. Both were found to be expressed and secreted by renal tubular cells. TIMP2 was predominately found in distal tubular cells, while IGFBP7 was typically found in proximal tubular cells [29]. During cellular stress or injury, renal tubular cells produce and release TIMP-2 and IGFPBP7. Both proteins block the effect of cyclin-dependent protein kinase complexes which results in G1 cell cycle arrest that prevents further cell division with DNA damage [30].

The selection of TIMP2 and IGFBP7 for the prediction of AKI was first reported in the Sapphire study [31]. This study demonstrated that multiplication of the two urinary biomarkers ([TIMP-2]*[IGFBP7]) or Nephrocheck® test (Astute Medical, San Diego, USA) is an outstanding metric in the prediction of moderate to severe AKI (KDIGO stage 2–3) within 12 h. Nephrocheck® showed an AUC of 0.80 in a heterogeneous sample of patients suffering from sepsis, shock, major surgery, or trauma. Subsequently another study demonstrated the cutoff 0.3 $(ng/mL)^2/1000$, with a sensitivity of 89% and a specificity of 53%, while for the >2.0 $(ng/mL)^2/1000$, the sensitivity was 44% and specificity was 90% for AKI prediction [32].

In 2014, the US FDA approved Nephrocheck® as the first biomarker to predict the risk of developing moderate to severe AKI within 12 h. In a recent meta-analysis [33] of Nephrocheck® for predicting cardiac surgery-associated AKI, findings revealed an AUC of 0.83. Nephrocheck® also performed well among patients with sepsis (AUC 0.85), congestive heart failure (AUC 0.89), surgery (AUC 0.84), cardiac arrest (AUC 0.91), platinum induced nephropathy (AUC 0.92), and patients

with CKD (AUC 0.91) [34, 35]. Nephrocheck® has been currently predicting subsequent AKI at the cutoff of 0.3 $(ng/mL)^2/1000$.

Recently, several studies (PrevAKI and bigpAK study) showed promising results using Nephrocheck® to guide preventive strategies with the implementation of the KDIGO bundle consisting of the optimization of volume status and hemodynamics, avoidance of nephrotoxic drugs, and prevention of hyperglycemia in high-risk patients [36, 37].

21.4 Innovation in the Management of AKI: Role of Electronic Alerts

Deficiencies in the identification and documentation of AKI often lead to failure to diagnose patients, inadequately assess patient status, and improperly adjust nephrotoxic agents. Just one-third of community-acquired AKI patients visited outpatient departments, and only half of them were diagnosed in an acute setting. These missed opportunities lead to progressive AKI and increased patient mortality [38]. In the emergency department, a mention of AKI in the medical records of patients with kidney injury occurred only half the time of actual cases. The identification was more common among severe AKI cases (46% at stage 1 vs. 88% at stage 3) [39]. This under identification may occur from a lack of recognition and understanding of the importance of diagnostic coding in administrative databases and hospital reimbursements [40].

Electronic alerts (e-alert) in electronic health records (EHR) provide clinicians, staff, patients, and other caregivers with knowledge and person-specific information to enhance healthcare management. The delivery of e-alerts to physicians can provide reminders, enhance clinical recommendations, generate data reports, and assist with documentation and diagnostic approach.

Several studies demonstrated improved outcomes after implementation of an e-alert system. The probability of overlooked AKI events was found significantly lower and the likelihood of an early consultation with a nephrologist increased under an e-alert system. Severe AKI events were reduced after implementation of the alerts. Furthermore, the likelihood of AKI recovery improved in the alert group [41]. The DETECT-H project reported found e-alert systems as a useful tool to improve renal outcomes in hospital-acquired AKI [42]. Moreover, this technology fueled a decrease in hospital mortality, dialysis use, and length of stay among AKI patients [43]. However, some recent studies did not find an association between the use of automated AKI e-alerts and a change in the rate, severity, or mortality of AKI. A small reduction in occupied hospital bed days was found [44, 45]. A summary of studies comparing hospitals that introduced an e-alert system with those without e-alert in AKI are shown in Table 21.3.

Table 21.3 Studies of using of electronic alerts compared with no electronic alert in hospitals

Study	Park [41] (2017)	DETECT-H [42] (2019)	Al-Jaghbeer [43] (2018)	Baird [44] (2021)	ELAIA-1 [45] (2021)
Study design	Cohort	Retrospective observational study	Observational study	Cohort	RCT
Country	Korea	Spain	USA	UK	USA
Number of patients or AKI episodes	1309 vs. 1884	1241 alerts from 11,022 admissions	346,412 vs. 181,696	32,320 episodes	6030 (3059 vs. 2971)
Mean follow-up (days)	Discharge	Discharge	N/A	90	14
Mean age (years)	68 vs. 64 ($p < 0.001$)	77	59 vs. 59	N/A	71 vs. 71.3
AKI incidence (%)	N/A	45.3	12.8 vs. 11	100	70.0 vs. 63.0
AKI stages (%) 1 2 3	Stage 2 and 3 27.1 vs. 31.6 [OR 0.75 (0.64–0.89), $p < 0.001$]	49.8 24.5 25.8	N/A	67.2 15.1 17.7	77.7 vs. 77.5 13.5 vs. 13.6 8.7 vs. 8.8
AKI progression (%)	N/A	N/A	N/A	N/A	15.9 vs. 15.5
RRT (%)	N/A	57.1	0.5 vs. 0.7	N/A	3.5 vs. 3.1
Mean LOS (days)	N/A	1.52 (1.11–2.08)	5.7 vs. 5.7	N/A	4.3 vs. 4.2
In-patient mortality (%)	HR 1.07 (0.68–1.68)	3.18 (1.8–5.59)	2.4 vs. 2.4	N/A	N/A
Follow-up mortality (%)	N/A	N/A	N/A	30-day mortality 15.9 90-day mortality 22.8	14-day mortality 8.9 vs. 8.9

21.4.1 E-alerts and AKI Care Bundle

By the time creatinine rises, significant kidney damage may have already occurred. It is imperative to identify patients at risk of AKI and immediately modify their care to maximize the window of opportunity for intervention [40]. KDIGO proposed the care bundle for a stage-based management approach in order to decrease incidence of AKI [3]. The use of e-alerts can link to a clinical decision support (CDS) system which can improve quality of care by impacting provider behavior, enhancing patient safety, improving clinical care efficacy, and increasing provider and patient satisfaction. A real-time electronic alert system known as an "AKI sniffer" increased the number and timeliness of early therapeutic interventions. The ADQI workgroup also described automatic alerts and CDS features that can affect performance [46].

Studies have reported disparate clinical outcomes from CDS systems. One study did not show an improvement in clinical outcomes [47]. In another study, complete care bundle delivery within 24 h after an alert was associated with decreased

mortality and decreased progression of AKI [48]. The Tackling AKI study demonstrated that although complex hospital-wide interventions (e-alerts, care bundle, and education program) did not reduce 30-day AKI mortality, it did reduce the length of stay and improved quality of care [49]. A cost-effectiveness analysis in the Tackling study found an incremental cost savings of £732 per admission with the care bundle. Using a willingness to pay threshold of £20,000 per quality-adjusted life year, the probability of the intervention being cost-effective compared with standard care was 90% at the National Health Service in the United Kingdom [50]. The ICE-AKI study investigated acute medical admissions of a multi-modal intervention which improved in-hospital outcomes [51]. The NINJA program observed a significant and sustained decrease in nephrotoxic AKI rates after using a CDS system in multiple pediatric institutions [52]. A recent meta-analysis showed that CDS systems significantly reduced mortality, increased AKI recognition, and prompted investigations, leading to improved patient-centered outcomes and care processes [53]. A summary of studies of e-alerts and AKI care bundle compared with usual care is shown in Table 21.4.

Table 21.4 Studies of using electronic alert-based KDIGO care bundle compared with usual care

Study	Wilson [47] (2015)	Kolhe [48] (2015)	ICE-AKI [51] (2018)	Tackling AKI [49] (2019)
Study design	RCT	Prospective cohort	Controlled before-after	Multicenter RCT
Country	USA	UK	UK	UK
Number of patients or AKI events	2393 (1201 vs. 1192)	2500 AKI in 2297 patients	14,673 (7881 vs. 6792)	24,059 AKI (10,017 vs. 14,042)
Mean follow-up (days)	30	134	Discharge	30
Mean age (years)	60 vs. 61	76.9 vs. 76.6 ($p = 0.713$)	74.2 vs. 74.1	76.6 vs. 75.4
AKI incidence (%)	0.0 vs. 0.6% (p = 0.81)	12.2 vs. 87.8	7.73 vs. 6.67 [OR 0.990 (0.981–1.000), $p = 0.049$]	49.4 vs. 53.8 [OR 0.94 (0.88–1.0), $p = 0.06$]
AKI stages (%) 1 2 3	N/A	($p = 0.0001$) 41.2 vs. 55.9 32 vs. 24.2 26.8 vs. 19.9	N/A N/A 6.57 vs. 5.52 ($p = 0.592$)	64.5 vs. 60.6 19.8 vs. 21.4 15.7 vs. 18.0
AKI progression (%)	N/A	3.9 vs. 8.1 ($p = 0.02$)	6.1	N/A
RRT (%)	7.2 vs. 5.9 ($p = 0.18$)	N/A	N/A	5.3
Mean LOS (days)	N/A	11.2 vs. 12.5 ($p = 0.098$)	15.0 vs. 13.7 ($p = 0.194$)	9.0
In-patient mortality (%)	5.9 vs. 5.1 ($p = 0.40$)	18 vs. 23.1 ($p = 0.046$)	21.67 vs. 24.72 [OR 0.924 (0.858–0.99), $p = 0.038$]	N/A
Follow-up mortality (%)	N/A	30-day mortality 25.2 vs. 28.5 ($p = 0.219$) 60-day mortality 27.1 vs. 30.7 ($p = 0.205$)	7-day mortality 10.51 vs. 12.58 [OR 0.907 (0.859–0.957), $p < 0.001$]	24.5

21.4.2 Pros and Cons of E-alert Systems

The advantages of an e-alert system include providing real-time information, standardization of practice, improving documentation, long-term cost savings, facilitating information gathering, providing a platform for quality measures, facilitating the teaching of trainees, and enhancing patient safety [51]. Potential limitations of this system consist of monitoring only serum creatinine which is a lagging indicator, false alerts leading to response fatigue by physicians, ineffective methods of alert delivery, and technical issues such as network connectivity problem [54]. The disadvantages of the system may also lead to the replacement of clinical judgment and over-reliance on computerized monitoring, lack of universal patient and physician acceptance, lack of user-friendliness, cost of system installment and maintenance, unsettled legal and ethical issues, lack of robust findings of benefits, and system inflexibility. CDS systems neither replace the work of clinical providers nor have superior performance when compared to clinicians, but can help improve the decision-making of care providers.

21.5 Innovation in Post-AKI/AKD Clinic and Role of Telemedicine

AKI is estimated to occur in up to 20% of patients in the hospital and up to 50% of patients admitted to the ICU. In addition to poor outcomes during hospitalization, AKI survivors are at increased risk of albuminuria, CKD, ESRD, mortality, and cardiovascular events. A systemic and meta-analytic study found that AKI was associated with a ten-fold risk of CKD, three-fold risk of ESRD, and a two-fold risk of mortality [55]. AKI survivors also had an increased risk of albuminuria, which is one of the markers of CKD progression. A large multicenter prospective cohort study in North America showed that elevated UACR 3 months after discharge was associated with a higher risk of kidney disease progression (HR 1.53; 95% CI 1.43–1.65) [56]. AKI survivors also experienced increased cardiovascular events including heart failure, stroke, and lower quality of life [57]. Post-discharge care in post-AKI clinics among AKI survivors is essential to improve long-term outcomes.

Currently, there are no standardized guidelines for the selection, timing, frequency, and methods for follow-up care for patients following an episode of AKI. Previously, post-AKI survivors were rarely followed up with after discharge despite the KDIGO recommendation that they have a kidney function measurement 3 months after discharge to determine renal resolution, new onset, or worsening of pre-existing CKD [3]. The ADQI Workgroup consensus suggests timing and frequency of post-AKI follow-up depends on the severity of AKI [58]. Patients with more severe AKI should receive early and frequent follow-up. Recovered and non-recovered AKI patients should be seen by a nephrologist within 1 week, while patients with stage 1 AKI may be followed-up in 3–6 weeks or several months.

An additional consideration is who should assume responsibility during the follow-up (nephrologist versus primary care physician). A retrospective study of 3877 patients who survived AKI-D showed improved all-cause mortality with nephrologist follow-up compared with those without any follow-up (8.4 vs 10.6 per 100-patient years, HR 0.76 (95% CI 0.62–0.93)) [59]. Recently, two randomized controlled trials tried to find the intervention to improve outcome of AKI survivors. The FUSION trial [60] randomized post-AKI survivors into nephrologist follow-up versus usual care. The study found major adverse kidney event (MAKE) at 1 year, which was no different between groups. The author [61] randomized post-AKI survivors into either follow-up by a multidisciplinary care team (MDCT) (nephrologist, pharmacist, nutritionist, and renal nurse) or usual care by internists. Patients treated by the MDCT showed a significantly greater decrease in albuminuria and greater blood pressure control. However, MAKE at 1 year, CKD progression, and mortality were not different between groups.

An example of the process of care of post-AKI/AKD bundle was proposed in the Quality Improvement Goals for Acute Kidney Injury [58]. The recommendations were comprised of KAMPS (Kidney function, Advocacy, Medications, Pressure, Sick day protocol) for all patients with AKI and WATCH-ME (Weight assessment, Access, Teaching, Clearance, Hypotension, Medications) for patients with AKI who require dialysis. The intervention of any post-AKI clinic requires cooperation between multidisciplinary care teams (Fig. 21.2). However, adopting this standard of care might not be feasible for every center due to limited resources or healthcare policies.

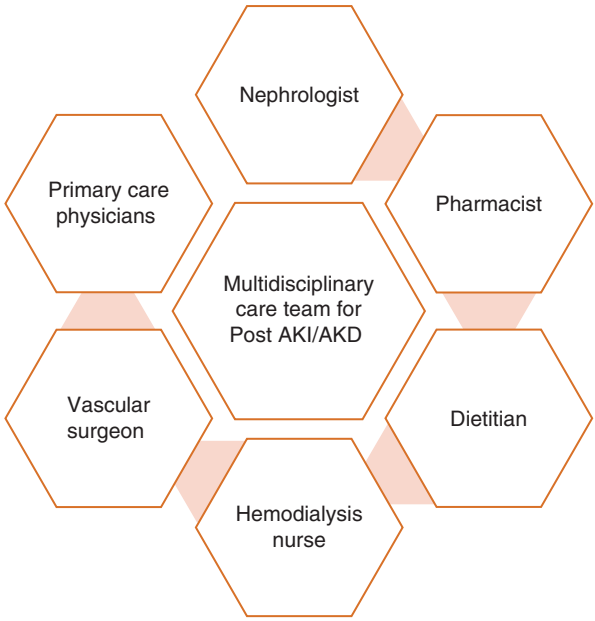

Fig. 21.2 The intervention of any post-AKI clinic requires cooperation between multidisciplinary care teams

Importantly, patients tend to be unaware of their disease and are often lost to follow-up, especially those with renal recovery and those who are not dialysis-dependent. Telemedicine and virtual software platforms such as videoconference, telephone, or smartphone applications can play a pivotal role in mitigating these losses. Telemedicine facilitates communication between patients and doctors and reduces waiting times, contact times, and cost. It is recommended that telemedicine should be implemented in post-AKI clinics.

21.6 Innovation in Continuous Renal Replacement Therapy: Role of Blood Purification

CRRT is the modality for renal support for critically ill patients in situations where intermittent RRT is inadvisable, such as hemodynamic instability or acute brain injury [3]. In recent decades, extracorporeal cartridges have been developed for use with CRRT circuits, especially for extra-renal indications such as sepsis. This blood purification uses hemoadsorption or hemoperfusion. These devices remove circulating cytokines, inflammatory mediators, and endotoxins and return purified blood to patients to restore their immune function. However, severe sepsis may cause a "cytokine storm" characterized by severe systemic inflammation, rapidly progressive shock, multiorgan failure, and death. This condition may need several sessions of treatment until an infection is cured. The sorbents are summarized in Table 21.5.

Table 21.5 Current device for adsorptive therapy in continuous renal replacement therapy

Devices	Company	Composition	Substance elimination
Polymyxin B	Toray, Japan	Polymyxin B bound to polystyrene derivative fibers	Endotoxin
LPS adsorber	Alteco, Sweden	Synthetic polypeptide bound to porous polyethylene discs	Endotoxin
oXiris®	Baxter, USA	AN69-based membrane, surface treated with a polyethyleneimine and grafted with heparin	Endotoxin Cytokines
CPFA	Bellco, Italy	Polyethersulfone plasma filter with adsorption on an unselective hydrophobic-resin cartridge, and a synthetic high permeability polyethersulfone hemofilter	Cytokines
Cytosorb®	Cytosorbents, USA	Polystyrenedivinyl benzene copolymer beads with biocompatible polyvinylpyrrolidone coating	Cytokines
HA-330®	Jafron, China	Neutral macroporous resin cartridge	Cytokines
MG-350®	Biosun, China	Neutral resin	Cytokines

21.6.1 Polymyxin B or Toraymyxin®

Polymyxin B (PMX) or Toraymyxin® (Toray, Tokyo, Japan) is a synthetic membrane coated with polymyxin B that binds endotoxin, making it useful in Gram-negative bacterial sepsis [62]. The EUPHAS [63], ABDOMIX [64], and EUPHRATES [65] studies showed an improvement of hemodynamic parameters along with monocyte and neutrophil function but still reported mixed results on survival outcomes. Post-hoc analysis of the EUPHRATES trial showed benefits in patients with multiorgan dysfunction syndrome (MODS) score > 9 and endotoxin activity assay (EAA) in the range of 0.6–0.9 [66]. Srisawat et al. also demonstrated that PMX could improve mHLA-DR expression in severe sepsis patients [67]. A recent meta-analysis demonstrated that a potential survival benefit of PMX can be observed when the control group mortality rate is >30–40% [68].

21.6.2 Cytosorb®

Cytosorb® (Cytosorbents, Monmouth Junction, NJ, USA) is porous polymer beads which adsorb pro- and anti-inflammatory cytokines, myoglobin, free hemoglobin, bilirubin or bile acid, pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs) with removal rates of the molecules >90–95% at 120 min [69]. Evidence supporting a favorable outcome on hemodynamic parameters and blood lactate levels was limited to case studies [70]. A RCT comparing CytoSorb® hemoperfusion with normal care demonstrated substantial elimination of cytokines during treatment sessions only and no reduction in mortality or long-term IL-6 plasma levels [71]. A small RCT of patients without RRT concluded that vasopressor use, procalcitonin levels, and big-endothelin-1 levels were reduced with Cytosorb® [72]. CytoSorb® can also be applied in other conditions generating inflammation, such as severe pancreatitis or cardiopulmonary bypass [73, 74]. A recent study of critically ill patients reported no noteworthy declines in sequential organ failure assessment (SOFA) scores, but IL-6 levels decreased significantly after treatment with CytoSorb® [75]. In summary, CytoSorb® has shown benefits in hemodynamics and reducing circulating IL-6, but impact on survival remains questionable.

21.6.3 oXiris®

oXiris® (Baxter, USA) consists of three layers including (1) a membrane of AN69, which is a copolymer of acrylonitrile and negatively charged sodium methallyl sulfonate to assist in diffusion, convection, and cytokine adsorption; (2)

polyethyleneimine (PEI), a surface treatment for reducing activation of bradykinin from the AN69 membrane and plays a role in endotoxin adsorption and inhibition of contact phase activation; and (3) heparin grafting, which is negatively charged and reduces local thrombogenicity. Both in vitro and clinical studies demonstrated that oXiris® has a similar endotoxin adsorption to that of PMX, similar adsorption to CytoSorb® for the elimination of most inflammatory mediators, and greater efficacy than normal filtering [76–78]. oXiris® also provided beneficial hemodynamic effects, reflected in cardiovascular SOFA scores or vasopressor doses. These results may be associated with the highly effective removal of inflammatory mediators [79]. Additionally, significant improvement in organ function has been shown in recent studies [80, 81]. The consensus from European experts specified septic shock as the most appropriate indication for oXiris® based on the recognition that stabilizing hemodynamic parameters is its most remarkable function [82]. By facilitating early removal of both endotoxins and cytokines, oXiris® can lead to improved outcomes for patients in critical care settings.

21.6.4 HA-230®, HA-280®, HA-330®, HA-380®

HA-230®, HA-280®, HA-330®, HA-380® (Jafron, Zhuhai City, China) are neutral macroporous resins which adsorb cytokines, complements, and free hemoglobin. These cartridges may be used as single therapy or in combination with other adsorption therapy techniques such as double plasma molecular adsorption system (DPMAS) for both liver and renal support. Many studies of the use of these resins point to improvements in hemodynamics, organ dysfunction, shortened ICU stay and reduced ICU mortality [83, 84].

21.6.5 Lipopolysaccharide Adsorbers

Lipopolysaccharide (LPS) adsorber (Alteco, Sweden) is a synthetic polypeptide bound to porous polyethylene discs that adsorb endotoxins. A number of case studies in patients with Gram-negative sepsis reported improvement of hemodynamics and decreased endotoxin levels, but no effect on survival.

21.6.6 Coupled Plasma Filtration Adsorption (CPFA)

CPFA (Bellco, Italy) is a combined plasma separation with adsorption and hemodialysis technique which can remove inflammatory mediators, especially in both liver and renal support. However, low-power landmark studies including COMPACT and ROMPA showed no survival benefit, clotting issues, and high cost [85, 86].

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Chapter 22
Innovations in Kidney Transplantation

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22.1 Introduction

Since the 1960s, dialysis and kidney transplant (KT) have successfully treated patients with end-stage kidney disease (ESKD). Over the decades, both the renal replacement therapies (RRT) have evolved and incorporated new technologies, resulting in notable improvement in their results [1]. Compared to dialysis, KT provides better results for ESKD patients, including higher survival, reduced ratios of cardiac events, hospitalization and infections, and better quality of life [2].

The remarkable improvements in patient and allograft survivals after KT over the years was mainly a consequence of the advances in surgical techniques, a better understanding of transplant immunology, development of techniques for pre- and posttransplant immunological monitoring, the availability of new immunosuppressive drugs, and better management of infections [3, 4].

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Despite these advances, the improvements in long-term patient and graft survivals in the last three decades were incremental [3, 4], suggesting that sustaining and disruptive innovations are required to uptrend the good results.

22.2 Promising Innovations in Kidney Transplantation

The main current challenges in transplantation are the suboptimal access to this therapy [5], the burden of chronic immunosuppression, and subclinical immunological events impacting graft and patient survivals [6]. Some innovations have been evaluated to deal with these challenges, and results are encouraging. Table 22.1 summarizes these technologies and their application are following discussed.

Table 22.1 Main innovations in kidney transplantation

Unmet need	Innovative strategies
Access to KT: Access to the waiting list	Social media for improving education and outreach about transplantation for CKD patients and general nephrologists
	E-health and telehealth to "shorten the distance" to the transplant center and optimize pretransplant evaluation
Access to KT: Organ supply and allocation	Social media for providing information about transplantation for health professionals and overall society
	E-learning and telehealth for patients and potential donors' education
	Machine perfusion: Organ "resuscitation" and assessment of organ quality
	Telepathology and artificial intelligence for interpretation of donor biopsy slides
	Tools for optimizing donor risk assessment and support decisions on organ acceptance
	Tools for predicting CKD after living kidney donation
	Tools for optimizing organ allocation
	Tools for predicting the waiting list time
Access to KT: Faster organs´ shipping	Shipping organs by drones
Access to KT: Expanding organ source	Xenotransplantation
	Kidney bioengineering
	Artificial-implantable renal devices
Organ preservation	Techniques for deceased donor maintenance
	Hypothermic and normothermic pulsatile perfusion
	Ex-vivo kidney perfusion with oxygenation and delivery of drugs or cellular and genetic therapies
Immunological evaluation	Epitope evaluation
	Identification of non-HLA antibodies

Table 22.1 (continued)

Unmet need	Innovative strategies
Transplant surgery	Minimally invasive surgical techniques: Laparoscopic, robotic-assisted, minimally invasive video-assisted, minimal-access, and minimal skin incision techniques
Immunosuppression and other immunomodulatory treatments	Regenerative medicine—immune tolerance
	Development of new drugs
	Precision medicine to customize immunosuppressive regimen and long-term strategy
	Gene therapy to modulate genes involved in allograft damage processes
Posttransplant follow-up and monitoring	Biomarkers for early detection of allograft injury
	Tools for predicting outcomes
	Telemedicine and telemonitoring
	Tools and technologies to support patients with medication adherence

CKD chronic kidney disease, *KT* kidney transplant, *HLA* human leucocyte antigens

22.2.1 Access to Kidney Transplantation: Access to Waiting List

Despite robust evidence that KT is better than dialysis for most ESKD individuals [2, 7], a significant proportion of dialysis patients do not have access to this treatment. The main barriers in access to KT involve suboptimal referral and enlistment to KT and imbalance between supply and demand for organs [5]. The suboptimal referral to pretransplant evaluation and waiting list enrollment results from educational and socioeconomic barriers [8]. Therefore, technologies for providing accessible information are valuable.

Social media has shown to be a powerful tool to reach patients with chronic diseases, fostering health literacy. These platforms enable the transmission of scientifically relevant content in an easy-to-understand language to an unlimited number of patients [9, 10]. Beyond patient health literacy, general nephrologists and other healthcare providers assisting chronic kidney disease (CKD) patients on predialysis and dialysis must recognize that KT is the best treatment option for ESKD [11, 12]. Social media is also an interesting web-based tool for health professionals' education, providing access to updated information, connection with experts, experience exchange, and engagement in scientific debates [13]. Importantly, misleading and erroneous information are usual in social media. Therefore, both patients and healthcare professionals should be warned about avoiding platforms whose content is not validated by an expert professional or an academic institution [14, 15].

In addition to social media, e-health and telehealth are potentially valuable tools to help CKD patients access the waiting list, "shorten the distance" to the transplant center, thus supporting and streamlining the pretransplant evaluation process [16–18].

22.2.2 Access to Kidney Transplantation: Organ Supply and Allocation

The main barrier to KT is probably the organ shortage. ESKD prevalence is growing worldwide, and the number of kidney donors has not risen to match the demand [19]. A multifaceted approach is required to break down these barriers to reduce the organ-supply imbalance.

New technologies are promising in supporting this fundamental step. Trustworthy social media are valuable tools to provide education for the general population and health professionals, impacting potential donor notification, improving donor maintenance, encouraging living organ donation, and reducing family refusal of deceased organ donation [20, 21]. E-learning and telehealth are also potentially effective tools for educating potential donors [22].

Another critical step to increase organ supply is to reduce organ discard. Machine pulsatile perfusion is routinely used to reduce delayed graft function (DGF) [23]. Beyond this classic use, evidence suggests that machine perfusion favors organ acceptance by providing the assessment of organ quality and allowing organ "resuscitation" [24, 25].

Telepathology and digital pathology using artificial intelligence are new strategies to ensure faster scanning times and more reproducible biopsy reports, potentially impacting on organ acceptance rates [26].

Using traditional statistical models or machine learning techniques, risk-prediction equations have been developed to optimize donor and recipient risk assessment and support the decision-making process. Currently, the most widely used predictor is the ***Kidney Donor Profile Index (KDPI)*** calculator, which combines ten donor-related variables and summarizes the likelihood of graft failure after a deceased donor kidney transplant. The formula is available on: https://optn.transplant.hrsa.gov/data/allocation-calculators/kdpi-calculator/

In the website http://www.transplantmodels.com/ (Copyright Johns Hopkins University, 2020), other predictive formulas are currently available:

- ***ESRD Risk Tool for Kidney Donor Candidates***: predicts the estimated risk of ESKD after living kidney donation.
- **Kidney Donor Risk of ESRD**: predicts the risk of ESRD in individuals who have already donated a kidney.
- **Live Donor KDPI Calculator**: calculates the risk score for a recipient of a potential live donor kidney.
- **KT Candidacy Calculator for Patients 65+:** estimates the probability of 3-year survival after KT in patients aged ≥65.
- **Johns Hopkins IRD Kidney Transplant Calculator**: estimates recipient mortality after receiving an Infectious Risk Donor (IRD) kidney.
- **Order of Deceased Donor and Living Donor Kidney Transplantation in Pediatric Recipients**: compares long-term patient survival after living and deceased KT in pediatric recipients.

- **KDPI-EPTS Survival Benefit Estimator**: predicts the 5-year survival benefit for receiving a kidney, based on candidate's Estimated Post-Transplant Survival (EPTS) and the kidney's Kidney Donor Profile Index (KDPI).
- **Kidney Transplant in the Context of the COVID-19 Pandemic**: estimates 5-year survival after KT and on the waiting list during COVID-19 pandemics.

Recently, using machine learning techniques, Brazilian authors developed a calculator to predict the waiting list time in São Paulo State: https://gustavomodelli.shinyapps.io/time_list_in_tx/ [27]. In addition to predictions, new technologies can be used to perform donor-recipient matches, whether for a living or deceased donor transplant.

22.2.3 Access to KT: Faster Organ Shipping

Prolonged cold ischemia time is a risk factor for DGF and graft loss. Thus, strategies to reduce this time are desirable. Depending on the territorial extension of the region and the country allocation model, a complex transportation network is necessary, and a long time is required for the organ to reach its destination.

Dramatic advances in unmanned aircraft systems (drones) allow for high races and long distances covering autonomous, monitored, and pilotless travel. As well as in other areas, drones have been presented as a cheaper and safer alternative for organ shipping [28]. The first successful kidney travel for transplantation was recently reported. The kidney was effectively transplanted and showed promptly reperfusion and function [29]. Barriers and concerns related to this technology should be individualized and further discussed.

22.2.4 Access to KT: Expanding Organ Source

Even in a hypothetical scenario of optimizing the supply of organs from living and deceased donors, it is likely that this supply of organs will not meet the growing demand. Therefore, it is necessary to evolve in developing alternatives for renal replacement.

Xenotransplantation and kidney bioengineering are promising strategies to expand organ offer, potentially providing an unlimited and ready-to-use supply of transplantable organs. The main barriers to kidney xenotransplantation are immunological events and organ-derived infections. Genetic engineering techniques have overcome these barriers with good preclinical data [30]. Recently, a kidney grown in a genetically altered pig was successfully implanted in a brain-dead human patient at the N.Y.U. Langone Transplant Institute. The allograft was not immediately rejected and produced urine for at least 54 h, encouraging scientists [31, 32].

Another potential source of organs is bioengineering and regeneration technologies, manufacturing kidneys. Techniques to obtain acellular extracellular matrix scaffolds (decellularization) and 3D printing using biomaterial (polymers) have been studied and improved over the last decades. However, the production of regeneration-competent cells is still challenging. Probably closer to becoming a reality is using stem cells to repair and regenerate poorly functioning organs and reduce the need for immunosuppressants after transplantation [33, 34].

Also encouraging, but with no forecast of becoming viable in the coming years, is replacing kidney function using artificial-implantable renal assist devices. Pioneered by UC San Francisco researchers, the equipment is based on microelectromechanical systems technology, with two chambers containing silicon-nanopore membranes: a hemofilter to remove toxins, water, and salts; and a bioreactor seeded with renal proximal tubule cells to reabsorb water and salts [35].

22.2.5 Organ Preservation

Significant advances have occurred in organ preservation since the 1960s, including a better understanding of the impact of optimizing organ preservation before harvest, the development of increasingly better preservation solutions, and the use of pulsatile perfusion [36, 37]. Despite these advances, innovations on organ preservation are still required to ensure organ quality, supporting the decision-making process on the acceptance or refusal of kidneys, reducing DGF rates, and improving kidney function and survival.

In this regard, promising attempts to improve preservation have been carried out. As an example, researchers at the University of California have demonstrated that the use of mild hypothermia (34 to 35 °C) in brain-dead deceased kidney donors reduced DGF among recipients. Notably, the study was prematurely stopped after the interim analysis of 370 of 500 planned donors on the recommendation of an independent data monitoring committee [38].

As for pulsatile perfusion, incremental innovations have been progressively described since the 1970s. In addition to traditional hypothermic pulsatile preservation techniques [23], promising results have been described with ex vivo normothermic kidney perfusion, gas delivery, such as oxygen, and delivery of drugs, polymeric nanoparticles, stem cells, and genetic therapies [39, 40].

22.2.6 Immunological Evaluation

The compatibility between donor and recipient Human Leukocyte Antigens (HLA) is a major determinant of acute rejection and graft survival and remains the core of kidney allocation. Many advances have occurred in past decades since their identification, mainly in HLA typing techniques, but also in clinical interpretation of anti-HLA antibodies before and after (de novo) transplantation. Recently, much effort

has been made to identify better allocation by advancing from HLA to epitope matching [41]. An epitope is defined as the polymorphic amino acid configurations recognized by activated B cells, so previous antibodies against an epitope can actively initiate the rejection. A computer algorithm program HLAMatchmaker (http://www.epitopes.net/index.html) made available an extensive panel of HLA alleles and their respective antibody reactive patterns (eplets) to identify epitopes that can react to specific antibodies. Applications, such as EpVix (https://www.epvix.com.br/), uses HLAMatchmaker to provide a useful and fast automated epitope virtual crossmatching at the beginning of organ allocation [42]. For highly sensitized patients, this free platform could be helpful in the allocation of suitable organs applying the virtual crossmatch by finding the acceptable HLA mismatches. An acceptable mismatch is a mismatch at antigen level but involves structural and functional compatible eplets, which, in turn, are of low risk to initiate rejection. Although this technique is part of allocation policy in some transplant programs, we need further larger studies to recommend its widespread use in clinical practice [43].

Although not common, there have been reported acute antibody-mediated rejection associated with non-HLA antibodies. The surveillance of these antibodies should be suspected in cases of absence of anti-HLA antibodies since they are not routinely tested. The reports cite antibodies against Major Histocompatibility Complex class I related chain A antigen (MICA); angiotensin type 1 receptor (AT1R); endothelin-1 type A receptor (Anti-ETAR); FMS-like tyrosine kinase 3 (FLT3); epidermal growth factor-like repeats and discoidin I-like domain 3 (EDIL3); intercellular adhesion molecule 4 (ICAM4) [44].

22.2.7 Transplant Surgery

In high-risk patients, minimally invasive surgical techniques have been attempted to reduce post-operative complications, resulting in shorter hospitalization and lower costs and morbidity. The most undesirable perioperative outcomes are wound dehiscence and infection, incisional hernias, longer analgesic need, and worse cosmesis. Minimally invasive techniques described in kidney transplantation include laparoscopic, robotic-assisted, minimally invasive video-assisted, minimal-access kidney transplantation, and minimal skin incision techniques [45].

One of the most promising options is the robotic-assisted kidney transplant (RAKT), first performed in the early 2000s [46]. Since then, robotic urological platforms and specific technical modifications were progressively developed, accumulating much experience. First aimed for obese patients (body mass index higher than 35–40 kg/m^2) planned to living donor transplants [47], this technique evolved for deceased donor transplants [48], and initial experiences were limited to patients without surgical challenges, that is, without severe atherosclerotic disease in iliac vessels, highly complex graft anatomy, or multiple abdominal surgery. Prolonged cold ischemia, re-warm, and total surgery time are potential disadvantages. Currently limited to high-volume and academic transplant centers, initial RAKT reports are promising, potentially providing favorable surgical and functional results [45, 46].

22.2.8 Immunosuppression

One of the most desired goals of transplantation researchers is to induce operational tolerance. Given its immunomodulatory properties, stem cells have been tested for decades, but the good preclinical results are yet to be reproduced in clinical studies [49]. While we await advances in clinical studies on operational immunotolerance, the use of long-term immunosuppressive medications remains mandatory.

The development of cyclosporine was probably the most disruptive innovation in kidney transplant immunosuppression. Since then, new drugs incorporated into the therapeutic arsenal have brought incremental improvements in the safety and efficacy profile, ensuring the current low rates of early acute rejection and a good safety profile. Since 2010, with the approval of everolimus and belatacept, no new drug was approved for use in the maintenance immunosuppressive regimen. Currently, clinical studies at more advanced stages are with iscalimab, an anti-CD40 monoclonal antibody that blocks the costimulatory pathway. For the prevention and treatment of antibody-mediated rejection in sensitized patients, studies have been carried out with drug repositioning, such as eculizumab and C1 esterase inhibitors, complement pathway inhibitors; imlifidase, an IgG-degrading enzyme of *Streptococcus pyogenes*; tocilizumab, an interleukin-6 inhibitor; daratumumab, a humanized monoclonal anti-CD38 antibody; and belimumab, a humanized antibody that inhibits the activity of B-lymphocyte stimulator [50].

Beyond the persistent quest for more effective and safe drugs, less nephrotoxic, and for providing a better quality of life, a fundamental challenge is to match the ideal immunosuppressive regimen for each patient, that is, individualization. In this regard, personalized precision medicine emerged as an up-and-coming innovation. By combining clinical data, omics (genomics, proteomics, metabolomics, and transcriptomic), and big data analytics, this strategy promises to support better decisions about the initial immunosuppressive regimen, drug exposures (ideal doses, and concentrations), and long-term strategy [51].

In addition to drugs and cell therapy, the clinical application of gene therapy is also promising in kidney transplantation. By using vectors (plasmids, nanostructured, or viruses) for delivery of extrachromosomal material to target cells, this therapy has the potential to modulate genes involved in kidney damage processes. Currently, most studies are focused on identifying the mechanisms and target genes involved in allograft damage, such as ischemia-reperfusion injury, immune response resulting in acute and chronic rejection, and fibrosis [52].

22.2.9 Posttransplant Follow-Up and Monitoring

Knowing what inflammation process is dominating the allograft was always a challenge. Graft invasive biopsy is the gold standard and fully available method to get the best answer. However, the possibility of accessing the signature of DGF, acute and chronic rejection by examining the blood or urine is now and ever in the

pipeline. Many biomarkers were raised and failed, but the aim of identifying biomarkers that early detect allograft injury remains pursued. Since graft damage is often multifactorial and multigenic, an isolated biomarker probably cannot predict or detect deleterious events. However, each biomarker might add information to understand the injury [53].

Recently, personalized precision medicine has emerged as a potential tool to individualize posttransplant immunosuppressive strategies. Genomic (DNA analysis) and transcriptomic (RNA analysis) biomarkers have been increasingly explored to contribute to this strategy [51].

As an example, a urinary panel of six cell-free microRNAs (miRNAs) (miR-9; miR-10a; miR-21; miR-29a; miR-221; miR-42) showed promising results in predicting DGF when analyzed in the first urine and within 5 days after kidney transplantation [54]. mRNA transcripts, called gene signature, in blood, urine, or graft biopsy has been investigated for predicting acute rejection or long-term outcomes. The findings reinforce the hypothesis that a gene expression profile can reflect the renal tissue immune pathways and act as an adjuvant tool for diagnosing and monitoring graft rejection. The number of genes included in this diagnostic "packages" varies from 3 to 19, and englobe genes involved in T-cell response (e.g., IFN-γ), chemokines (e.g., CXCL-10), and transcriptional factors (e.g., TIMP1) [53].

Other recently proposed biomarkers are small fragments of cell-free DNA (cf-DNA), derived from donor (dd-cf-DNA) graft cells, identified in the recipient blood due to cell death or injury. Despite some controversy about the method standardization, a dd-cf-DNA level greater than 0.34% of total cf-DNA is found in acute rejection episodes and DGF. A recent metanalysis showed that higher titles of dd-cf-DNA were found in patients with antibody-mediated rejection (ABMR) but not in T-cell-mediated rejection. These findings highlight that it should be of preferential utility in highly sensitized patients [55].

Also used by personalized precision medicine are big data and tools for predicting posttransplant outcomes. The validated risk-prognostication system (integrative Box/iBox) (http://www.paristransplantgroup.org) is another valuable attempt to define early surrogate endpoints to help identify patients at high risk of future graft loss and then design potential therapeutical interventions. The risk is evaluated at the time of a graft biopsy. Measurements included in the model are estimated glomerular filtration rate (eGFR), proteinuria, patterns of histopathology, and circulating anti-HLA Donor Specific Antibodies (DSA). The resulting score provides an estimated graft survival in the next 3, 5, and 7 years, which has shown accurate performance in validation cohort in Europe, at different times post-transplant, at different clinical settings such as immunosuppressive regimens, and randomized controlled trials [56]. In addition to the iBox, several predictors have been developed in recent years to predict other post-transplant outcomes, such as DGF, CMV infection, COVID-19-related death, among others [57–59].

The long-term follow-up of a KT recipient precludes close and prolonged clinical and laboratory monitoring. Access to conventional care should be limited for persons who live in rural areas, with multiple comorbidities, and difficult to travel or live in developing countries [60].

Telemedicine and telemonitoring are hopeful strategies to overcome the physical barriers and have been progressively and widely accepted worldwide [61]. Because of some translational, legal, and operational issues, telenephrology was not widely used in clinical practice before the coronavirus disease 2019 (COVID-19) pandemic. Inaccuracy of symptoms report, limited physical examination (video-dependent inspection only), ethical questions, reimbursement policies, lack of specific healthcare laws are some issues that require attention and improvement [62].

The recommended social distancing to avoid COVID-19 infection challenged the pretransplant evaluation and the post-transplant follow-up. The transplant community rapidly adapted to clinical practice toward adopting telenephrology strategies through the available technology, such as a mobile phone. The number of KT drastically fell after the COVID-19 pandemic in some countries. However, satisfactory experiences have been related to promoting access to pretransplant evaluation and in chronic follow-up care (clinical consultation, professional training, reminders, and self-monitoring) [60, 62]. Patients reported telehealth was convenient and minimized time, financial, and overall treatment burden [63]. Despite the limitations to broadly implement in all services, telehealth would be part of the COVID-19's legacy [62, 63].

Finally, tools and technologies to support patients with medication adherence are necessary, and they have been tested. Nonadherence to immunosuppressives is a major risk factor for worse kidney allograft outcomes. Non-adherent patients have a seven-fold increased risk of graft failure and acute rejection episodes and consequently higher costs to health systems [64]. Nonadherence is a multilevel behavior, which involves factors associated with the patient (sociodemographic profile, details of previous CKD treatment, psychosocial aspects, type of donor, immunosuppressive regimen), healthcare professionals (trust, satisfaction, communication quality), transplant center (composition of the team, patterns of care), and finally, with healthcare system (financial burden of immunosuppressives) [65]. Nonadherence is a potentially modifiable factor for poor outcomes [66]. However, strong evidence indicating the best strategies to reduce it are still lacking [67]. Recently reports supported measures directed to the patient to enhance the self-care and self-monitoring. Electronic devices and applications (eHealth) are being further employed to help patients adhere to post-transplant care. A recent metanalysis of randomized controlled trials of eHealth interventions showed a 34% increase in medication adherence. The type of intervention with the best results is multifunctional, defined by a strategy including two or more functions such as reminder, self-monitoring, educational, behavioral counseling, and clinical decision support system. Most interventions involved professional clinical support and a pre-defined delivery dose regimen [68].

Another perspective is to move the intervention focus from the patient to higher levels of care toward provider-related and system-related factors. It is mainly because the effect of published reports is small and collected from low-quality evidence [66, 67]. Toward this direction, a Brazilian multicenter study showed, for the first time, that a characteristic of post-transplant care, a more convenient treatment, assessed by the patient's satisfaction with the frequency of consultations, was associated with better adherence to immunosuppressives [69].

22.3 Conclusion and Future Perspectives

Notwithstanding the significant disruptive and incremental innovations developed in the last decades, some barriers and unmet needs remain relevant, affecting transplant access and allograft survival. Innovations and new technologies are mandatory to overcome these barriers and meet these unmet needs. Noteworthy, for these technologies to become a global reality, it is essential that pharmacoeconomic studies are carried out, especially in low-income countries, where resources are scarce.

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Chapter 23
Development and Implementation of Unmanned Aerial Vehicles for Donor Organ Transportation

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23.1 Introduction

Although the number of annual transplants has steadily risen in the United States, so too has the number of end organ disease patients on the waiting list for donor organs. In response, the systems for allocating donor organs have continued to evolve alongside improvements in surgical and pharmacologic interventions [1]. Yet, current logistics and transportation networks remain inefficient, costly, and hazardous.

The availability of deceased donors for transplantation has notably increased (38%) between 2015 and 2020, with a continuous upward trend since 2010 [2]. In 2020, 39,036 solid organ transplants were performed in the United States, 85% of which were from deceased donors [3]. Despite this upward trend, as many as 3,500 viable organs are discarded per year [4], and it has been estimated that approximately 20% of potential donor kidneys in the US are discarded [5].

Over the past several decades, donor organ allocation systems have continued to evolve, prompted primarily by efforts to distribute this precious resource more fairly. In 2019, the United Organ Sharing Network (UNOS) revised the Kidney Allocation System (KAS) for the second time since 2014 in an attempt to limit bias stemming from donor service area, expanding its match algorithm to a 250 nautical mile radius around the donor [6]. While the new system afforded proximity points to potential recipients close to the donor, the algorithm could potentially match recipients as far away as 2,500 nautical miles [6]. Despite the expanded distances over which donor organs could be matched, the modes of transportation and logistics services have remained largely unchanged over the past several decades.

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Currently, donor organs are transported via car, helicopter, as well as commercial and non-commercial fixed wing aircraft. In 2009, 26% of procurement transports utilized aircraft (fixed-wing or helicopter) [7]. Given increasing distances for donor organs to travel, increasing transport times have resulted in increased cold ischemia time (CIT) and instances of delayed graft function [8, 9]. Furthermore, since the 2014 KAS revision, the average air travel distance for a donor kidney has increased from 440 miles to 706 miles[5]. Reliance on air travel has presented several issues. First, organ transport by commercial aircraft is dependent on the availability of an aircraft, crew, and local airport resources. Second, non-commercial flights are expensive, putting a financial strain on insurance carriers, transplant centers, and OPOs [10]. A cost analysis of donor liver transport found that fixed wing aircraft transportation incurred an average cost of $7,875 per organ, compared to $118 per organ for ground transport [11]. Third, surgeons and clinical team members face increased personal risk with air transport. A 2009 retrospective analysis found that air travel associated with procurements constituted 56% of all travel-related accidents among transplant surgeons and that the risk of fatality for a non-commercial procurement flight was estimated to be 1,000 times greater than a commercial procurement flight [7]. Tragically, entire procurement teams have perished in air accidents [12]. Ground transport, on the other hand, is of course limited by traffic patterns and local speed regulations and is also susceptible to accidents.

Unmanned aerial vehicles (UAVs), or drones, have become increasingly utilized in both commercial and military projects and present an innovative approach to donor organ transportation. Their small size, ability to integrate sensors for organ monitoring, and remote piloting capability may pave the way for rapid roof-to-roof delivery of transplantable organs with continuous monitoring between donor and recipient hospitals. This chapter will describe the history, state of the art, and future of UAVs as a viable method for donor organ transportation.

23.2 UAV Technology, Current State of the Art

The medical field has already begun to adopt drones to deliver supplies and human tissue. Zipline, a biomedical distribution company, began using drones in 2016 to transport medicines and blood products in Rwanda, which they subsequently expanded to Ghana, Nigeria, and the United States [13]. United Postal Service (UPS) received Federal Aviation Administration (FAA) approval in 2019 for UAV delivery of packages containing blood and tissue samples across the United States [14]. Trials of UAV donor organ delivery have only recently begun, starting in 2019 with the transport of a donor kidney and subsequent transplantation in the United States [15].

Scalea et al. used a remote-controlled DJI M600 Pro drone model, which utilized six vertically oriented rotors with battery-powered motors positioned directly underneath to shield the payload from heat generated by the aircraft [15]. The drone requires a 5-min warm up period prior to active flight and can carry a payload of up

to 9.1 kg [5]. It can reach a maximum speed of 64 kmh (notably, drones developed by the military can achieve speeds ranging between 217 and 740 kmh) [16]. This model can tolerate wind resistance up to 8 m/s while hovering and travel through winds up to 32 kmh. The drone has a maximum service ceiling of 4.5 km above sea level, operating at temperatures between −10 °C and 40 °C [17].

In 2021, MissionGo, a newly formed company that develops and implements UAVs for biomedical and commercial cargo, performed its first test flight of a human pancreas in collaboration with Midwestern OPO, Lifesource [18]. The test utilized a newly designed MissionGo MG Velos 100 drone with integrated real-time flight telemetry that communicates with ground teams through Bluetooth and cellular signals [18]. Velos Rotor drones have cruising speeds between 30 and 70 kmh with a maximum speed of 130 kmh. It is also engineered to carry multiple payloads, totaling 10 kg [19].

23.3 Donor Organ Monitoring and Impact of Transit Conditions

UAV implementation has prompted a re-evaluation of the impact that transit-related stressors and environment have on donor tissues. During transport, organs experience shifts in temperature, acceleration, vibration, and pressure [15]. There is no currently standardized method for monitoring donor organs once they have been packaged on ice for transport. This dearth of data on donor organ transit conditions has potential ramifications for the overall quality and outcomes of donor allografts.

Hypothermic conditions are necessary for the preservation of the donor organ prior to transplant, which decreases the rate of cellular metabolism and cell death following aortic cross clamp [20]. The optimal temperature for organ preservation was found to be 4–8 °C. Temperature variation above this range resulted in increased cellular metabolism and, thus, more rapid hypoxia-induced cell death. Lower temperatures resulted in ice crystal formation with subsequent tissue architecture damage and protein denaturation [21].

Vibrational forces have been shown to trigger a host of changes at the cellular and tissue level through shear stress [22]. Damage to red blood cells was directly proportional to the magnitude and duration of acceleration [23]. The combination of warm ischemia and shear stress on lung tissue triggered depolarization of pulmonary endothelial cells and activation of a mechanotransduction cascade, leading to nitric oxide synthase-mediated oxidative injury [24]. Further, in bovine aortic endothelial cells, shear stress triggered changes in cytoskeletal assembly, cell conformation, and red blood cell adhesion [25]. Vibrational stress also affected tissues at the genetic level by altering expression of genes involved in cell cytoskeleton architecture, leading to disruptions in cell adhesion and conformation in murine embryonic fibroblasts [26]. Additionally, at the immune regulatory level, rat spinal cord exposed to tissue-specific resonant vibrational frequency increased the secretion of both pro- and anti-inflammatory cytokines (IL-2, IL-6, TNFα, VEGF, IL-10 and IL-4) compared to non-resonant frequencies [27].

Apart from vibrational forces, hypobaric conditions have been associated with tissue damage, showing increased hemolysis in an in vitro static blood model [28]. Fixed-wing aircraft maintain reduced cabin pressures relative to sea level (0.7 Atm) [29]. Hypobaric pressure has also been associated with decreased cerebral perfusion and mean arterial pressures in swine [30] and exacerbated preexisting cerebral axonal injury in rats with subsequent motor impairment [31]. Furthermore, fixed-wing air transport has been negatively associated with graft and patient survival compared to ground transport. Huang et al. showed that livers transported by fixed-wing aircraft had significantly higher CITs than those using ground transport, which was attributed to the time lost from aircraft availability, flight delays, and route diversions [29]. While there are no published studies yet assessing direct cellular, gene level, or functional changes in whole explanted organs related to fixed-wing and ground transport, these aforementioned studies provide a warning that forces experienced during transit may negatively impact donor organs and subsequently affect transplant outcomes.

Researchers have also begun studying the forces experienced during drone transport [32]. Scalea et al. assessed the effects of 14 drone flights, an aggregate of 62 min of flight time, on an explanted human kidney [5]. The organ experienced 1.5 m/s of acceleration and a peak velocity of 67.6 kmh. The temperatures remained stable and low during flight (2.5 °C) within a built-in cooler, and pressure decreased with altitude, ranging from 0.37 to 0.86 kPA. Drone travel vibration was <0.5 G, lower than that observed with fix-wing air flight (>2.0 G). Kidney biopsies done immediately prior to and following the flight were negative for changes in the degree of glomerular sclerosis, cortical scarring, and hyalinosis [5]. These findings increase confidence in UAVs as a safe mode of transportation. Further study is needed to assess the histologic and functional impact of transport stressors (temperature, pressure, and vibration) on whole explanted organs [1].

23.4 Clinical Utilization

The first drone-delivered donor organ was transplanted in April 2019, flying a roughly 5 km path over the city of Baltimore to the University of Maryland, where it was implanted into a 44-year-old patient (Fig. 23.1). She had end-stage renal disease secondary to hypertension and had been on dialysis for nearly a decade. This delivery using a custom-built LG-1000 4-arm octocopter was preceded by 44 test flights assessing various aspects of aircraft performance including take-off, communication, parachute function, and landing. Throughout its course, temperature, vibration, and pressure readings showed no significant difference compared to organs transported by ground. The craft carried a total of 4.4 kg through a clear 20 °C spring day, with 16 kph winds and gusts up to 26 kph. Thirty days after successful transport and transplant, the patient remained off dialysis and her creatinine had fallen to 2.36 from 9.06 mg/dL at the time of transplant [15, 33].

Fig. 23.1 LG-1000 four-arm octocopter drone carrying the first donor kidney for human transplant landing at University of Maryland Medical Center, April 2019

The following year in September 2020, Nevada's OPO, Nevada Donor Network, and MissionGO completed two test flights in the greater Las Vegas area using another drone model. The first carried research corneas from Southern Hills Hospital and Medical Center in Las Vegas, NV to another urban hospital 3.2 km away. The second flight, the longest transport to date, carried a research kidney over 16 km to a small town outside the city [34, 35].

In May 2021, MissionGO and Lifesource, OPO for the upper Midwest, conducted the first drone test flight carrying an explanted pancreas. The organ was flown in a 16 km loop over the Mississippi river with take-off and landing at Mercy Hospital in Anoka County MN, 23 km outside Minneapolis, MN. Pre and post flight biopsies of the pancreas were negative for changes in tissue architecture [36].

Then in September 2021, a pair of donor lungs were transported by drone from Toronto Western Hospital to Toronto General Hospital in Canada, over 1.2 km. The double lung allograft was transplanted into a 63-year-old recipient with pulmonary fibrosis. This was performed as a collaboration between Unither Bioélectronique Inc., a subsidiary of United Therapeutics Corporation, and Toronto's University Health Network [37, 38].

Although there have been only four major sets of drone-based donor organ transport testing in North America to date, they represent a willingness of transplant centers and OPOs to integrate this technology into the care of their transplant patients. These test and donor transport flights also point to the safety of UAVs as well as a noninferiority to piloted aircraft in terms of vibration, temperature, and pressure changes measured during transport.

23.5 Current Limitations of UAV Implementation

Despite the promise drone use holds for improving efficiency, personnel safety, and organ monitoring, there are several limitations to implementing this technology. Transplant surgery in general, and so too drone use, relies on mutual trust between the recipient and donor surgical teams. Many transplant centers prefer to procure donor organs for their own recipients, forgoing the option to allow for import of that organ via drone. A 2019 survey among transplant surgeons revealed a 50% acceptance of donation after brain death pancreata procured by surgeons from other transplant centers. On the other hand, 67% of respondents had no preference regarding which team procured donor kidneys [39]. Although this survey represents a stated willingness to accept import allografts procured by other teams, documented import practices reflect a significantly lower tolerance for this practice. Eighty-five percent of respondents of a 2009 survey noted that they would allow another, nearer, team to procure a donor liver for them, when only 14.8% of transplanted livers represented actual imports [7].

Commercially available drones have relatively limited cruising speeds, with average speeds ranging from 64.3 to 96.5 kmh, making them slower than land-based transport. Drone stability is also exceedingly weather dependent given their relatively small size. Heavy winds and rain affect air turbulence and may result in the grounding of drone flights compared to other forms of air transportation.

There are also regulatory hurdles to drone implementation for donor organ transportation. First, FAA regulations pertaining to drone usage restrict public drone flight to a maximum altitude of 120 m and a visual line of sight to the pilot, creating a buffer from low-flying aircraft which cruise at 150 m [40]. This currently prohibits long distance deliveries where line of site to the drone is not possible. However, the FAA is currently reviewing drone regulations particularly as they apply to donor organ transport flights.

23.6 Future Applications

While in its very early stages, biomedical UAV technology presents an opportunity for developing and integrating a constellation of interventions to improve solid organ transplant outcomes. On top of shipping local donor organs to avoid traffic congestion within metropolitan centers, drones may one day be able to fly faster and longer distances to deliver locally procured organs to out of state recipients without need for transporting a surgical procurement team. Drones may also be implemented for transport of other transplant-related tissues and samples such as lymph nodes and spleen for crossmatch assays, donor vessels to be used in reconstruction, or blood samples. Implementation of drone transport has encouraged a redesign of organ transport and tracking infrastructure, paving the way for medical technology startups to attempt to integrate communications and tracking of donor organs from match to procurement to delivery route to recipient hospital. The first of these

companies, MediGo, has begun to forge partnerships with OPOs [41]. Furthermore, organ transport via UAV has necessitated the development of technologies to more closely monitor the conditions of the organ while in transit, such as temperature, pressure, vibration, altitude, and geolocation [5]. These sensors, although developed for UAVs, can be incorporated into traditional organ transport packaging in order to study and standardize preservation conditions. This has also spurred research into the impact transit-derived vibrational forces exert on the functional and immunologic outcomes of donor organs. While drones and improvements in logistics integration make longer distance travel feasible, resulting in subsequently longer transit times, UAVs may also come to integrate machine perfusion technology. Placing donor organs into hypo- or normothermic perfusion devices for transport may facilitate increased CIT tolerance or rehabilitation of marginal organs while in transit [42]. UAV research has permitted the integration of technologies and experience from specialties outside of transplant, allowing the field to continue to evolve, improving access and outcomes for those with end organ disease.

23.7 Conclusion

Despite the increasing complexity and matching radius of organ allocation systems in the United States in the service of equal opportunity for recipients, the modes of transporting these organs over ever greater distances have not significantly changed. Air- and land-based transport systems continue to present an increased risk of injury or even death to procurement teams. UAVs present an alternative delivery system allowing procurement teams to forgo long distance travel while also integrating real-time monitoring of the donor organ environment, improved tracking and logistics, and ultimately the opportunity to add modular perfusion technologies to the payload system. While still in its infancy, the use of drones for donor organ delivery presents a rich landscape for innovation of transportation logistics, organ monitoring, and organ preservation.

Conflicts of Interest Dr. Scalea holds patents in organ preservation and monitoring through the University of Maryland. Dr. Scalea founded MediGO, Inc. and MissionGO, Inc. companies which increase access to transplants using efficiency-oriented and innovative transportation models.

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Chapter 24
Implementation and Development of a Robotic Surgery Program

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24.1 Introduction

The idea of using "robots" for the surgical specialties in the last century and contributions from the military came up with new concepts of telepresence and telemanipulation. Prototypes were developed in a standard model, where robotic arms accurately replicate the movements performed by the surgeon and surgical instruments mimicking the human hands. Several systems are being developed and tested in several countries around the world and in the future will have new options on the market, with improvements in hardware, software, new tools, and big data associated to artificial intelligence [1].

Robotic surgery has been growing steadily all over the world and offers technical advantages and benefits over other surgical techniques [2], and so many institutions are evaluating the possibility of acquiring this technology. In urology, the technique is already well established as a successful surgical technological innovation, and in nephrology, robotic surgery has contributed to the performance kidney transplants [3], facilitating vascular anastomoses [4].

After so many factors, the decision whether to implement a robotic surgery program in a hospital institution must be evaluated regarding innovation and marketing. The development of a business plan, deep analysis of the market share,

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evaluation of the program's objectives, definition of the population that will be served, assessment of the institution's infrastructure [2], and a financial feasibility study might analyzed [5, 6]. The high investment for the acquisition of the robotic system and other types of equipment and the implementation of the program also require adaptation of the physical area of the operating room (OR) and Central Sterilize Supply Department (CSSD). Creation of a training center is mandatory, and robotic supplies, maintenance contracts [7], are crucial to build up a specialized team for the successful of the project. Marketing and training costs must also be considered.

Based on the experience of implementing four platforms Da Vinci robotic, from the company Intuitive Surgical® and simulation centers in private hospitals in Brazil, this chapter will describe how to carry out the implementation and development of a robotic program in a hospital institution, using tools and best practices for sustainability, quality of care, and patient satisfaction.

24.2 Implementation and Development of a Robotic Surgery Program

The implementation of a robotic surgery program requires planning and structuring new processes within the hospital institution. In this section, we will cover recommended practices during the accomplishment of a new robotic program.

The Technical Director and Nurse Coordinator must create the processes, flowcharts, attendance care, and training protocols. These guidelines need to be established to ensure maximum safety in implementing the program. Further, an operating room layout for each type of surgical procedure should be implanted, as well as, checklists of pre-per-pos procedures and the elaboration of forms for all surgeries. Postoperative care, and discharge, should be planned and implemented together with CSSD; material flow and robotic tweezers have to be driven safely [5, 8].

To develop a robotic surgery program, the goals of the clinical staff and the surgical team must be very well aligned with the protocols of the institution and the hospital rules. There should be a substantial financial investment in the initial phase of the program with a focus on developing best practices, continuing education, data monitoring, and care security. These steps are crucial, so the program can go on looking for the initial learning curve with quality and get positive outcomes.

24.2.1 Robotic Surgery Committee

During the implementation and development of the program, a robotic Surgery Committee must be created with a multidisciplinary team together with the institution's board. This Committee will establish the process timeline that must be developed before the arrival of the system. The Committee must contemplate: institution's

director, program's technical director, nurse coordinator, anesthesiology coordinator, surgical specialties coordinators, marketing coordinator, and clinical engineer.

The business plan and the evaluation of costs and resources must be available after defining the robotic system and simulator model. In this step, it is recommended to list all the system supplies, disposables, and tools to be purchased to begin the program.

Implementation demands a schedule establishing steps to initiate and will depend on the institution's infrastructure and processes [9]. Once the first case is scheduled, a timeline of steps and activities should enroll the staff to guide the surgery.

The Committee must create the robotic surgery internal guidelines, the attendance care, training protocols, and the short- and medium-term goals. It is recommended that the duties and responsibilities of each member of the robotic Committee must be defined and the medical coordinators of each specialty selected have to gather every other month. The training/certifications of surgeons should be monitored, and rules to monitor the program's performance data should be developed [5]. The Committee must evaluate the outcomes and suggest improvements for the program. It is also recommended to define the processes that need to the program [10].

At the first meeting of the Committee, it is recommended to present the investment spreadsheet with the equipment and supplies that must be purchased and the deadline schedule for each activity. Based on this, the nurse coordinator must drive the purchase of all disposables and supplies for OR and CSSD to perform the first robotic surgeries. The first surgeries to be performed in the program should also be discussed and planned in the first meetings [11], and the selection of the first patients should also be carried out [2].

Frequent meetings have been held to monitor each stage of the timeline, verifying each stage and the establishes deadlines, monitoring the arrival and installation of the equipment to be used in the OR, CSSD, and at the training center. All the stages of the schedule have to be completed before the robot arrives to avoid to financial losses and system idleness.

The Robotic Committee must be aware of the improvement issues, the performance of the teams, and the strategies to be traced for the development and growth of the program. The institution must promote a culture of great quality [9], establishing a performance evaluation of surgical, execution, and financial departments. This performance follow-up should be reported every other month for the Committee implement best practices.

Reports should provide data on program performance, surgical times, the productivity of each surgeon and each specialty, the average cost of surgeries, the length of stay of robotic patients in the hospital, and surgical outcomes, such as reapproaches and readmissions [9]. Based on the data and reports presented, the program coordinator and director have to get metrics and scores to point out improvement in the robotic surgery parameters. The Robotic Committee is responsible for analyzing all these data and index, developing financial strategies, and monitoring the clinical staff for the program's growth [9]. In the committee meetings, the goal of the management team must be very well aligned, and strategies must be discussed with transparency for the continuous improvement of the program.

24.2.2 Infrastructure

A robotic surgery program requires the creation or adaptations of the following sectors:

- Adequately sized operating room, electrical installations, no breaks, and slab reinforcement
- CSSD decontamination area for installation of a specific ultrasonic washer
- Simulation room
- Administrative office

The Nurse Coordinator with the institution's clinical engineer and the company's engineer representing the robotic system must assess the operating room in the OR, where the system will be installed and the decontamination area in the CSSD. This assessment is necessary to verify the need or not for renewal and adequacy of the facilities where the robotic system, the ultrasonic cleaner, and other equipment will be installed. Based on the assessment made, the institution must carry out the modifications to receive the equipment [11, 12]. During this visit to the operating room, the location of each component of the robotic system and the ultrasonic cleaner should be defined. It is recommended to carry out an architectural project before and a structural planning of the room. In some institutions, it is necessary to reinforce the operating room slab to support the weight of the robotic system.

The installation guide provided by the representative companies must be completed to plan the system arrival. Dimensions (width, height, length), weight, and the electrical/hydraulic recommendations must be evaluated so the institution can provide the necessary adjustments in its structure. It is recommended that the floor structure support the weight of 750 kg/m^2. This instrument's guide also requests an internet provider net for the operating room, as the robotic system needs to be always connected to the internet (on-site system) for remote monitoring of the robot's operation.

To tun the robotic system, the routes at the institution must be evaluated, the width of the corridors measured, the unevenness of the floor and the structure of the elevators must be checked, to provide maximum safety when receiving and transporting the system to the OR. The size of the truck that will transport the robot, the appropriate place to park at the institution, and the schedule of the best date and time for receiving it must also be evaluated.

Installation of the robotic simulator in the simulation room must also be planned. An exclusive room for training, with temperature control, auxiliary monitors, installation of no breaks, and dedicated outlets, must be provided for the installation of the simulator. Forms must also be created to monitor training performance.

The installation and testing of the robot must be monitored by the institution's Clinical Engineering, Nurse Coordinator and Medical Technical Director. Accessory materials and supplies that come with the robotic system must be checked and sent to the destination sectors (CSSD, OR, or clinical engineering).

24.2.3 Training and Continuing Education

Acquiring a new technology requires training the entire staff to ensure patient safety and execution success [10]. Training must be carried out for the operating room staff that will be dedicated to the program, including nurses, technicians, scrub nurses, anesthesiologists, and surgeons. For the team to feel prepared and safe for the most diverse situations in the operating room, training must be periodic due to the high investment in the program, the complexity of the system, and the necessary updates [8, 13].

The institution must create accreditation criteria for the clinical staff, for surgical team's learning curve improvement carrying out the structuring and standardization of the program's training protocols to offer the maximum quality and safety of care [11]. To plan the implementation of robotic programs, it is highly suggested that the training and qualification of the technical director, the coordinating nurse, and the head of anesthesiology is necessary before the arrival of the robotic system.

It is recommended that the technical director be a robotic surgeon [2] and that the nurse coordinator has experience in Surgical Center [5, 10]. The technical director, the head of anesthesiology, and the nurse coordinator must carry out technical visits and monitor surgeries in institutions that are a reference in robotic surgery. Regarding the nurse coordinator, it is recommended to complete the *Da Vinci Surgical System® Coordinators Course* provided by the company Intuitive Surgical® and also to accomplish benchmarking in hospitals with a large volume of robot-assisted surgeries. There are also other courses and specializations in robotic surgery for the nursing area on the market today.

After the installation of the Da Vinci system and the ultrasonic cleaner, training (theory and practice) must be scheduled with the OR and CSSD teams. The first practical training at the OR and CSSD is provided by the company representing the robotic system and other training must be provided by the Nurse Coordinator and the Technical Director.

The initial theoretical training is carried out on the Intuitive Surgical® website, which contains learning plans with videos and tutorials. As completing the training modules, an online assessment should be carried out to test the acquired knowledge.

To achieve practical training in the OR, the robotic system, the simulation abdomen, the training tweezers, and other robotic materials must be used. Training should include how to connect the system, draping, marking portals, positioning the patient cart, docking, endoscope, robotic tweezers, electrocautery, set up on the console, and the functionalities of the components of the system and system troubleshooting.

Theoretical and practical training at the CSSD (tweezers, endoscopes, and system accessories) includes how to conduct the transport of materials from the OR to the CSSD, precleaning, manual cleaning, automated cleaning, drying, lubrication, sterilization, and storage of materials.

Robotic simulator introductory training must be performed by the representative company engineer. The monitoring of training in the simulator must be carried out by an employee of the institution trained for this activity.

As soon as the installation and training on the robot and simulator, the coordinating nurse and the technical director of the program must improve the flows, processes, administrative, technical, care, and training protocols. According to the specificities of robotic surgery and robotic materials, it is recommended to train all the sectors involved with the program: financial analysts, administrative assistants, medical implants and prosthetics sector, form center, warehouse, patient attendance care, and marketing.

It is suggested to create a well-structured training protocol for surgeons, with all the steps that the surgeon needs to take to become a surgeon skilled in robotic surgery. The entire journey of the surgeon in the institution, from its accreditation to its certification, must be monitored and evaluated, including simulation, case observation, monitoring of surgeries as an auxiliary surgeon, practical training in the OR (in-service), and monitoring by proctors [14]. A training and follow-up program for residents should also be created so that they can develop basic knowledge of robotic surgery [2].

Once the program increases the number of robotic surgeries, the learning curve of the team tends to decrease, improving surgical times and optimizing the operating room. Surgical growth must be linked to the quality, and to achieve this goal, continuous training of the technical team is necessary.

A multi-specialty program is essential to increase surgical volume [2], and the program director plays a key role in encouraging other specialties to become involved in robotic surgery, thus increasing the program's productivity and consequently contributing to the training of local proctors of each surgical specialty.

The organization can form its *proctors* as the program's surgeons move along their learning curve and demonstrate productivity and a satisfactory clinical outcome from patients. *Proctors* trained by the institution can contribute to the training of future surgeons in the program.

Robotic technology comes with the necessity to develop new skills and greater responsibilities. It requires a lot of planning, high vigilance, and predictability from the director and nurse coordinator of the program. Thus, to keep up with this evolution in the surgical area, the robotic team needs to overcome challenges and constantly update itself to provide better surgical outcomes for patients [8, 15].

A well-trained, steady, and exclusive operating room team brings numerous benefits to the program. Among them, it allows effective communication between the team, faster room turnover, generating optimization of surgical times, high performance, reduction of adverse events, cost reduction, medical team satisfaction, and greater patient safety [7, 11–13, 15].

24.2.4 Robotic Team

The sizing of staff must be attained to meet the administrative and assistance demands. It is recommended to hire exclusive collaborators for the program: technical director, coordinating nurse, assistant nurses, nursing technicians, scrub nurses, administrative-financial collaborators, and simulation technicians.

24.2.5 Safety and Quality in Robotic Surgery

The first objective for the program might be safety and quality of care and is linked to patient satisfaction and experience with robotic surgery [9]. Tools and a policy for monitoring the patient on their surgical journey must be implemented to ensure that the robotic patient is treated in its entirety and individuality. The assessment of dissatisfaction and the observations that the patient makes serve as subsidies for opportunities for improvement in the program.

To manage the quality of care provided and offer maximum safety to patients, continuous analysis of data, metrics, and index is suggested. Forms can be implemented to collect information from robotic procedures. Monitoring of surgical times and checklists of materials used in surgeries should be recorded to monitor the team's learning curve and to obtain material costs for each procedure. The development of institutional metrics should be encouraged to monitor the productivity of the clinical staff, satisfaction, and patient outcomes.

As soon as the first surgery is scheduled, the checklist created in the planning phase should be used to guide the room layout, checking all materials and equipment necessary for the surgery and the positioning of the patient. The surgeon's *proctor* and the system representative company can assist with materials, how the patient should be positioned, and what the operating room layout should be. As specialties are being implemented and surgeries are scheduled for the first time, new checklists must be validated and standardized at the Committee.

After all materials and equipment have been checked, a pre-procedure simulation must be carried out 1 day before the surgery in the operating room with the entire multidisciplinary team and the company representatives leaders. Patient positioning, docking, and room layout should be simulated. A realistic simulation must always be performed before each new robotic procedure.

It is also recommended to build up a positioning patient protocol and a docking position for each type of robotic procedure. The protocol must include how to position the patient on the surgical table, as well as all the materials needed for the surgery. Covers and positioners must be used to prevent pressure injuries and electrocautery damage ensuring maximum safety for the patient during the surgery.

It is highly suggested to implement a specific care line for each robotic specialty, to monitor the patient from the pre- to postoperative period. As an example, it is suggested for urologic patients that a multidisciplinary team monitor the erectile function and continence rehabilitation of these patients [6].

To also ensure the quality of care, preventive and corrective maintenance contracts for the robotic system and other equipment must be signed to ensure their proper functioning during surgeries. The management of equipment used in robotic surgery must be performed by the institution's Clinical Engineer together with the Program Coordinator. Monitoring system maintenance and robotic equipment are some of the most critical points of the program to prevent their malfunction and, consequently, the cancellation of surgeries. Program success depends on fully functioning equipment.

Another important subject to fulfill in the program's implementation phase is to establish the inventory control and robotic materials management. The program coordinator nurse must manage the inventory together with the institution's warehouse sector and establish the rules for future purchases of robotic supplies. The inventory should preferably be weekly, and a safety reserve of supplies should be kept at the institution for any type of unforeseen event. Inventory management is one of the key points of the program, avoiding shortage of robotic materials and cancellation of surgeries, directly impacting the teams' productivity.

To manage the program, the medical director must count on the daily support and commitment of the coordinators of each surgical specialty. Monitoring their team and stimulating the surgeons to enroll to Da Vinci platform from the accreditation of doctors will turn the program scalable and profitable. Intensive training in the robotic simulator is suggested for the first cases and evaluation of the team's difficulties and patient outcomes.

Periodic self-assessment will help determine which areas of the program need improvement. Benchmarking with other robotics services is indicated to compare institutional performance with robotic hospitals that are already a reference in the market [2].

24.2.6 Robotic Surgery Department

An administrative, financial, and care team dedicated to the program is essential for the management and administration of the service [5, 6, 9]. It is recommended to create a robotic surgery department within the institution, with an administrative headquarters for managing the flow of robotic surgeries and monitoring the journey of patients from the preoperative to the postoperative period.

The management team must acquire all OR and CSSD materials and equipment necessary for use in robotic surgeries, and establishing the processes and protocols that need to be created for the implementation and success of the program. In addition to managing equipment and materials, the team must standardize material checklists, patient positioning, and room layout by procedure, coordinate the robotic

surgical map, manage the surgical data collected, create the receipt flow and hospitalization of robotic patients in the institution and establish the processing flow of robotic materials in the CSSD [5, 8].

In the planning phase, the costs of robotic inputs need to be analyzed to build the surgical packages that will be charged to the patient. Negotiations must also be carried out with health plans [2], and models of patient contracts and the specific informed consent form for robotic surgery must be eligible.

Employees of the administrative and financial sector of the program must provide the budget for the surgery to the patient and provide all preoperative guidance, direct the consultations and tests that he must undergo before the surgery, and request the signing of contracts and terms of the surgery robotics.

Audit of robotic accounts should be performed after surgical procedures to allow cost savings, program sustainability, and analysis by managers [9]. It should be considered that in the initial phase of the program, longer room times and longer surgical times are expected, negatively impacting the Surgical Center parameters [9].

24.2.7 Marketing and Patient Education

The implementation of a robotic surgery program in an institution naturally becomes a marketing strategy for the hospital, attracting patients and surgeons who wishes to have access to this technology [10]. While the technology clearly benefits the patient, this technique also offers advantages for the surgeon, such as ergonomic comfort, three-dimensional vision, and a ten-fold increase in the image [7]. Thus, a marketing plan for patient education and program visibility is recommended. It is important to develop marketing strategies targeting patients and the clinical staff, such as the creation of digital content in social media, scientific events, and educational material [2, 6].

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Chapter 25
Robotics and the Avant-Garde Role of Urologic Surgery

Tomás Bernardo Costa Moretti, Ricardo Miyaoka, and Wilmar Azal Neto

25.1 Introduction

Robot-assisted surgery is a consequence of the technological evolution of minimally invasive surgery and consists of the coupling of a robotic interface between the laparoscopic instruments and the main surgeon. This technology brought together characteristics such as precision, range of motion, strength, resistance and a privileged three-dimensional view of the operative field. Until now, robotic technology only interprets the surgeon's movements applied in a unit called "console" and projects them onto dedicated instruments with articulations capable of faithfully reproducing them (Fig. 25.1).

These characteristics allowed us to revisit the conventional surgical technique, as the capacity for more delicate and precise dissections forced urologists to improve their previous anatomical and functional knowledge. With this, it was possible to change the paradigm where the objective is not only to cure the disease but also to reduce surgical damage to the urinary tract, preserving its function.

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Fig. 25.1 DaVinci™ robotic operating system. (**a**) Surgeon console where the movements are performed and sent to the unit attached to the patient. (**b**) Robotic unit that performs the surgeon's movements, consisting of articulated robotic arms with instruments introduced into trocars located in the abdominal wall under supervision of an assistant. (Photo: with permission from Ricardo Miyaoka, 2021)

From the point of view of optimizing surgical techniques, robotic surgery is at the forefront and is the main representative of an inevitable path in the development of urology. It is noteworthy that it is not only a natural consequence of the evolution of diagnostic methods but also responsible for boosting the development of other areas in patient care, adding various technologies in care.

As robotic devices can become costly in many scenarios around the world and because they are at the frontier of knowledge along with other technologies, their acceptance and proof of effectiveness by the scientific community takes time. The evidence slowly becomes more robust and can prove the cost-effectiveness of robotic surgery.

In this chapter we will describe the main innovations in robotic surgery in urology and consequently in nephrology, as well as the prospects for the future. Thus, the focus will be on the description of techniques that impact the maintenance of renal function, whether preserving the renal parenchyma, restoring the functionality of the urinary tract or even in renal replacement therapies.

25.2 Nephron Sparing Robotic Surgery

The development and greater access to imaging methods has increased the incidence of small kidney lesions called incidentalomas, which are generally smaller than 4 cm in diameter and can be endophytic, exophytic, solid, or cystic.

The main concern of the urological community is to preserve renal parenchyma without compromising oncological safety. Solid and cystic lesions, until proven otherwise, should be evaluated for the possibility of resection with preservation of the affected kidney called partial nephrectomy [1].

The innovations of partial nephrectomy are moving toward achieving the following goals: preserving the renal parenchyma and reducing the time of warm ischemia in the intraoperative period, all these predictors of postoperative renal function.

25.2.1 Preservation of Renal Parenchyma

Partial nephrectomy is the gold standard technique for the treatment of localized renal cell carcinoma smaller than 7 cm (T1) and involves resection of the tumor with a safety margin, where it may be necessary to remove extensive areas of remaining

renal parenchyma [2]. However, as many lesions have a pseudocapsule, enucleoresection of the renal lesion is currently being sought without compromising nephrons with oncological safety. Surgery that respects this precept and uses methods to do so is known as nephron-sparing surgery.

Even in a setting with larger tumors (>4 cm), close to the renal hilum and endophytic, robot assisted partial nephrectomy (RAPN) had similar positive surgical margin (PSM) and estimated glomerular filtration (eGFR) rates when compared to open and laparoscopic surgery [3]. Although robotic surgery is more costly, recent studies have shown no differences in other outcomes such as PSM, estimated blood loss (EBL), warm ischemia time (WIT), postoperative complications, and length of stay [4–6] when compared to other techniques. In another analysis, Choi et al. analyzed 2240 patients in a meta-analysis where no difference was found between EBL, operative time, PSM, but RAPN had lower WIT, conversion to open surgery and change in postoperative eGFR [7]. These results are attributed to the facilitation that the robot allows during dissection and reconstruction movements as well as the magnification of the 3D image and the tremor filter which optimizes time at a crucial moment in the surgery [8].

Several resources were being used to achieve the goal of preserving the greatest amount of healthy kidney tissue and robotic surgery is a consequence of this technological development that involves both pre- and intraoperatively.

Preoperatively, the correct staging allows for a more adequate operative planning allowing for a more precise and detailed approach. At this point, robotic surgery stands out as it allows the surgeon to more faithfully reproduce the previously planned tactic within a minimally invasive context.

The characteristics of solid lesions and their relationship with the rest of the kidney can be previously evaluated with scores that classify their complexity. Several methods of nephrometry have been described (R.E.N.A.L., PADUA score, c-index score, ABC scoring system) and are widely used in urological surgery studies as well as in clinical practice. These classifications aim to predict the chance of unfavorable outcomes in an attempt to preserve the organ such as PSM, perioperative complications, and prolonged WIT, as well as the volume of renal preservation [9, 10].

Still in the context of operative planning there are image reconstruction techniques to facilitate tumor lesion classification and surgical planning. Among the technologies used we have 3D printing models with representation of the main and segmental renal vessels [11] (Fig. 25.2), as well as the use of holograms with 3D vision, both capable of providing greater understanding and application of nephrometry described above [11, 12].

These reconstruction technologies can also be used during surgeries. Currently, robotic surgeons use ultrasound probes coupled with the robot's vision to facilitate

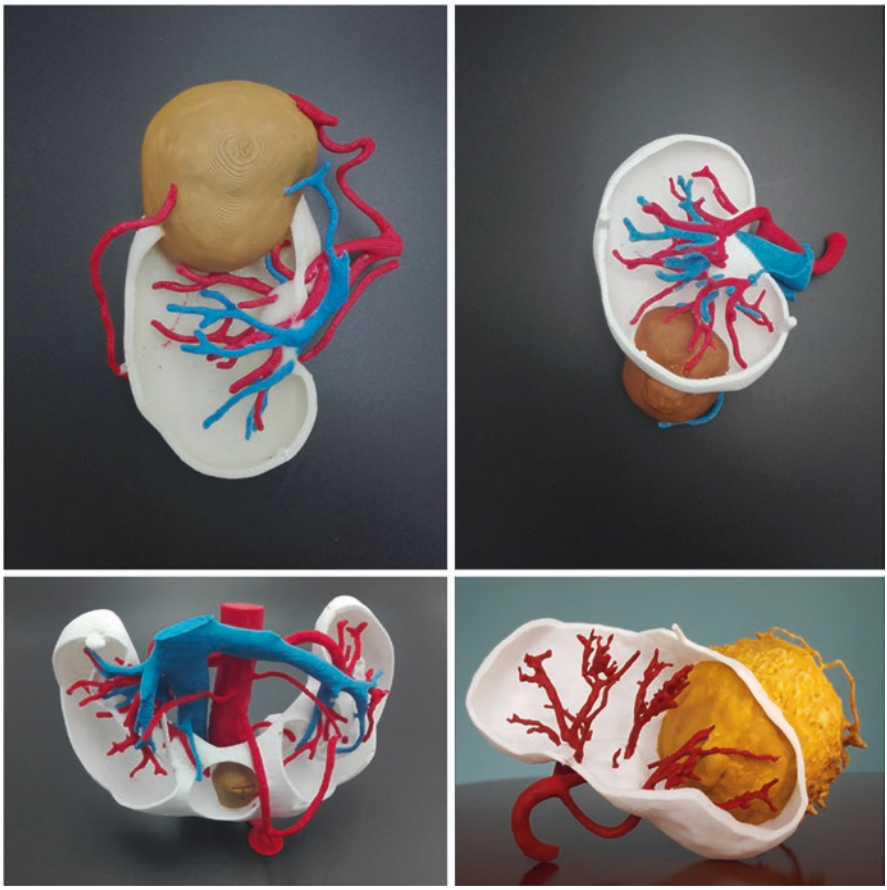

Fig. 25.2 Examples of reconstructions in 3D printed models of kidneys with renal tumors and their vascularization. (Photo: with permission from Francesco Porpiglia, 2021)

the location of lesions (Fig. 25.3). A trend for the future is to couple the image of the 3D reconstruction with the intraoperative image of the robot, and through the augmented reality with stereotaxic synchronization of the kidney, it allows real-time visualization of the renal vascularization and the tumor even for more complex cases [13] (Fig. 25.4).

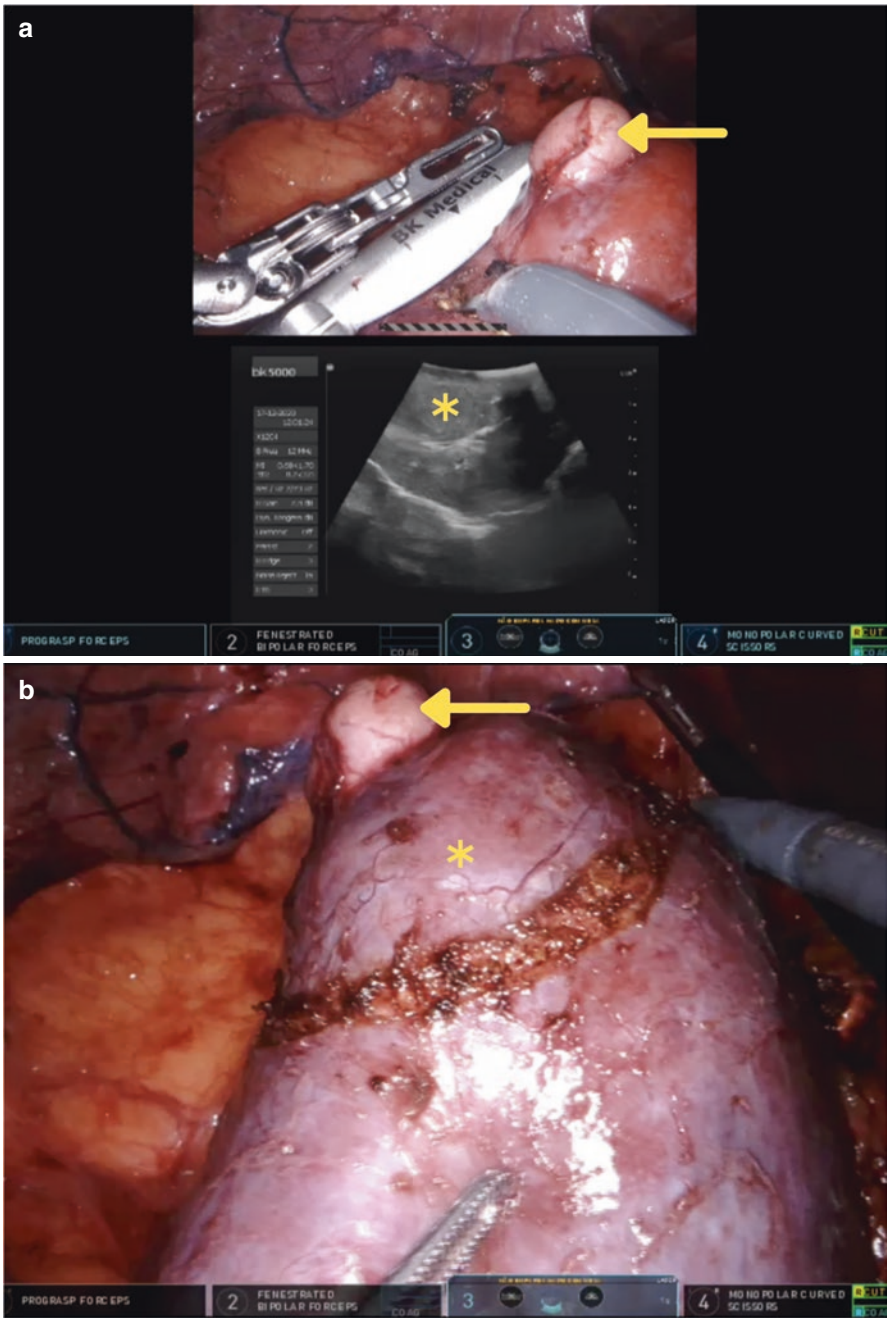

Fig. 25.3 Intraoperative image of robotic partial nephrectomy. (**a**) Real-time intraoperative ultrasound of right kidney upper pole lesion. (**b**) Demarcation of the resection area after identifying the extent of the lesion. Solid arrows: exophytic portion of renal tumor; asterisk: endophytic portion of the renal tumor. (Photo: with permission from Tomás B. C. Moretti, 2020)

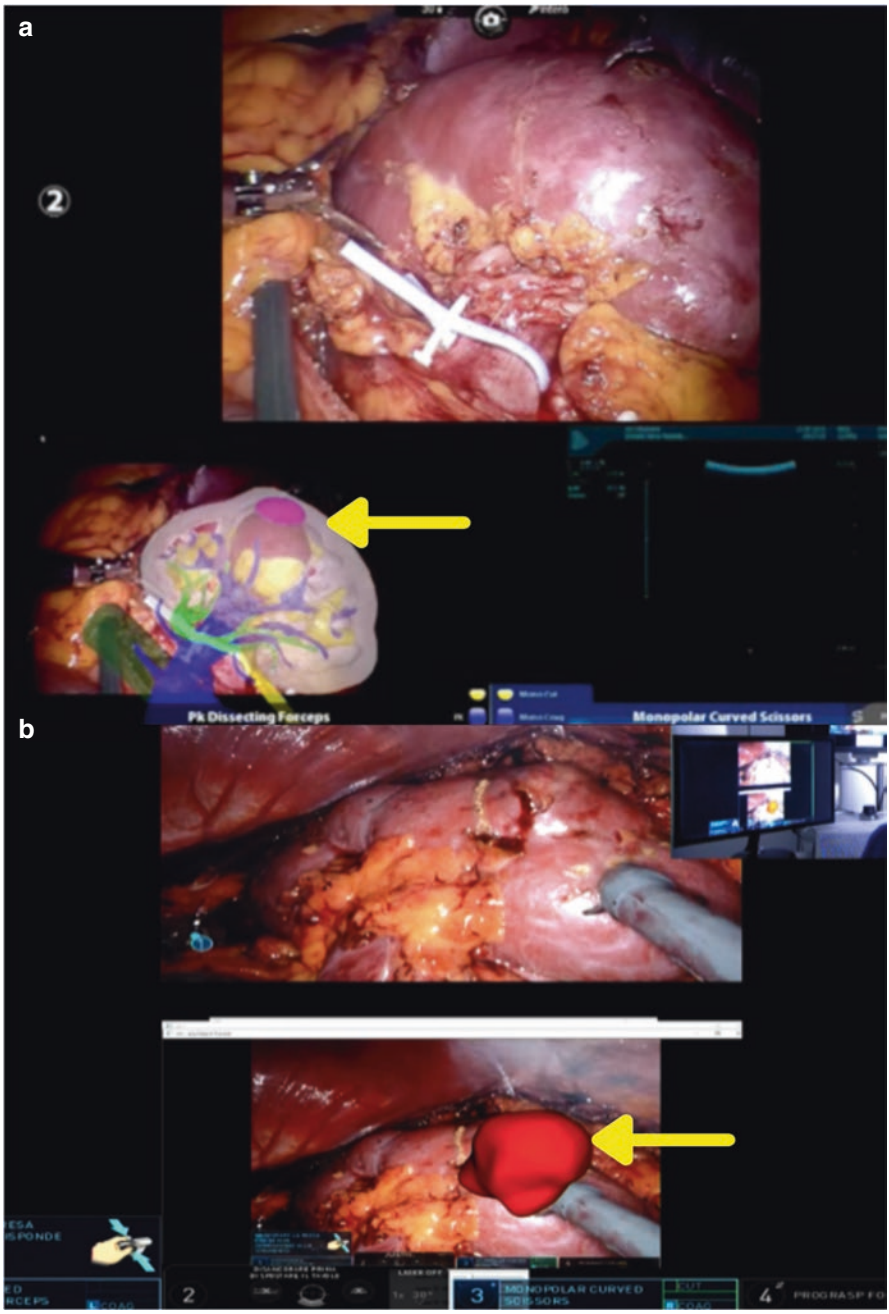

Fig. 25.4 Intraoperative image of robotic partial nephrectomy with augmented 3D reconstruction performed. (**a**) Intraoperative image fusion, 3D reconstruction, and intraoperative ultrasonography with renal vascularization. (**b**) Evidence of endophytic renal lesion on the renal surface with stereotaxic synchronization in augmented reality. In detail, image of a surgeon with 3D glasses for viewing a hologram for preoperative planning. Solid arrows: endophytic renal lesion. (Photo: with permission from Francesco Porpiglia, 2021)

25.2.2 Reduction of Warm Ischemia Time

Such pre- and intraoperative planning technologies, in addition to allowing a more precise resection of the lesion and preserving nephrons, enable the understanding of tumor vascularization, which, associated with the precision and delicacy of the robot's movements, allow for better dissection of the renal hilum and super-selective clamping of the affected renal area.

Segmental vascularization can be confirmed using perfusion markers such as indocyanine green. This substance is injected intravenously after clamping the artery and the camera with a fluorescence filter indicates the real area of ischemia confirming the tumor area to be resected and maintaining kidney perfusion (Fig. 25.5). Super selective clamping of secondary or tertiary branches of the renal artery is useful in patients with chronic kidney disease who have an earlier return of glomerular function postoperatively when compared to total clamping [14].

The preoperative study can allow the dissection of lesions without clamping the renal artery or vein since the ease of dissection with robotic technology allows better visualization of the dissection bed, reducing bleeding and maintaining renal perfusion, called off-clamp partial nephrectomy. For more complex tumors, total interruption of circulation has a better benefit, however, patients with smaller and superficial lesions can benefit from off-clamp surgery by reducing the WIT and with early recovery of renal function [15]. In a meta-analysis of late evaluation after 5 years, no difference was found in eGFR between the total clamping and off-clamp techniques [16].

Another approach is a mix between the total clamping technique and the off-clamp called partial clamp. Two suture planes are usually performed, one for the medullary layer and collecting system and the other for the cortical (Fig. 25.6). As usually the largest vessels are in the medullary layer, after suturing it, the renal artery can be unclamped and the cortical layer can be sutured without excessive bleeding, preserving the vitality of the kidney and less WIT. Pneumoperitoneum can cause kidney damage and the stability of the robot's arms, associated with devices that were developed with laminar gas injection flow as well as continuous aspiration of smoke from the electrocautery, allows the use of intra-abdominal pressures of less than 12 mmHg, which reduces exposure to CO_2 as well as less renal damage [17, 18].

For the future, robotic surgery finds itself in an era where new devices are in development making the technology more accessible. In addition, the emergence of new data transmission technologies, such as 5G, may allow remote surgeries to be performed, unifying practices and techniques around the world, as well as the fusion of technologies.

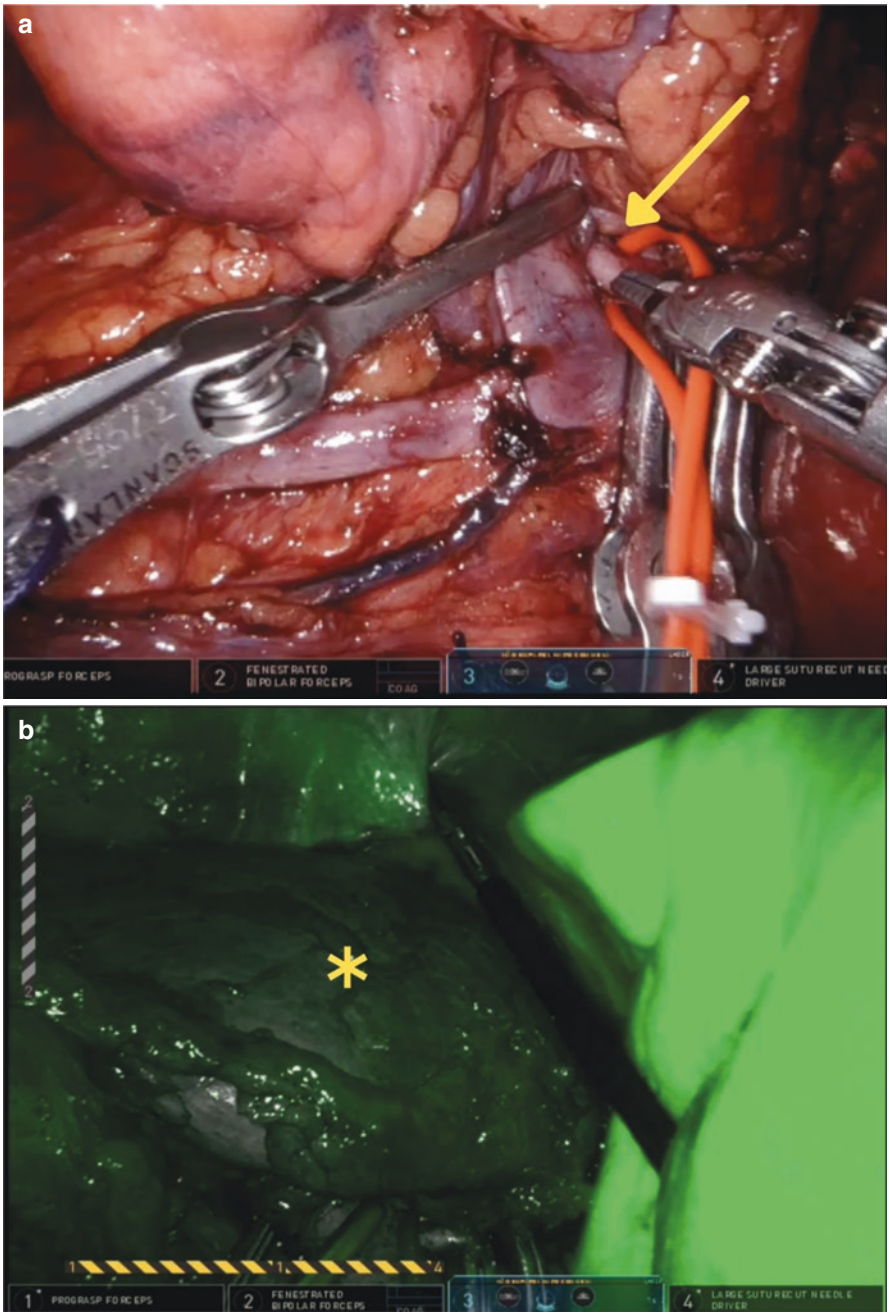

Fig. 25.5 Intraoperative robotic partial nephrectomy with study of renal perfusion with intravenous indocyanine green and fluorescence imaging. (**a**) Renal hilum dissected with clamping of veins and renal artery. (**b**) Fluorescence filter after indocyanine green with liver showing high uptake and kidney without perfusion after hilum clamping. Solid arrow: clamped renal hilum. Asterisk: kidney without indocyanine green uptake. (Photo: with permission from Tomás B. C. Moretti, 2020)

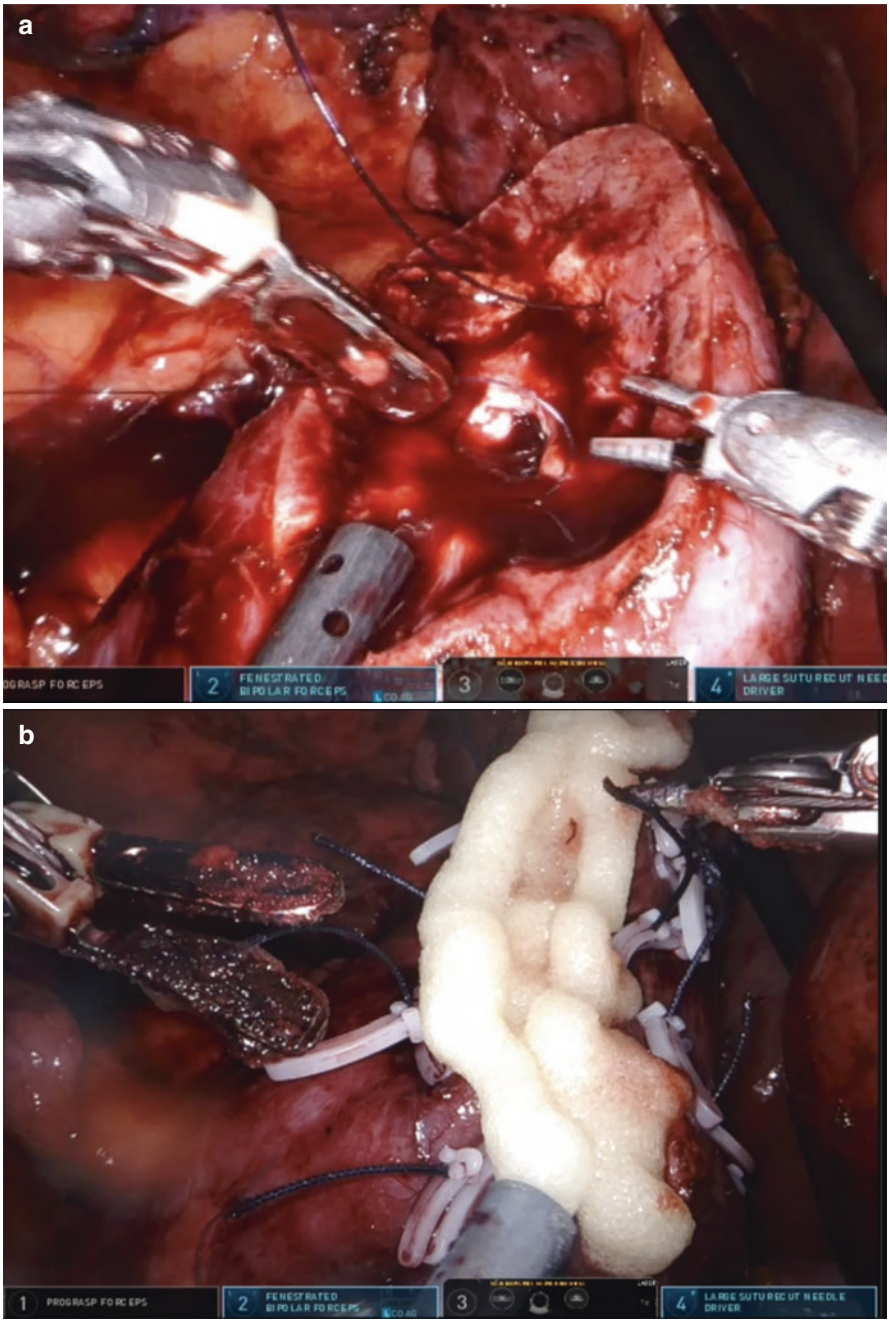

Fig. 25.6 Intraoperative image of robotic partial nephrectomy after tumor resection. (**a**) Appearance after tumor resection in the upper pole of the right kidney. (**b**) Final appearance after suturing and closing the defect with clips and hemostatic foam. (Photo: with permission from Tomás B. C. Moretti, 2020)

25.3 Robotic-Assisted Kidney Transplantation

Kidney transplantation is the gold standard treatment in patients with end-stage kidney disease. Robot-assisted laparoscopic surgery has been able to overcome many restrictions of classical laparoscopy, particularly in complex and demanding surgical procedures [19]. In 2016, the first robotic-assisted kidney transplant (RAKT) was performed in Europe, which is considered one of most challenging urological procedures due to its technical aspect [20]. Thanks to this robotic assistance, minimally invasive surgery in kidney transplantation growing progressively, not only in the most common living donor, but also in deceased donor scenario [21].

25.3.1 Surgical Technique

The patient is positioned in supine Trendelenburg and the robot is docked between the parted legs of the patient. Peritoneal flaps are raised, creating a peritoneal pouch over the psoas muscle. A small Pfannenstiel incision is made to insert the previously wrapped in an ice-packed gauze kidney allograft. The graft renal vein anastomosis is performed in an end-to-side continuous manner to the external iliac vein. Afterwards, the arterial anastomosis is accomplished in the same way to the external iliac artery (Fig. 25.7). Then, reperfusion of the graft is carried out followed by the ureteroneocystostomy (usually in a Lich-Gregoir technique) [22].

In order to reduce the vascular anastomosis complications, urologists prefer to implant left kidneys (longer vein) and single artery ones. However, some series report RAKT using right and multiple artery kidneys varying from 12.2 to 15.4% [23, 24].

Different techniques from the previously described, however, are possible. Felip et al. in 2021, published the first five cases of a robotic transvaginal-assisted living donor kidney transplantation. They describe the insertion of the graft through the vagina and, since they had no complications and a good median operative time (220 min), conclude that this new technique is feasible and safe [25]. Moreover, Adiyat et al. described a series of 34 RAKT with total extraperitonealization of the graft, reproducing closely the technique of the open renal transplantation, with good graft function [26].

25.3.2 Comparison to Open Kidney Transplantation

Open kidney transplantation is still the gold standard for renal transplant. However, as the number of RAKT grows, an increasing number of studies comparing both techniques emerge and some already conclude that the robotic operation is not

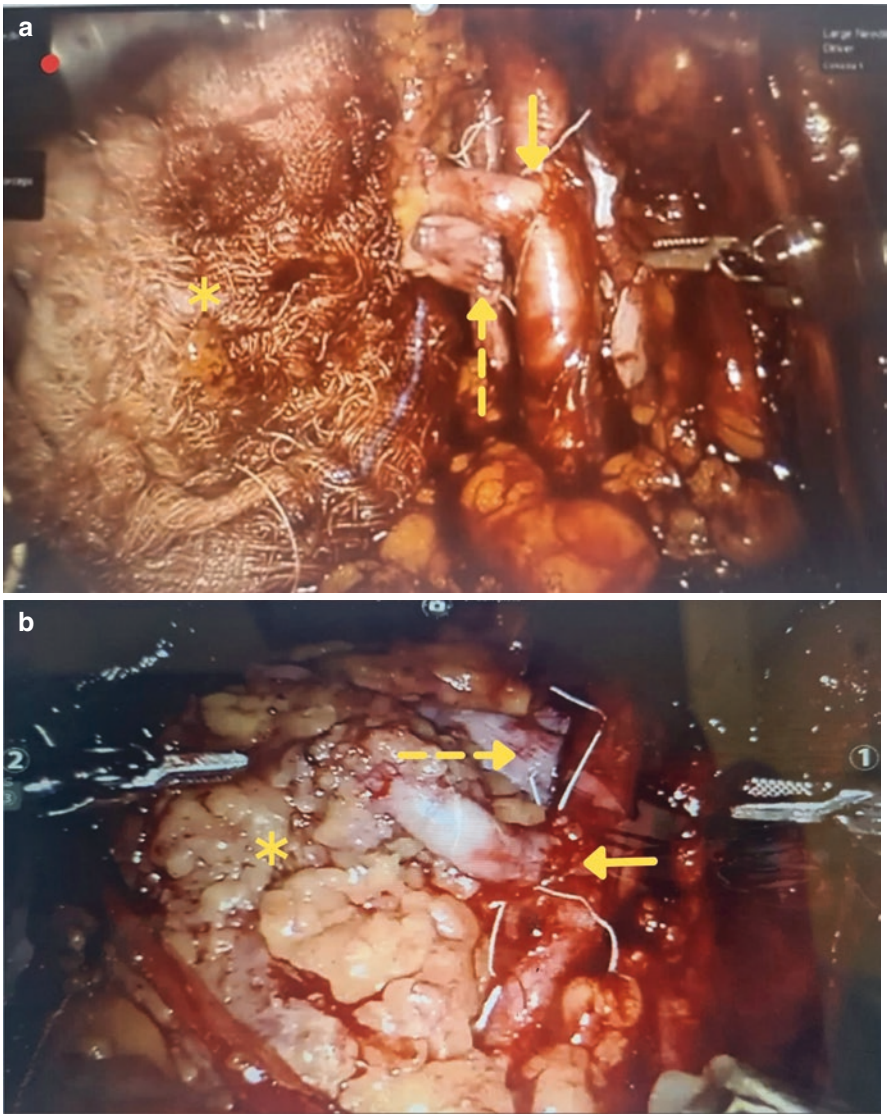

Fig. 25.7 Intraoperative imaging of robotic-assisted kidney transplantation. (**a**) Aspect of the arterial and venous anastomosis with the graft wrapped in a wet compress. (**b**) Aspect of the arterial and venous anastomosis with the perfused graft. Continuous arrows: arterial anastomosis; discontinued arrows: venous anastomosis; asterisk: graft. (Photo: with permission from Rafael F. Coelho, 2020)

inferior to the open approach [20]. A systematic review published in the European Urology evidenced not only no difference in graft or patient survival but also that minimally invasive surgery had lower site infection and incisional hernia rates; nevertheless, showed a prolonged cold and warm ischemia time, as well as total operation time [27].

A meta-analysis coordinated by Liu in 2020 demonstrated similar results: RAKT had significant higher rewarming time and total ischemia time compared to conventional operation, with a lower rate of surgical site infection. Furthermore, on an average follow-up of 31 months, patients had similar functional and clinical efficacy, besides similar all-cause mortality [28].

Moreover, other publications report similar outcomes. Mean total operation time was higher, as well as lesser lymphocele and wound healing disorders in RAKT than in open access; besides, they observe excellent short- and midterm results in graft function [20, 29, 30].

25.3.3 Learning Curve

Since RAKT is a relatively new procedure, there are no papers specifically about its learning curve. However, some authors do comment about their experience and results toward time.

Musquera et al. after analyzing 82 living donor RAKT published about the "lesson learned." They noticed a significant reduction in time between the first 20 cases versus the following ones (248 vs. 189 min, $p < 0.05$) [23].

The European Robotic Urologic Section (ERUS) published in 2021 a multicenter prospective observational study with 291 living-donor patients, in which the groups concluded that the learning curve for RAKT is relatively short. They compared the first 120 cases versus the following 171 and reported a significant reduction in surgical time (265 vs. 230 min, $p < 0.05$) [24].

25.3.4 Surgical and Functional Results

Regarding the functional outcomes of RAKT, studies report that it can already be considered an attractive minimally invasive method for kidney transplantation, but that further investigation must be done to consider it the standard approach. Recent paper observed a mean creatinine of 1.52 mg/dL and a renal graft survival of 98% after an average time of 1.8 years [23]. The ERUS publication, above

mentioned, concluded that RAKT performed in wide experienced centers have good surgical and functional results, competitive with open kidney transplantation [24].

Several studies evaluate robotic renal transplant's surgical complications. Musquera et al. after 82 RAKTs, reported five conversions to open surgery due to abnormal graft vascularization, two embolizations for subcapsular and a hypogastric artery bleeding without repercussion, and one venous thrombosis leading to loss of kidney [23]. Moreover, the groups that participated in the ERUS series, with a total of 291 living-donor RAKT, observed 17 cases of postoperative bleeding (six required re-exploration due to hematoma), while one patient presented venous thrombosis, two arterial stenosis, three incisional hernias, six ureteric stenosis and nine lymphoceles [24].

25.3.5 Pediatric RAKT

Kidney transplantation (KT) is the gold-standard treatment for end-stage renal disease (ESRD) in children [31]. Applying techniques of minimally invasive surgery may contribute to the improvement of clinical outcomes for the pediatric transplant patient's population and help mitigate the morbidity of KT.

However, many challenges remain ahead. Minimally invasive surgery has been consistently shown to produce improved clinical outcomes as compared to open surgery equivalents. Despite the presence of these improvements, many challenges lie ahead, such as: anesthesia aspects (tolerance for cavity insufflation), robotic instruments specific for adults, anatomic aspects (small abdominal cavity) and no standard trocar placement (need to adapt to each child) [19]. Cost-effectiveness still is a barrier to overcome.

Finally, in this scenario, RAKT should be performed by a multidisciplinary experienced team, supported by a pediatric nephrologist, urologist and anesthesiology team. And although data on this procedure in children is still scarce, it seems a safe and feasible surgery, with excellent results in graft function [31]. Further studies to better determine the benefits of the robotic approach as compared to the laparoscopic and open approach are necessary.

25.4 Reconstructive Urology

Reconstructive urology can be defined as a subspecialty field that manages and treats genitourinary conditions that affect normal voiding and sexual function [32]. The principles of robotic reconstructive surgery are similar to open surgery whilst offering the advantages of reduced tremor, better visualization and ergonomics, unlimited freedom of movement with improved dexterity and longer reach with

better access to structures, and an assumed less steep learning curve than the one required for pure laparoscopy [32, 33].

There are multiple conditions that may require a reconstructive intervention. From a nephrological perspective, the main goal of any intervention would be maintenance of renal function which can be jeopardized by recurrent urinary tract infections (caused by urinary stasis following urethral stenosis, bladder neck stenosis or VUR), complete or partial obstruction of the upper urinary tract (ureteral stenosis, UPJ obstruction, ureteral compression by endometriosis, oncological pelvic pathologies or postsurgical adherences, etc.), or both.

We will review the main robotic surgical approaches to address these situations.

25.4.1 Pyeloplasty

Ureteropelvic junction (UPJ) obstruction consists in a congenital obstruction of the ureteral transition segment between the renal pelvis and proximal portion of the ureter. Regardless of the exact etiology, UPJ obstruction is most commonly treated with a dismembered technique (Anderson-Hynes). In this technique, UPJ is fully dissected off the surrounding tissue until it is clearly visible. If a crossing vessel is identified, it should be spared and ureter transposed anteriorly before anastomosis is performed (Fig. 25.8). The approach is exact the same of the one used for laparoscopy but offering the advantage of a more intuitive, ergonomic and easy-to-perform suture.

The first robotic pyeloplasty was described in 2002 by Gettman et al. [34]. There are no prospective studies comparing open, laparoscopic, and robotic approaches in adults, only case series suggesting very similar outcomes equally very effective with success rates over 93% even for beginners [35, 36].

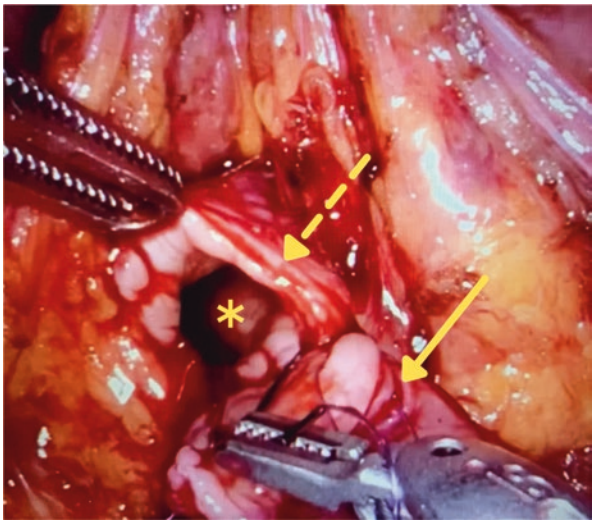

Fig. 25.8 Intraoperative image of robotic pyeloplasty for correction of ureteropelvic junction obstruction. The surgery is at the time of reconstruction with an anastomosis between the ureter and the renal pelvis. Solid arrow: ureter. Discontinued arrow: renal pelvis wall; asterisk: lumen of the renal pelvis. (Photo: with permission from Ricardo Miyaoka, 2021)

25.4.2 Ureteral Reimplantation

Ureteral reimplantation is required in cases of ureterovesical reflux, distal ureteral stenosis or trauma. Open approaches have historically been associated with high success rates (95–99%) but robotic technique offers a less invasive approach with comparable outcomes with decreased morbidity [37]. The first robotic reimplant was reported by Patil et al. in 2008 [38]. A small case series by Muffarij et al. reported 100% success for robotic ureteral reimplantation after a mean follow up of 31.5 months [39]. Stricture-free rate and operative time seem to be very similar for open, laparoscopic, and robotic techniques, although minimally invasive techniques are associated with a shorter hospital stay and reduced blood loss [40].

25.4.3 Boari Flap

The Boari flap technique consists in using a flap of the bladder to repair longer segment distal ureteral strictures which may be as lengthy as 15 cm. The first robotic procedure was described by Stolzenburg who reported on 8 cases with 100% success rate with 12-months follow up [41]. Although these initial results are promising, further studies with larger cohorts and long term follow up are needed comparing robotic versus open and laparoscopic approaches.

25.4.4 Ureteroureterostomy

End-to-end anastomosis may be an alternative to repair short (<3 cm) proximal or middle ureteral stenosis that fail endoscopic treatment (Fig. 25.9). Results are comparable for open, laparoscopic, and robotic approaches regarding outcomes, surgical time, and complications, both in pediatric and adult series. There may be a trend toward shorter OR time and less blood loss for the robotic approach, but data is still very scarce [42, 43].

25.4.5 Buccal Ureteroplasty

The buccal ureteroplasty is a resource for those with longer or multifocal strictures of the proximal or middle ureter that cannot be repaired with primary ureteroureteral anastomosis. Buccal mucosa is an excellent alternative when blood supply to the reconstruction site is at risk.

Recently, Lee and Zhao reported on a multi-institutional experience with a success rate of over 90% at a median follow-up of 24 months for robotic ureteroplasty with buccal mucosa [44].

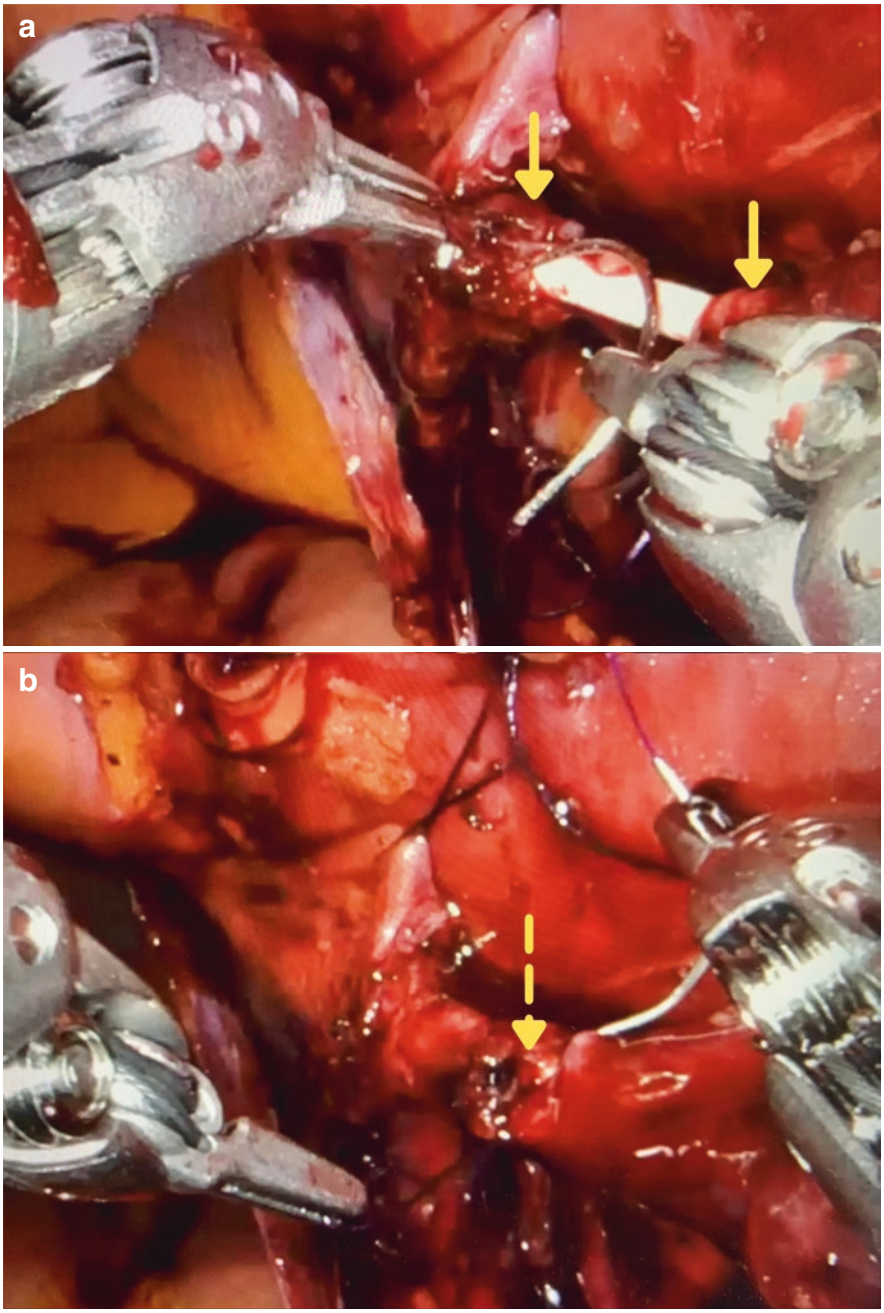

Fig. 25.9 Intraoperative image of robotic ureteroureterostomy for correction of short ureter stenosis. (**a**) Isolation of the ureteral stumps (continuous arrows) over a ureteral catheter. (**b**) Final appearance after anastomosis (discontinued arrow). (Photo: with permission from Ricardo Miyaoka, 2021)

25.4.6 Appendiceal Flap

Appendiceal flap ureteroplasty provides some advantages including relatively easy appendiceal mobilization, well-defined blood supply through the mesentery of the appendix, negligible absorption of urine, ability to replace totally obliterated ureteral segments, and lack of donor site morbidity (when compared with buccal mucosa graft). A case series from Reggio et al. [45] reported on six patients with no recurrences at 16 months of follow-up. All cases were right-sided with strictures averaging 2.5 cm.

25.4.7 Ileal Ureter

Ileal ureter should be used as a last attempt to replace an irreversibly damaged ureter when all previously described techniques do not apply or fail. Technique consists in interposing an ileal segment harvested approximately 20 cm before the ileocecal valve and attaching distally to the bladder or spatulated distal ureter and proximally to the proximal ureter, renal pelvis, or lower calyx as feasible [46].

Robotic ileal ureter was first described in 2008 by Wagner et al. [47], but to date, only few small case series with short follow-up are reported. Most reported cases provide encouraging satisfying outcomes.

25.4.8 Augmentation Cystoplasty

Augmentation cystoplasty (AC) was first described in 2008 in children by Gundeti et al. [48]. Since then, there have been description of only few case reports and one small case series involving 19 patients who underwent AC for different indications including low compliance, refractory detrusor hyperactivity and bladder pain syndrome. This series reported on no major complications and very good long-term outcomes [49]. The technique seems safe and feasible. Prospective comparative series are desirable but very difficult to become a reality as botulinum toxin and electrical nerve stimulation can resolve a significant number of cases that might have an indication for AC otherwise.

25.4.9 Bladder Neck Reconstruction

Bladder neck reconstruction is devoted to resolve bladder neck contracture (BNC) which usually develops after a radical prostatectomy for prostate cancer treatment but may also derive from simple prostatectomy for benign prostatic hyperplasia,

transurethral resection of the prostate (TURP), thermal ablation by high intensity focused ultrasound (HIFU), or pelvic trauma. Robotic Y-V plasty consists in identifying the lesioned segment, incising it ventrally and creating a Y-V advancement flap on the anterior surface of the bladder.

The Kroepfl group reported on a series of consecutive adult male patients who underwent robotic Y-V plasty for recurrent BNC. At a median follow-up of 23 months, 10 patients (83%) had clinical success and no evidence of recurrence [50].

25.5 Conclusion

Since its inception, robotic surgery has remained at the forefront of technological development in urology. The reproducibility of robotic techniques is becoming more and more acceptable, with the economic barrier as a brake on this development. With the emergence of new robotic platforms, this technology becomes more accessible and allows its application in different settings.

The cost-effectiveness becomes more evident in more prevalent pathologies, such as partial nephrectomy and thus, allowed greater acceptance in the urological community and allowed better results to be achieved in a shorter time. In the case of reconstructive urology, as it has a lower incidence, it still needs more studies and greater volume to overcome costs, as well as kidney transplantation.

Thus, robotic surgery is an important technological link that brings together a common interest of urologists and nephrologists, preserving renal function.

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Chapter 26
Caregiver Robots in Nephrology: Is It Feasible?

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26.1 Introduction

26.1.1 From the Dawn of Humanity to Technological Evolution and Healthcare

The history of humanity has always been marked by discoveries, struggles, and achievements. From the dawn of civilization until today, the ancient hominids have been adapting to the environment, fighting for their survival, and, subsequently, creating, inventing, innovating, mastering fire, elaborating tools, evolving physically and mentally toward hominization, during millions of years, until today. In this slow process of their development, their brain elements have also evolved, and, consequently, their mind, their cognition, their knowledge, and their intelligence have also been furthered.

The use of tools and utensils allowed them to perform various daily activities related to their survival needs [1].

In the evolutionary process, with the development of the mind, reasoning, logic, affections, religious aspects, life in cooperation, culture, attention, and the recognition of the needs of others have emerged. Then, both the process of assisting their fellows and the technological process started being acquired and developed, permeating the transformation of this hominid into a human being; healthcare started being part of their capabilities, and the sick started being cared for, instead of being merely abandoned to die.

Human life expectancy has also been increasing throughout history. The evolutionary and developmental process that took many thousands of years has brought humanity to its current stage of evolution and technology.

The concept of human evolution is directly associated with the degree of technological development acquired over time, through the improvement of tools and

utensils [1]. The existing technology, which is currently in wide expansion, has evolved exponentially in basically all fields of knowledge; from the invention of the primitive wheel, today transportation to other planets and prototypes of driverless cars are available; computers have replaced primitive machines; ships feature high technological density; the Internet is an indispensable reality worldwide; various high-tech devices are useful in everyday life. Three-dimensional (3D) printers have been gathering attention in recent years, virtual communication is necessary in contemporary times, and robotic technologies are being created to meet human needs and interests.

All these transformations have contributed to the emergence of Society 5.0, in which an attempt will be made to balance economic advancement with the solution of social issues; in this novel form of existence, computers and hyperconnectivity will allow citizens to lead a more sustainable and efficient lifestyle [2, 3]. This 5.0 or 5G society represents the fifth generation of wireless mobile Internet, which promises a major technological breakthrough, as it will greatly enhance current Internet speeds without the need for wires. In the healthcare field, bureaucracy will be reduced; the use of smartwatches and other monitoring gadgets will have a significant expansion; this technology will allow "smarter" devices that can monitor indicators such as hormones or nutrients in the body, allowing for immediate application or extraction; medicine and nutrition will possibly acquire a preventive character, no longer focusing on diseases, but rather on the maintenance of people's welfare [4].

In the slow process of human development, knowledge in the field of healthcare started to establish specifications/specializations, which rendered the guidance of assistance provided to those who were sick more feasible. Among the range of specialties, nephrology has become one of the most important for the maintenance of human health, and technologies have been used in this field to provide care to patients.

The use of technologies in healthcare is known as Digital Healthcare, envisioned by the Brazilian Ministry of Health since 2015, with the publication of a document entitled "Digital Healthcare Strategy for Brazil 2020–2028" [5].

However, despite all the technological breakthroughs, to this day many still lack access to healthcare or refuse to adhere to it, despite increased investments; therefore, it becomes a requirement for technology directed at healthcare to be reproduced free of charge in communities in an attempt to save millions of lives and to meet the plans of the United Nations in Agenda 2030 [6, 7].

The technological developments achieved in the healthcare field to date should be consolidated within the guidelines of ethics, reliability, safety, sustainability, efficiency, effectiveness, and especially with solutions that meet the demands of all: citizens, patients, physicians, nurses, dentists, pharmacists, that is, those who consume digital healthcare products [8].

Regardless of the use of technology that assists healthcare providers with patient care, these providers are paramount to the success of healthcare in its entirety, irrespective of whether or not the use of robotic equipment is included.

26.1.2 Nephrology and the Specialties It Encompasses

Nephrology is a relatively recent medical specialty, albeit Hippocrates had first observed urinary alterations as signs of kidney disease. Its emergence occurred only in the 1940s; in 1950 the first hemodialysis machines and the first homologous kidney transplantation occurred, respectively [9].

The Brazilian Society of Nephrology was founded in August 1960. It was only in 2006 that the International Society of Nephrology established World Kidney Day (on the second Thursday of March) to celebrate and encourage initiatives to prevent kidney disease [10].

This fascinating medical specialty prevents and treats non-surgical renal diseases, and is composed of the following subspecialties: renal pathophysiology, clinical renal diseases (chronic, acute, hypertension, hydro-electrolytic, acid-base metabolism, lithiasis, urinary tract infections, hereditary, and others), dialysis (hemodialysis and peritoneal dialysis), and kidney transplantation (from living and deceased donors) [11].

26.2 Theoretical Input

26.2.1 Healthcare Assistance

In recent years, regarding the care provided to patients and health promotion, both physical exercises and therapies have undergone constant changes, which allow the development and creation of opportunities for personalized and life-changing interventions for patients, in order to obtain effective responses toward recovery, adaptation, prevention, and/or health promotion [12]. These aspects include the care provided to patients presenting kidney alterations.

Concerning dialysis centers, for instance, although clinical guidelines of best practices recommend the implementation of physical exercises for patients as part of routine activities, the factor that promotes effective exercise programs is centered on the professional commitment of a multidisciplinary team, composed of physical therapists, nephrologists, geriatricians, social workers, and nurses, in addition to sufficient physical space and equipment. With no relevant causes of serious injuries from participation in exercise programs, there is evidence indicating the incorporation of exercises into the care routine of dialysis patients; however, it is necessary to determine the ideal training program according to patients' personal characteristics to ensure the adequate application of said exercises [13].

Interdisciplinary care aims to maximize the quality of life during treatment, slow the progression of renal failure, reverse and/or reduce uremic syndrome, and avoid complications during and after treatment. Thus, initially, the goal is to perform activities that correspond to the needs of musculoskeletal disorders (MSD) management, motor control, postural changes, and complex regional pain syndrome (CRPS)

management. It is also necessary to perform activities to prevent the effects of general body disorders and maintain the trophic effect of exercises on both the musculoskeletal system and the autonomic nervous system. Finally, it is necessary to implement activities that allow the use of available time, allowing the body adequate stimuli for stabilization, conscious and unconscious use of the body through recreational and therapeutic physical activities [14].

The application of such interventions in any phase improves muscle strength, physical endurance, functional independence, increased mobility, improved functionality, and perceived improved quality [15].

The treatment of intradialytic hypertension, performed during hemodialysis, offers advantages, such as the following: greater adherence to treatment, the convenience of schedules, reduction of monotony during sessions, in addition to the ease of medical follow-up. It is recommended to start the exercises in the first 2 h of hemodialysis, in order to avoid physical effort in the second half of the session. When patients' hemodynamic conditions are unfavorable, cardiovascular instability and drops in blood pressure may occur as of the third hour, rendering this practice impossible [16].

More studies are required to evaluate the improvement in lung function of these patients, in terms of lung volume and capacity, given that despite the various pulmonary repercussions resulting from kidney disease, the physiological effects and changes generated by its treatment are barely known [17].

Several secondary issues are centered on physiotherapy, since its effects have demonstrated that it improves longevity and reduces morbidity, in addition to delaying hospitalization [18].

In this sense, with or without the adjuvant use of technological equipment, the evolution in recent years regarding physical and therapeutic exercises has provided the execution of individualized and early interventions, obtaining good responses in recovery, adaptation, prevention, and health promotion. Possibly, with the current technological breakthroughs, the care provided by healthcare teams to patients presenting kidney alterations will generate greater added value.

26.2.2 Technological Breakthroughs in Education and Healthcare

The technological breakthroughs in healthcare are numerous, covering both teaching and care, and some are commented upon in the sequence.

– The teaching of First Aid will become more accessible to children and adolescents; the development and validation of technologies for educational health resulted in the creation of a comic book for parents/guardians to teach their children and adolescents. This technology was developed and validated by expert judges and was considered relevant and appropriate to promote this educational process [19].

- A video for educational intervention was produced regarding care aimed at the prevention and management of syphilis; the product was submitted to the process of development and validation, and the validated video constitutes a significant technological production, which should be used in the context of healthcare [20].
- The WhatsApp application has proven to be an invaluable asset for undergraduate nursing students and professors in addressing demands in the subjects offered in the undergraduate course [21].
- The influence of technological innovation in teaching leads to a dynamic, innovative, and proactive aspect to students, which requires changes in the format of the teaching–learning process. Such changes have been encouraging students to be active and builders of their knowledge, which directly contributes to the process of developing critical-reflective thinking, autonomy, and safety to perform procedures [22].
- 3D printers contribute to surgical planning and the preoperative stages of surgeries; they also produce molds of organs for educational purposes and custom implants and prosthetics. 3D printing is a technology with potential applications in different fields of healthcare; however, its use still encounters difficulties, including its high cost and long processing time, elements that, for the time being, diminish the possibility of its mass application. More collaborative efforts and advances in printing technologies can speed up and shorten the process, which will bring a paradigm shift in planning different clinical applications in the not-so-distant future [23].
- Patients presenting with heart failure are being treated with ventricular assistance devices, which improve their short- and long-term survival and their quality of life, leading to a progressive reduction in the rate of complications. Despite these benefits, some limitations are still related to the cost of these devices, durability, complication rate, and application to a limited spectrum of patients, although with advances in current technology it is possible to mitigate such issues [24].
- Telehealth has been advancing and assisting speech therapy and technological developments. Evaluated international studies were distributed in telecare (telerehabilitation and telediagnosis) and distance education (tele-education), demonstrating positive results with the use of technological resources. This field has been striving, then, to improve the quality of the services it offers and the ease of access to these services, generating a more effective impact on the prevention, diagnosis, and treatment of communication alterations between people [25].
- The Brazilian Ministry of Health has regulated telemedicine as of 2020, foreseeing the exercise of medicine through the use of interactive audiovisual and data communication methodologies, with the objective of providing assistance and education, and encouraging research in the field of healthcare. This use of interactive technologies, such as videos and applications, is an essential and safe alternative to protect the health of physicians, healthcare teams, and patients. It also contributes to the reduction of the overload faced by healthcare units and avoids the mobility of people; therefore, telemedicine is a practice that can guarantee a more permanent increase in the use of technology in healthcare [26].

– In the context of Primary Health Care (PHC), the objective is to expand the population's access and provide comprehensive healthcare to people; therefore, the tools used by physical therapists in this level of care are the following: individual consultations, home visits, and group work. Performing health promotion efforts and implementing relational technologies are challenges faced by physical therapists, but several healthcare providers already recognize these practices to promote integral care [27]. This proposal should be expanded to other fields within healthcare and the COVID-19 pandemic raised the need for the advancement of telehealth worldwide.
– Free technologies could assist the population in need of them. Among such technologies are the following: hand prostheses, printed with 3D technology and delivered to vulnerable people; open-source, low-cost therapies offered for the minimization of neglected diseases; magnetic resonance imaging (MRI) scanners that can be built and maintained at a low cost; probes for organ visibility inserted into a smartphone, that is, a "pocket" scanner; wearables and software that can be used to analyze biological data from epileptic patients; emerging technologies that assist in diagnosing, monitoring, and rehabilitating changes in mental health; sensors for respiratory health, and so forth [6].
– In turn, the WhatsApp application has proven to be an essential asset in the population's routine and has also contributed to the resolution of administrative demands and in the surveillance of patients already monitored by the family health teams, assisted by the Unified Health System (*Sistema Único de Saúde* [SUS]) [28].

However, significant technological breakthroughs have been obtained with robotic equipment to aid in healthcare. With technology becoming part of daily life in the healthcare field, the inclusion and use of such equipment have increased considerably in recent years. The pressure for consumption is exerted as much by their manufacturers as by the healthcare providers themselves and, in many cases, even by the patients who increasingly desire innovative and less painful procedures in their treatments [29] as well as more agile ones.

26.2.3 The Use of Robotics and Robots in Healthcare

Robotics is a field of technology that encompasses mechanics, electronics, and computing, which deals with systems composed of machines and automatic mechanical parts which are controlled by integrated circuits, making motorized mechanical systems, controlled manually or automatically by electrical circuits. In the early twentieth century, in face of the need to increase productivity and improve product quality was the time when robots found their first applications in industries, with George Devol being considered the father of industrial robotics [30].

But when examining the history related to the use of robots, Jules Verne (1828–1905) is the name most people come across, who is considered the "father of science fiction,"

having written publications on traveling underwater, around the world, from the Earth to the Moon, and to the center of the Earth, with many of his ideas becoming reality with the increasing technological advancement of humanity [31].

In this historical review, not excluding other important names throughout the years, there is Isaac Asimov, born in 1920, of Russian-Jewish origin, but who was raised in the United States since he was a child. He had a brilliant academic life and made several projections, including a series of predictions of the future, projecting it 50 years ahead of his time. Among his predictions are the existence of microwaves, fiber optics, the Internet, microchips, flat-screen televisions, and so on. He wrote several works, both scientific and science fiction, including the Robot Series. In the book *I, Robot*, he idealized and presented the three rules or laws of robotics, as a condition for the coexistence of robots with humans, in order to prevent any possible danger that artificial intelligence could pose to humanity. They are as follows: "a robot may not injure a human being or, through inaction, allow a human being to come to harm"; "a robot must obey orders given to it by human beings except where such orders would conflict with the First Law"; "a robot must protect its own existence, as long as such protection does not conflict with the First or Second Law"; in the book *The Robots of Dawn*, he later added the "Zeroth Law," above all the others—"A robot may not harm humanity, or, by inaction, allow humanity to come to harm" [32]. These laws of robotics proposed by Asimov are still recognized and esteemed due to their specifications of preventing human harm, stipulating obedience to humans, and incorporating robotic self-protection [33].

In any case, technological progress has allowed the existence of industrial robots, which have been around for years and have had a significant impact on manufacturing, moving on to service robots, which operate in a semi or fully autonomous manner to perform tasks that are useful to humans or equipment, and to the so-called artificial intelligence (AI), a type of software that enables technology to adapt itself through learning, with the objective of making systems capable of feeling, reasoning, and acting in the best possible way [31].

Subsequently, these concepts state that a robot is an automatic device created to perform tasks, which features feedback connections between its sensors, actuators, and the environment, relieving it of direct human control; however, robots that are partially controlled by humans may exist [34].

These machines are imitations of life; however, these machines are wires and mechanisms that collectively constitute a robot. They are increasingly used to perform tasks; robots do not dream, feel, or get tired. This technology, now being implemented by several factories and industries, has been generally successful in issues raised regarding cost reduction, increased productivity, and the various labor issues related to employees [30]. They are then considered reprogrammable and multifunctional devices designed to move materials, parts, or tools by using programmed movements to perform a variety of tasks; they can perform mechanical work, normally associated with humans, in a more efficient manner and without endangering human life [1].

Several technological breakthroughs already exist in the healthcare field; robotic technologies are present in health-oriented software and artificial intelligence. Microrobots initially tested in laboratories are able to navigate through the human

body, transporting therapeutic compounds to target destinations and reducing the side effects of these medications, thus treating several diseases [35].

In the field of robotics applied to healthcare, there are robots that organize and deliver medications, that assist in daily activities such as dressing and feeding patients, those that assist patients in hygiene habits and rehabilitation processes. There are also those that assist in patient mobility by helping in the transportation from bed to chair and vice-versa. In addition, there are social robots that assist in rehabilitation therapy and those employed in the transportation of medications, food, and communication resources [36].

There are benefits and advantages in the integration of robots in routine healthcare, as long as this implementation is conducted ethically and responsibly. The following can be identified: a reduction in surgical risks, infections, bleeding, and sequelae, such as scars, due to the precision of robot surgeons; faster recovery for patients who spend less time in the hospital; and better quality of the images during the procedures, due to the robots' micro-cameras and 3D vision. In addition, there is the democratization of access to healthcare, since patients can be cared for at a distance by using robots; high-quality ergonomics, since surgeons can perform a procedure more safely and accurately with the assistance of robots; a better experience for patients, who not only receive humanized care from healthcare providers, but also the best of technology; less invasive procedures, with smaller and more precise incisions; and the performance of tasks such as receiving patients, assisting in mechanical and repetitive activities, and so on [37].

The technological development related to robotics increased exponentially, starting in late 2019, following the COVID-19 pandemic. Robots with humanoid aspects were used in China to deliver food in a hotel designed to house individuals infected with SARS-CoV-2; in Romania, robots disinfected rooms using ultraviolet rays, communicated with patients to provide relevant information regarding their clinical condition, and delivered food. In Italy, a robot programmed to operate in a hospital allowed the checking of vital signs and the communication between patients and the healthcare team, by sending messages. In Brazil, a telepresence robot used in a hospital environment avoided the high exposure of healthcare providers to contact infected patients during the healthcare process. However, it is known that the use of robots in healthcare had already been growing long before the current health emergency [38].

Social Robots are designed to interact with humans by following social behaviors and rules bound to their role. Many are humanoid and try to imitate some human aspects and, in this way, manage to interact. Some examples are as follows: robots that play soccer, dance, play instruments, talk by speaking in sentences, and even those that display human-like emotional behaviors. There are also the social robots classified as anthropomorphic companions, such as the following: those that imitate babies, animated dolls, pets, or others, and therefore interact with humans. There are also virtual social robots, which are not seen in person but perform interactions, usually via computer [39]. In any case, they seem to contribute to reducing depression symptoms in some patients, and the possibility of their implementation in intensive care units and dialysis centers may be considered.

Some examples already being implemented in several healthcare centers are highlighted as follows.

The development of robots that perform surgery seems to have started in France in 1988 with Carpentier and Loulmet, who were responsible for the first surgeries using robotics. Technological breakthroughs have since enabled computer modeling and virtual reality simulation to train surgeons in preoperative patient care. To assist in working with patients, the Robear robot lifts and moves them with ease, assisting hospital staff who typically require assistance in moving patients several times a day [37].

The R1T1 telepresence robot was developed in Brazil and since 2014 it has been part of the care routine in a national hospital, assisting healthcare providers and patients to perform remote consultations, collaborating with entertainment activities, and enabling interaction between patients and their families [40].

The Da Vinci Robot is a portable piece of equipment that can aid in robot-assisted laparoscopic surgery, in surgical interventions undergone by patients. Controlled by a physician at a long distance from the site, its first test took place in 2006, in the United States. This robot brings benefits in relation to laparoscopic surgery by allowing a greater range of movement (in relation to the human hand), eliminating rest tremors, and having a 3D vision, among other features. It is used in urological surgeries, especially prostatectomies; however, its use has already been tested in other fields, such as digestive, gynecological, and proctological surgeries, to name a few. Since its incision is smaller, even in more complex cases, robotic surgery induces a quick recovery of patients [37, 41].

Another type of robotic equipment is the Xenex LightStrike Germ-Zapping Robot, which eliminates pathogens, reducing the risk of infections. It is a portable full-spectrum ultraviolet disinfection robot; it uses ultraviolet light that protects against pathogens, destroying germs that cause healthcare-acquired infections; it deactivates bacteria that cause infections. It is placed in a room after it has been cleaned; it emits a 5-min cycle of pulsed xenon ultraviolet light, which is more intense than sunlight and differs from the light created by mercury lamps; in just two or three cycles, the light destroys viruses, bacteria, mold, fungi, and bacterial spores that cause infections; it eliminates microscopic germs on surfaces and in hard-to-reach places [42].

Autonomous Mobile Robots perform delivery and transportation tasks in hospitals, deliver pharmacy medications and lab samples, and heavier loads such as meals, bedding, and environmental services, safely; they work 24/7 allowing the healthcare team to remain focused on patient care [43].

The Pepper Robot is 1.2 m tall and humanoid in appearance. It is autonomous and moves by itself with its wheels, interacting with people through voice and with its arms. It is pleasant and friendly, designed to communicate in the most natural and intuitive way with humans [44]. It has been trained to be sociable and works as a receptionist in a hospital in Belgium. During the current pandemic, its use is invaluable; it does not get tired, can store thousands of data files without mixing any of them up, and can be integrated with medical software. It recognizes the human

voice in 20 different languages, knows if it is interacting with adults and/or children, accompanies patients around the hospital so they do not get lost, smiles and greets them [37]. Currently, this robot has been programmed to detect whether people are wearing a mask to prevent the spread of COVID-19 and is able to prompt individuals to do so [44].

The Laura Robot, from Brazil, uses its cognitive technology to identify and reduce deaths caused by sepsis. It was developed with machine learning; it learns, understands situations, talks to patients, and is able to assist healthcare providers. The Adam Robot, who is also Brazilian, assists patients suffering from visual impairments; it uses AI to identify in no more than 5 min eye diseases such as presbyopia, astigmatism, hyperopia, and myopia [37].

Animal-shaped robots can make for interesting interactions. The Huggable Robot resembles a teddy bear and talks to people, becoming a social robotic companion; its movements, gestures, and expressions display a character that is rich in personality rather than a simple robotic artifact [45, 46].

One existing device that promotes assistance to patients is the PARO Robot, designed to improve quality of life during recovery from surgery or treatment for depression or other mental disorders. The PARO Therapeutic Robot is an interactive [36] device that resembles a baby seal and is designed to provide the benefits of animal therapy without relying on live animals. It has been used with elderly patients suffering from dementia and has been proven to reduce stress and provide comfort to anxious patients. Its memories of previous interactions allow it to develop a form of pleasant personality; it also naps, blinks, wiggles its fins, and makes small noises, especially to its owner. It was designed in Japan in 1993 and introduced to the public in 2001. It was classified as a class 2 medical device by US regulators in the fall of 2009 [47].

The Somnox Sleep Robot is a pillow-shaped robotic device; it assists rough sleepers with validated, medication-free sleep solutions to reduce stress and restlessness, resulting in a better, deeper sleep. It works with the body's natural rhythms to help people sleep, without unpleasant side effects; it uses continuous and accurate breathing simulation to calm a racing mind, guiding the person into a deep restorative sleep; its sounds are subtle and its breathing movements assist in relaxing, calming the mind [48].

As described, several robots with different applications are available in various healthcare specialties, including nephrology.

26.3 Study Objective

To present the application proposal of a care-providing robot in the specialty field of nephrology.

26.4 Method

In the development of this text, the Theoretical-Reflective Trial was used, concerning the use of a robot to provide care to nephrology patients. Due to the characteristics of this study, it was not necessary to submit it to the approval of a Research Ethics Committee.

With this methodological option, a literature search was carried out and the following controlled descriptors were selected from the Descriptors in Health Sciences—DeCS/*Medical Subject Headings*—MeSH, in Portuguese, English, and Spanish: Robótica, Robotics, Robótica; Cuidadores, Caregivers, Cuidadores; Diálise renal, Renal Dialysis, Diálisis Renal; Hemodiálise no domicílio, Hemodialysis, Home Hemodiálisis en el Domicilio; Transplante de Rim, Kidney Transplantation, Trasplante de Riñón; Nefropatias, Kidney Diseases, Enfermedades Renales; Insuficiência Renal Crônica, Renal Insufficiency, Chronic, Insuficiencia Renal Crónica; Insuficiência Renal, Renal Insufficiency, Insuficiencia Renal. However, in the search performed, without year limitations, in CINAHL (via EBSCO), COCHRANE, EMBASE, Latin American and Caribbean Health Sciences Literature (LILACS), Medical Literature Analysis and Retrieval System Online (Medline) via Public Medline or Publisher Medline (PubMed) and Web of Science no articles were identified.

Therefore, for the contextualization of the theme and the elaboration of the guiding points of this text, contents approached in diversified materials, in specific sites of robotic technologies, in sites specializing in Nephrology, as well as in some studies carried out worldwide and in Brazil were used, although none was found on the use of robots as caregivers for nephrology patients.

Following this search and thorough reading of the materials/articles found, the most pertinent contents for reflection on this theme were selected; with this, some guiding points/categories were elaborated to support the Theoretical Trial and the Reflection of the study on robots as caregivers for nephrology patients. By the end of this step, the authors present a proposal of what they have envisioned for a nephrology patient's care robot.

26.5 Theoretical Reflection

For better understanding, this Theoretical-Reflective Trial was subdivided into the following parts: the use of robots in nephrology, examples of robots participating in surgeries and those used in kidney transplants, caregiver robots, and, finally, the nephrology Caregiver Robot (NCR) proposal.

26.5.1 The Use of Robots in Nephrology

The advances in the field of nephrology in recent years, with the use of more efficient, safer, and cycling hemodialysis machines for automated peritoneal dialysis, with disposable dialysis supplies, have not yet been accompanied by the use of robots and AI with the same intensity as seen in the healthcare field, in general.

Currently, there are expectations of having robots in the near future that can provide care to patients with kidney diseases. The high prevalence of diseases such as diabetes mellitus (DM) and hypertension (HTN) has increased the number of chronic kidney disease patients and the need for nephrological care worldwide [49, 50].

Similarly, in Brazil, epidemiological data highlight that HTN and type 2 diabetes mellitus (DM2) are significant risk factors responsible for a large number of cases of terminal chronic kidney disease (CKD) [51, 52].

As CKD progresses to more advanced stages, the needs for medical care, medications, and support in the fields of nursing, laboratory tests, nutrition, physical therapy, and psychology increase. The healthcare team specializing in nephrological care has to deal with the complexity of the metabolic picture that sets in, and the complications in different organs and body systems resulting from progressive uremia. Simultaneously, the team prepares the kidney patient for future dialysis and transplantation.

Dialysis is a type of procedure that greatly affects the lifestyle of those who need to undergo it [52, 53].

During the dialysis period, the need for kidney assistance intensifies, demanding providers trained in this field at an increasing rate. However, it has been observed that the number of these providers, such as nurses and physicians specializing in this field, has not been increasing in the same proportion as the progressive increase in demand for nephrological support. The specialists in nephrology constitute 1–2% of all work capacity in the medical and health fields and are concentrated in large urban centers, leaving areas of low demographic density without assistance [52–54].

Robotic evolution seems to have been greatly enhanced by the pandemics that have occurred in the world, and especially the current one, caused by SARS-Cov-2, which resulted in COVID-19. Robotic equipment has been introduced in various healthcare settings, especially in the phases that are considered the most intense and dangerous of viral transmissibility, to avoid human contact [38]. In the nephrology specialty, it was no different. The current pandemic has added yet another factor to the need to expand nephrological healthcare to patients living in more remote and low-income areas who do not have access to healthcare.

In this sense, the use of technologies such as telemedicine with synchronous video visits with collection and recording of medical information can assist in pre and post kidney transplantation and in the follow-up of patients on chronic dialysis programs at home [55, 56].

The development of machines that can, while dialyzing the patient, instantly record and report vital data (blood pressure and heart rate), urea clearance (*Kt*/*V*), as well as the need for fluid removal and immediately adapt the dialysis schedule according to patients' conditions is a possible dream. Such a form of online monitoring would allow unpleasant complications such as hyponatremia and its consequent cramps, hypotension, nausea, and vomiting to be prevented [57].

Therefore, the creation of a dialysis machine tailored to each patient by modifying their prescription instantly would make it possible for this procedure to be personalized for each patient. One such model of creating a portable intelligent dialysis machine was elaborated in 2019 [53].

The development of robots capable of providing renal care, particularly in the performance of surgical procedures such as nephrectomy for transplantation and for removal of renal and prostate tumors is already a reality [58]. The use of intelligent systems capable of identifying, through data collected from patients, the early diagnosis of kidney disease and its progression, indicating earlier referral to a specialist, is also already outlined [59, 60].

The development of robots capable of assisting chronic kidney disease patients in their needs at different stages of CKD is one of the future possibilities, and even performing dialysis assistance with intelligent machines [36, 61, 62].

As explained above, the assistance provided in healthcare to chronic kidney disease patients changes according to the phase of the disease in which each patient is found. Knowing that CKD is defined as abnormalities of the renal structure or function, present for more than 3 months and with health implications; therefore, it can be classified based on its etiology, glomerular filtration rate, and level of albuminuria [63].

Upon reaching the end stage of CKD, patients will require renal replacement treatments (hemodialysis, peritoneal dialysis, and kidney transplantation). Dialysis is a type of procedure that negatively affects the lifestyle of those who need to undergo it, and the need for support and assistance increases dramatically. In nephrological assistance to patients presenting terminal renal disease (*V*), hemodialysis (HD) procedures stand out since they are the most commonly used in several countries [64].

Patients on the chronic hemodialysis program have to undergo, several times a week, hemodialysis sessions and monitoring of their vital signs before, during, and after each session, with the collection of information regarding their health status. Monthly blood samples are taken for laboratory analysis. The installation of patients to the dialysis machine is a basic trained nursing procedure and involves puncture of the arteriovenous fistula or connection to the central venous catheter. The hemodialysis prescription by the nephrologist is based on the anamnesis and physical examination performed before dialysis, the patient's vital signs, and fluid removal needs, based on the interdialytic weight gain, among other parameters. According to

the patient's needs and clinical status, the HD programming is established, involving mainly the session duration, the blood and dialysis liquid flows (bath), the sodium concentration, and the ultrafiltration to be used. The prescription of medication such as erythropoiesis-stimulating agents or iron supplementation follows a scheme based on the patient's hematometric indexes [53].

In the case of peritoneal dialysis (PD), the specialized nurse or the patient or trained caregiver manually connects the line containing the dialysis bags to the peritoneal catheter (Tenckhoff) (CAPD) and may interpose the cycler in the case of automated peritoneal dialysis (APD). The number of bags, their glucose concentration, the volume of each bath to be infused, and the length of stay in the peritoneal cavity are all programmed, which are also based on the patient's clinical status and needs. Monitoring the patient's vital signs and symptoms during all dialysis procedures (HD or PD) should be done periodically and at any time intercurrences occur [53, 63, 64].

To date, these activities that are basically the job of trained medical and nursing staff in nephrology could not be replaced by robot technology. However, efforts have been made to develop an implantable hemodialysis machine capable of real-time monitoring of patient conditions and data, as well as therapy parameters and alarms, with real-time responses for immediate professional intervention [53].

However, AI has been playing an increasingly significant role, in assisting physicians in most stages of patient care; in nephrology, it can already be used to improve clinical care, hemodialysis prescriptions, and follow-up of transplant recipients. Artificial neural networks can predict the occurrence of progressive immunoglobulin A nephropathy (IgAN) more efficiently and accurately than experienced nephrologists; left ventricular dysfunction (LVD) is frequent in CKD patients, especially in the hemodialysis population; convolutional neural networks (CNN) have been used to predict the risk of LVD in a different cohort of 50,000 patients and the CNN predictions achieved a sensitivity of 86.3%, specificity of 85.7%, and accuracy of 85.7%, higher than those obtained traditionally [65].

As for dialysis, there are expectations for the development of wearable artificial kidneys, although their use is currently prevented by major safety concerns. In addition, dialysis patients with hemodynamic instability generally do not tolerate intermittent dialysis therapy due to their inability to adapt to a changing scenario of unforeseen events. Thus, developing new wearable dialysis devices and improving clinical tolerance will require contributions from new branches of engineering, such as the use of AI and machine learning (ML) for real-time analysis of equipment alarms, dialysis parameters, and related patient data with real-time feedback response. These technologies are capable of abilities normally associated with human intelligence, such as learning, problem-solving, understanding human speech, or planning and decision-making. Common applications of AI are visual perception (computer vision), speech recognition, and language translation [53].

In the Department of Urology at Johns Hopkins Medical Institutions dedicated to the development of new technologies for urology surgery, URobotics (Urology

Robotics) was developed in 1996; the program combined efforts and expertise from the medical and engineering fields through a close partnership of clinical and technical staff, and through this joint effort, a number of robotic devices, instruments, and systems were created, several of which have been used successfully in operating rooms [66].

Emerging technologies derived from AI, ML, electronics, and robotics will offer great opportunities for dialysis therapy, but much innovation is needed before there is an intelligent dialysis machine capable of analyzing and understanding changes in patient homeostasis and responding appropriately in real-time; efforts are being made in the fields of tissue engineering and regenerative medicine to provide alternative cell-based approaches to treating kidney failure, including bioartificial kidney systems and the deployment of bioengineered kidney constructs [53].

It is understood, therefore, that the use of such technological equipment can possibly be increased in nephrology, just as it happens, especially in the urology specialty.

26.5.2 Examples of Robots Participating in Surgeries and Those Used in Kidney Transplants

In nephrological surgeries, as well as in other fields, the afore mentioned Da Vinci robot-surgeon has been used. It enables surgeons to be ergonomically and comfortably positioned to view 3D images captured by a camera, improving the physician's perception and correcting errors related to involuntary hand tremors, besides being less invasive when it comes to kidney surgeries. This technology, despite its great benefits (less tissue manipulation, lower risks related to human-caused errors, customer satisfaction rate, fast recovery, and is minimally invasive), is still a challenge in the Brazilian context, due to high acquisition costs and few healthcare providers trained in this and other technologies [67].

This type of robot brought robotic assistance in minimally invasive surgeries in the 2000s, proving itself to be of value for recipient renal transplantation, enabling vascular anastomosis, which is considered a challenging point in laparoscopy, thus allowing a reduction in surgical time, this being a significant step toward organ transplantation, as well as in renal replacement therapies that bring more improvements in the lives of patients with renal disease undergoing dialysis, improving quality of life, increasing their survival and reducing the rate of cardiovascular complications from chronic renal failure [67–69]. For this procedure, open surgery is considered the gold standard, with a low incidence of complications and favorable results, in addition to its low costs [68].

Robotic surgery, which is minimally invasive, in recent years, in parallel with the consolidation of open surgery, has enabled a new concept for the surgical care of nephrological patients.

In Romania, a review study described the current research and impact of AI/machine learning (AI/ML) algorithms in dialysis and kidney transplantation. The published studies were presented from two points of view as follows: Which medical aspects were addressed? Which AI/ML algorithms were used? Four electronic databases or studies that used AI/ML in hemodialysis (HD), peritoneal dialysis (PD), and kidney transplantation (KT) were investigated. Sixty-nine studies were divided into three categories, as follows: AI/ML and HD, PD, and KT, respectively. Following careful analysis of the obtained material, the authors postulate that although guidelines are reluctant to recommend the implementation of AI in daily practice, there is ample evidence that AI/ML algorithms can predict better than nephrologists: volumes and hypotension or cardiovascular events during dialysis. Altogether, these studies report a robust impact of AI/ML on patients' quality of life and survival, and the coming years will likely witness the emergence of AI/ML devices that improve the management of dialysis patients, thereby increasing their quality of life and survival [57].

In Italy, a study aimed to report on the University of Florence's technique for robot-assisted kidney transplantation (RAKT) of living and deceased donors, highlighting the evolution of surgical indications and technical nuances in light of a single surgeon. A dedicated program for living donor RAKT was developed at the Institution in 2017 and subsequently implemented for deceased donors. All RAKT procedures were performed by a single, highly experienced surgeon. The experience confirmed the feasibility, safety, and favorable outcomes of RAKT from both living and deceased donors in appropriately selected recipients; however, the authors suggest that there should be further investigations to refine the RAKT technique and evaluate the benefits and risks of robotics for kidney transplantation, especially from deceased donors [70].

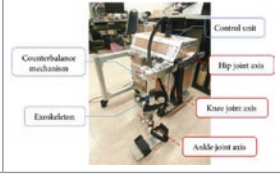
Kidney transplantation is the treatment of choice for patients with chronic kidney disease (CKD), but the shortage of kidneys and the disabling medical conditions these patients suffer make dialysis essential for most of them. Developing novel wearable dialysis devices and improving clinical tolerability will require contributions from new branches of engineering, such as artificial intelligence (AI) and machine learning (ML) for real-time analysis of equipment alarms, dialysis parameters, and related patient data with real-time feedback response [53].

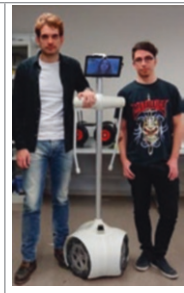
26.5.3 Caregiver Robots

Several robot caregivers have been reported in the literature and may be able to assist healthcare providers and CKD patients during home, outpatient, and inpatient care.

Table 26.1 describes the types of caregiver robots, which have been developed over the past two decades, that can promote health for these patients through collaboration in daily living activities, socialization, entertainment, physical rehabilitation, emotional support, and caregiving support [71–86].

Table 26.1 Description of caregiver robots, according to their applicability, type, name, origin, characteristics and purpose, and applications in healthcare

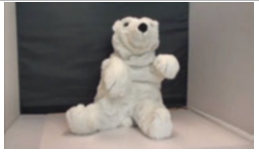
Applicability	Type	Name	Origin	Characteristics and purpose	Applications in healthcare	Figure
Home use	Assistance robot	4-DOF [71]	China, 2009	Robotic arm designed to assist the elderly with simple tasks, such as pouring a glass of water	To promote self-care and reduce anxiety	No figure
Home use	Social assistance robot—companion robot	AIBO [72]	Japan, 1999	Non-verbal, dog-like robot with a metallic appearance and the ability to see, walk, and interpret commands. It can learn, mature, interact with humans, and express responses	To bolster self-esteem, improve socialization, provide emotional support, and reduce anxiety	
Rehabilitation	Rehabilitation robot	*Anklebot* [73]	Thailand, 2017	Exoskeleton designed to assist hip, knee and ankle joint training with patients in the sitting position	To promote mobility and self-care	

Home use	Assistance robot	*Assistant Personal Robot (APR)* [74]	Spain, 2016	Remote-controlled mobile robotic platform with video conferencing capabilities. Its mobility is provided by omnidirectional wheels and its head is based on an Android tablet. The APR has two mobile arms that rotate 360° and can be used for gestural interaction. The purpose of this robot is to provide social assistance to the elderly	To promote self-care, improve socialization, provide emotional support, and reduce anxiety	
Rehabilitation	Assistance robot	*Baxter* [75]	The United States, 2012	A 100 cm tall industrial robot equipped with two arms capable of assisting patients in making certain movements with their upper limbs, such as reaching for a cup	To promote self-care, foster self-responsibility, bolster self-esteem, and reduce anxiety	

(continued)

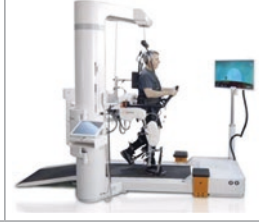
Table 26.1 (continued)

Applicability	Type	Name	Origin	Characteristics and purpose	Applications in healthcare	Figure
Home use	Assistance robot	*Care-o-Bot-4* [76]	Germany, 2015	A 148 cm tall mobile robot with a touchscreen head, microphone for speech recognition, cameras for facial and gesture recognition, a one-finger hand, and integrated sensors. It was developed to assist people in the home environment and in daily life activities, such as shopping at the supermarket, serving drinks, storing medical information, making phone and video calls	To reduce social isolation, promote self-care and foster self-responsibility	
Clinical use	Assistance robot	*Cody* [77]	The United States, 2009	A 155 cm tall robotic nurse, it is fitted with two arms attached to an omnidirectional mobility base. It was developed to assist disabled people to perform certain tasks, such as bathing using gentle compressions on the skin	To promote self-care and foster self-responsibility	

Clinical use	Assistance robot	*Compounding robot* [78]	The United States, 2013	Robotic equipment capable of preparing individualized doses of intravenous chemotherapy medications in a variety of infusion bags and syringes. It can be programmed to recognize the physical parameters of syringes and vials, and gravimetric measurements to ensure that the correct medication ingredients are combined and the final dose is accurate	Promoting safe medication preparation in healthcare facilities and integrating technologies with human resources	No figure
Home use	Social assistance robot	*CuDDler* [79, 80]	Singapore, 2013	A robotic teddy bear that interacts vocally, is able to move its neck, arms, and eyelids, and even recognize verbal and non-verbal communication interactions associated with the patient's emotional state	To bolster self-esteem, improve socialization, provide emotional support, and for the reduction of anxiety	

(continued)

Table 26.1 (continued)

Applicability	Type	Name	Origin	Characteristics and purpose	Applications in healthcare	Figure
Home use	Assistance robot	*Dinsow* [81]	Thailand, 2009	A 60 cm tall robot with a touch screen head and video camera. Its purpose is to monitor elderly patients, make video calls with family members, provide alerts to caregivers regarding patient activities, provide reminders for medication administration and blood pressure measurement, perform physical exercises with the elderly, and provide entertainment (games and karaoke)	To improve socialization, reduce anxiety, improve adherence to medication and non-medication therapies, and provide healthcare support through reminders	
Home use	Assistance robot	*Home Nursing Robot (HNR)* [82]	China, 2011	A robotic system, with a humanized cartoon appearance, capable of detecting and processing six physiological parameters (electrocardiographic, oxygen saturation, blood pressure, heart rate, breathing, and body temperature)	To promote self-care and monitor vital signs	
Rehabilitation	Assistance robot	*LoKomat* [83]	Switzerland, 2000	Robotic rehabilitation device capable of providing repetitive, physiological gait training to increase muscle strength and joint range of motion according to the patient's needs and performance	To promote body mechanics	

Clinical use	Assistance robot	*Quirubot* [84]	Spain, 2012	A robotic instrument operator with voice recognition capable of recognizing up to 27 surgical instruments and providing 82 verbal instructions. The robot locates the desired instrument, picks the instrument from the tray, and places it on an exchange tray in which the human operator can finally handle the object	To assist the instrumentation team during the execution of surgical procedures	No figure
Clinical use	Mobility robot	*RoNa* [85]	The United States, 2014	A robotic, humanoid-type nursing assistant capable of transporting patients weighing up to 500 kg	To prevent falls, assist in the transfer and positioning of patients	No figure
Clinical use	Assistance robot	*Vein-Finding* [86]	The United States, 2008	A robotic system in preliminary studies that aims to render intravenous catheterization more reliable and less painful. Such a system would enhance the sensory and motor skills of human providers while maintaining the human provider in full control of the insertion through a haptic device (a joystick with a high level of freedom)	To promote the safe insertion of intravenous catheters	No figure

26.5.4 The Nephrology Caregiver Robot (NCR) Proposal

The authors of this chapter have devised a proposal for the Nephrology Caregiver Robot, based on the reflections within the text, and this is presented below.

With the increase in home dialysis, either with CAPD or APD or even with Home Hemodialysis (Home Care Nephrology), care provided outside of clinics or hospitals has been growing in recent years. This increase requires healthcare providers, especially nurses and nephrologists, to travel to patients' homes to provide nephrological care, such as turning dialysis on and off and following all procedures for a few hours, which is quite inconvenient and costly for the healthcare system.

Similarly, care robots are already available which are capable of performing clinical and assistive care and storing information, monitoring the patient's health status, and assisting healthcare providers to provide patient care in inpatient and outpatient settings in different fields of healthcare, as described so far.

Therefore, it begs the question "Why not use a robot to collect all the clinical information and vital signs, interdialytic weight gain, and other information and transmit it to healthcare providers remotely, so that they can schedule the dialysis and the necessary medications?". Thus, it would be enough for a trained technician to install the patient on the machine and stay with the patient, in the clinic, home, or other care space.

On the other hand, with the exponential technological advance that is taking place, even if there is no need for dialysis, but for closer monitoring of nephrology patients, these robot caregivers could "turn on" and "off" automatically, stay in the homes or clinics and perform laboratory test collection, check vital signs, record the care provided, interact with patients, and transmit data to healthcare providers regarding patients' health status, among other functions.

Such a proposal hinges on a series of theoretical research and technological development. Therefore, this team of authors thought of inviting professionals in the field of information technology and robotics for this possibility, thus strengthening the properties of this product (Fig. 26.1).

Fig. 26.1 Interaction of the Nephrology Caregiver Robot with healthcare providers and patients

The idealized and proposed framework for the performance of the Nephrology Caregiver Robot (NCR) was as follows

1. The NCR interacts with the nephrology patient, who may be in their home, clinic, or other care space. The robot greets the patient, asks questions, and gets answers. These questions must be elaborate and follow a script: how is the patient feeling at that moment? Does the patient present any discomfort? Does the patient have any specific message that they wish to communicate or something similar? The robot records these answers in its memory. This care robot process requires special attention and monitoring by the healthcare team.
2. The NCR analyzes whether the patient is wet, has involuntarily lost urine or feces, and informs the family and/or clinic caregiver to change clothes to keep the patient dry and comfortable. In more advanced stages of robotic technology, such equipment could be capable of performing this care.
3. The NCR asks the nephrology patient for authorization to obtain their vital parameters. Once authorized, it obtains the values of blood pressure, respiratory rate, heart rate, and axillary temperature. It records this data in its memory and transmits it to the healthcare providers who are working remotely but receiving this data online and interacting with them.
4. The healthcare providers analyze the parameters obtained by the NCR and send some form of feedback, which can be that "everything is fine" and there is no need for further interaction with the patient or that "it is necessary to obtain physiological data" such as blood, urine, or another biological sample from the patient.
5. The NCR requests consent from the patient again and can obtain the necessary physiological data. It records what it has done, analyzes the data obtained, and relays it to the healthcare providers.
6. Healthcare providers then analyze the physiological parameters received and return information to the NCR, such as: "Offer the patient some type of medication with the specific dosage indicated", "Do not medicate the nephrology patient since it is not necessary yet and continue to observe them", "Offer them some liquid or solid food", among other possibilities. These remote nursing or medical orders are transmitted to the NCR that is close to the patient, who fulfills the prescription.
7. The NCR does as instructed, continuing to interact with the patient and recording their observations. After a specified timeframe, it obtains the patient's vital parameters again, records the data, and notes whether the procedure performed resulted in the patient's recovery.
8. The NCR relays the values found to the healthcare team a second time. If the result is positive, it ends the interaction and schedules itself to come back later and restart the cycle previously performed. If it is negative, it continues the interaction, receiving orders from the healthcare team, staying close to the patient, and even arranging for the patient to be moved closer to the team.
9. During all these procedures, the NCR tries to keep the nephrology patient calm and free of anxiety. This cycle is repeated as often as necessary.

10. The nephrology patient will be able to communicate with the healthcare team, regardless of the intervention performed by the NCR. However, it is assumed that with the performance of the robotic equipment and the confidence it conveys, the patient will tend to reduce this direct communication with the healthcare team.

The NCR could be a piece of equipment owned by the patients themselves or by the health institution, which would temporarily lend it to the nephrology patient, through healthcare plans or through the public health system in effect in the country, such as the Unified Health System in Brazil and others in different countries.

Since telepresence robots already exist, which assist family and/or healthcare facility caregivers by providing audio and visual feedback to them [87, 88], the NRC could be built based on this type of robotic equipment.

26.6 Conclusion

The use of robots is already a reality in the routine of several healthcare systems and providers, particularly among those in socioeconomically developed countries, and is becoming engaged in the systems of developing countries.

In nephrology, it has been used in nephrological surgeries, specifically in the surgical field and in urology. However, with the exponential technological breakthroughs, it is expected that in the near future, robots may also be performing directly in the care of patients presenting kidney alterations, interacting with patients, performing procedures such as collecting samples for exams, assisting in the administration of medication, after receiving feedback from the healthcare teams. It also highlights the need to implement new robotic technologies, such as the one presented by the authors in this study, in clinics, at home, and in other spaces outside hospitals.

It is urgent to develop new equipment, test and evaluate it in the field of knowledge of robotics in healthcare, especially in the field of Nephrology, since technological breakthroughs and the improvement of machines and processes should be aimed at achieving a prominent role of assistance in healthcare. Reflections are necessary among the interdisciplinary teams to ensure that these breakthroughs are consistent with the real needs of patients, considering the different phases that involve and require nephrological treatment. Research agendas are recommended to intensify investigations on the subject of robots, their monitoring in care since developments in the nephrological field lack greater implementations in practice.

The advancement of robotic caregivers in nephrology will surely be to the benefit of a faster and more controlled quality enabled by this form of equipment, as well as the recording of each step of care, and thus possibly rendering the lives of nephrological patients more pleasant during the course of their treatments.

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Chapter 27
Preclinical Randomized Controlled Trials in Nephrology

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27.1 Introduction

Preclinical animal studies are widely used to test candidate drugs for their capacity to modulate disease processes [1]. Preclinical animal studies are a fundamental part of translational research, which tries to carry over (Latin: "translatio") new knowledge from basic science to improve human health [2]. Hence, "Translational Nephrology" focusses on the unmet medical needs of patients with kidney disease. In the past few decades, a vast number of animal studies proved drug efficacy in small rodents, whereas only few translated into clinical research and finally benefit patients, referred to as "translational crisis" [3].

A factor contributing to this failure is the large gap between the design and conduct of animal studies versus that of clinical trials (Fig. 27.1) [4]. Compared with the established standards of clinical trials, which include dedicated ways of randomization, a controlled design with pre-specified endpoints, and blinded observational phase to ensure reliable results, animal studies are often conducted in a single-center setting, frequently conducted by early career researchers quite biased about the results of the study [5]. On a scale for the different quality levels of evidence, such studies rank low (Table 27.1). Therefore, there has been a call to reduce the gap

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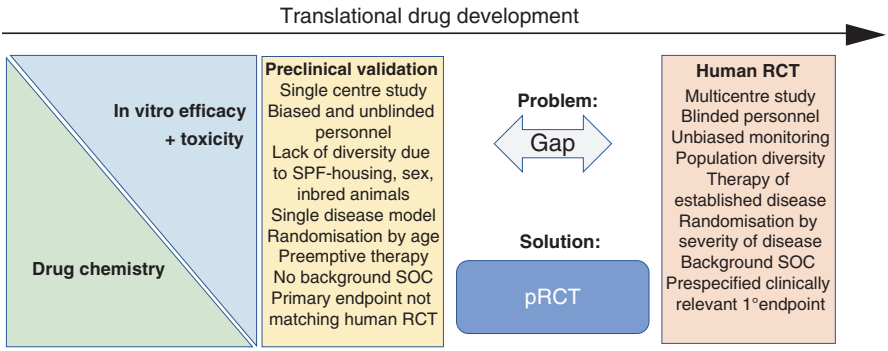

Fig. 27.1 Position of preclinical randomized controlled trials (pRCT) in the flow of translational drug development. The pRCT reduces the gap between preclinical studies and human RCTs. *SOC* standard-of-care

Table 27.1 Evidence levels in clinical and preclinical research

Level of evidence	Clinical studies	Preclinical studies
Highest	Systematic review, several multicenter RCTs	Systematic review, several multicenter pRCTs
	Multicenter phase 3 RCT in different world regions with heterogeneous populations	Multicenter pRCT with heterogeneous animals/ disease models
	Multicenter phase 2 RCT in a single world region with comparable populations	Multicenter pRCT region with comparable animals/ same disease model
	Single-center RCT with heterogeneous populations	Single-center pRCT with different disease models or non-SPF-housing conditions or outbred mouse strains analyzed at several time points conducted by unbiased personnel (at best, all of the five)
	Single-center RCT with homogenous populations	Single-center pRCT with single disease model or SPF-housing conditions or inbred mouse strains analyzed at single timepoint conducted by biased personnel
	Single-center longitudinal cohort study without intervention	Single-center descriptive longitudinal animal phenotype study without intervention under SPF-housing conditions in inbred mouse strains analyzed at single timepoint conducted by biased personnel
	Single-center cross-sectional cohort study without intervention	Single-center cross-sectional animal analysis at single timepoint without intervention under SPF-housing conditions in inbred mouse strains analyzed at single time point conducted by biased personnel
	Single-center cross-sectional case–control study without intervention	In vitro studies: 2D/3D culture of primary cells freshly isolated from human or animals
Lowest	Case series, case reports	In vitro studies: 2D/3D culture of cell lines

RCT randomized controlled trial, *SPF* specific pathogen-free

between animal research and clinical trials by testing novel drugs in preclinical randomized controlled trials (pRCT) (Fig. 27.1) [5]. Recently, the Animal Research: Reporting In Vivo Experiments (ARRIVE) recommendations have been endorsed by an increasing number of journals and scientists as an academic standard how to report animal studies. Although the ARRIVE recommendations mainly focus on how to minimize bias and thoroughly describe in vivo studies, they also provide advice and best practice for the upfront design and proper conduct of animal studies [6].

Another factor contributing to the translational crisis is related to the tested individual itself [7]. In a clinical trial, the participants are highly heterogenetic in terms of ethnicity, genetics, environment, treatment practice, healthcare settings, disease stage, and age. In contrast, in a preclinical trial, tested animals are most often inbred rodents, selected and bred to a maximum of homogeneity to minimize group size and housing costs [1]. Such narrow spectrum of diversity in terms of genetics, age, sex, and environmental factors has little to do with diverse human populations. Some efforts have been made to increase the heterogeneity of tested animals. One is to increase genetic variability by using defined outbred mice [8, 9]. Some researchers also suggested including models on different strains to increase the genetic variability [5, 10]. Another strategy is to introduce more environmental variability to increase the heterogeneity of tested animals as well as animal handling procedures by performing multicenter pRCTs [11, 12].

Though pRCT/multicenter pRCT is not an ultimate solution, it is believed to offer more robust and reliable results and to provide more valuable information for clinical researchers upon selecting potential candidates for clinical trials [5, 10]. When performing a pRCT/multicenter pRCT that follows the ARRIVE guidelines, preclinical researchers may confront issues related to study design, randomization, blinding, etc. In the following part we discuss these issues according to our limited experience and propose suggestions that may improve the quality of pRCT.

27.2 Study Design

In most drug-testing experiments, study groups receive either placebo or drugs without any background therapies. This study design is reasonable since the aim of the study is to explore the efficacy of certain drugs. It might not be sufficient, however, when we translate the result to clinical trials, in which most patients receive a standard therapy. It is possible that one compound fails to prove its efficacy in a study added with current standard therapies because the effects of this compound overlap with those of standard therapies. Thus, for a preclinical trial in an advanced stage, adding a standard background therapy might narrow the gap between preclinical and clinical trials. For instance, in a drug-testing experiment of diabetic kidney disease, we added metformin, ramipril, and empagliflozin as a background therapy to investigate, if bromoindirubin-3′-oxime, a compound promoting podocyte regeneration and survival, would have additional effects beyond this type of

background therapy [13]. Practically, it requires some pilot studies to adjust the doses of background therapies so that standard therapy not already totally diminishes the disease phenotype, leaving enough room to test potential compounds in an add-on design.

The International Society of Nephrology has launched the "Advancing Clinical Trials" (ISN-ACT), initiative to encourage existing infrastructures within ISN to improve the global nephrology community's participation in clinical trial research (https://www.theisn.org/in-action/research/clinical-trials-isn-act/). Information provided on this website may also help preclinical researchers to match their design of animal studies with the requirements of respective trials in humans.

27.3 Inclusion and Exclusion Criteria

The ARRIVE guideline recommends that all possible inclusion and exclusion criteria should be defined and documented in a preregistered protocol a priori [6]. For the inclusion criteria, there is a significant discrepancy related to age between preclinical and clinical trials. In a clinical trial aiming to test a new drug in adults with chronic kidney disease (CKD), any patient who meets the diagnosis of CKD, regardless of age, will be considered. Whereas in preclinical trials, tested animals are at the same or similar age. In some spontaneous disease models, such as MRL^{lpr} lupus mouse model, the age of onset varies [14]. Therefore, on such models an inclusion criterion based on the same age could increase the diversity of disease severity, which further implies larger group size. One possible strategy is to include mice based on disease severity rather than age. This requires to regularly screen the animals for the onset of disease, e.g., the onset of proteinuria as a sign of the spontaneously developing lupus nephritis of MRLlpr mice or for hematuria as a sign of the spontaneously developing Alport nephropathy in collagen 4A3−/− mice.

Also predefined exclusion criteria help to avoid reporting bias. The ARRIVE guidelines recommend reporting any exclusion of animals or data points in the analysis. Frequently, animals are excluded because they reached humane termination criteria during welfare monitoring, e.g., the body weight drops below a certain threshold. In an intention-to-treat analysis, such animals should not be excluded because they may provide useful information on possible adverse events. Some missing data points may be due to less sample volume, mishandling of samples, bad quality of histology, etc., which are evitable and place a higher requirement on practical issues.

27.4 Randomization and Blinding

The ARRIVE guideline recommends to employ appropriate randomization methods (e.g., web-based randomization tools and relative functions in various statistical software), instead of randomly allocating animals [6]. Randomization strategies include simple randomization, stratification (e.g., mice are stratified by a variable,

which might affect the endpoints), and minimization (each animal is allocated according to the already allocated ones; a method aims to balance important variables between groups and is suitable for small trials) [6, 15, 16]. It is important to carefully think about how to implement blinding into the following steps: randomization, drug administration (e.g., performing the oral gavage or injection), outcome assessment (e.g., evaluate the lymphadenopathy based on a semi-score method, histological quantification), and statistical analysis (e.g., during handling missing values and outliers) [6].

27.5 Prespecifying Clinically Relevant Endpoints and Outcome Measurement

All outcomes, dissected into primary and secondary outcomes, have to be specified a priori [6]. Of note, for an advanced-phase pRCT, only clinically meaningful endpoints should be chosen that match with those being analyzed in a putative follow-up clinical RCT. For example, in the kidney disease domain, clinically relevant endpoints are "kidney-related/uremic death," which can be studied in models of severe bilateral acute kidney injury or in Col4a3-deficient mice with progressive Alport nephropathy. Also, adenine- or oxalate-feeding can lead to progressive crystalline nephropathy and end stage kidney disease. GFR decline, GFR slope, or cause of BUN and serum creatinine levels can serve as surrogate markers for a decline in excretory kidney function, the clinically most relevant aspect in AKI and CKD research. Although frequently done, quantifying proteinuria is less relevant, because proteinuria is only a surrogate for the other surrogate markers. Histopathological lesion patterns such as degree of glomerulosclerosis or interstitial fibrosis may provide interesting mechanistic information but are irrelevant as primary endpoint from a clinical perspective because these parameters are never assessed as endpoints in clinical trial settings. Thus, to restrict the study analysis to a single clinically relevant primary endpoint and, possibly numerous, secondary endpoints that provide mechanistic clues or are hypothesis-generating helps avoid misinterpretations and *p*-value hacking, an important factor contributing to the reproducibility crisis. One has to remind that also animal studies are only powered for the primary endpoint and not for any of the secondary endpoints. Therefore, a definite conclusion can only be taken on the (prespecified) primary endpoint of the study and not on any of the other findings that provide only supporting information.

27.6 Protocol Registration

Human RCTs are registered at www.clinicaltrials.gov. It is recommended to register a study protocol before the pRCT commences [6, 17]. A protocol should include key aspects related to study plan such as hypothesis, study groups, primary and secondary endpoints, group size and corresponding calculation, randomization,

blinding, and study procedures. The adherence to preregistered protocol may avoid outcome reporting bias [17]. A free online platform that offers researchers to register their pRCT protocols is available at www.preclinicaltrials.eu. A temporal embargo tool is in place not to reveal sensitive information is necessary.

27.7 Summary

Currently, it is still unknown if pRCTs/multicenter pRCTs adhering to ARRIVE guideline can better address the translational crisis. They emphasis more on higher standards of scientific rigor and incorporate more clinically relevant features, which may provide more valuable and transparent information for both preclinical and clinical researchers in the domain of nephrology.

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Chapter 28
Alternative Clinical Trial Designs for Nephrology Research

Neeru Agarwal, Murilo Guedes, Roberto Pecoits-Filho, and Meg Jardine

28.1 Introduction

Randomized controlled clinical trials (RCTs) have traditionally been regarded as an official paradigm in the evolution of treatments in the medical field. Over the past few decades, several areas of medicine have conducted large-scale clinical trials with impactful changes in practice and patient-centric outcomes. At the same time the traditional clinical trial design often requires large resource expenditures and usually focuses on narrow research questions which are sometimes at odds with the current clinical uncertainties in nephrology.

Despite the high burden and cumbersome nature of kidney disease, there is limited reliable evidence to guide patient care [1]. In fact, the design and implementation of RCTs have been challenging due to the characteristics of patients with kidney disease, who are a heterogeneous and complex population with very high comorbidity burden. Challenges related to infrastructure, workforce, and resource

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allocation in the field of nephrology research have also been identified as limitation to the implementation of RCTs [2]. Global availability of national funding for nephrology trials is scarce, as well as formal physician training in clinical research [2]. Unmet and outstanding disparities remain across countries to the execution of RCTs in kidney diseases [2].

Although improvements in clinical trial development have been noticeable in recent years, particularly in the areas of diabetic and glomerular kidney disease, nephrology lags behind other areas of medicine in number, size, and quality of RCTs [1, 3]. In this chapter we will describe alternative and innovative trial designs that can be used to overcome the challenges and accelerate innovation to improve evidence generation in nephrology. We also discuss the incorporation of routinely collected data through patient registries and electronic health records to facilitate the conduct of efficient and cost-effective pragmatic, comparative effectiveness clinical trials.

28.2 The Conventional Approach to Clinical Trials

The key features of traditional RCTs include random allocation of participants to the control and intervention arm, allocation concealment and blinding of the intervention to participants and investigators, and strict inclusion and exclusion criteria to enhance internal validity of the study. The process of randomization ensures balance between known and unknown confounders in participant groups, thereby enabling the estimation of the cause–effect relationship of an intervention with minimized bias.

RCTs can be classified based on the type of intervention being evaluated: (1) explanatory RCTs demonstrate the efficacy in "ideal" circumstances, while pragmatic RCTs test the comparative effectiveness of the intervention in everyday clinical practice; (2) superiority RCTs determine if the new treatment is better than placebo or standard of care, while equivalence trials aim to determine that the new treatment is no worse; and (3) clinical trials evaluating a new drug can be classified as phase I, II, or III, with phase III trials most comparable to RCTs.

Following randomization, common study designs for RCTs include parallel, crossover, and factorial designs (Fig. 28.1). In parallel-group designs (Fig. 28.1a), study groups remain fixed during follow-up, and sample size and power are predefined. In crossover RCTs (Fig. 28.1b), each participant receives an intervention in a random sequence and serves as his or her own control. The factorial design is a variation of the traditional parallel RCT but allows comparison of two or more simultaneous interventions (Fig. 28.1c).

These traditional trial structures, however, can be costly, resource-intensive, and time-consuming [4, 5]. Extrapolating results of these trials to broader population groups is also not strictly valid; therefore, the results may have limited "real world" applicability [6]. In fact, RCTs recruiting kidney transplant recipients and dialysis

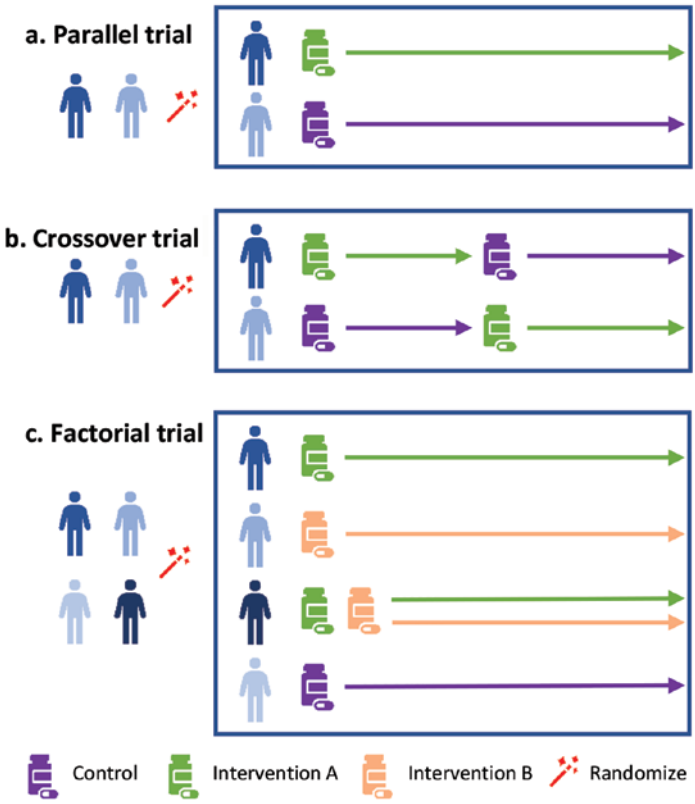

Fig. 28.1 Traditional RCT designs structures: parallel (**a**), crossover (**b**), and factorial (**c**)

populations have been shown to enroll healthier participants than their representative population, potentially restricting generalizability of these traditional clinical trials [7, 8]. To address these problems, a large spectrum of alternative clinical trial designs have emerged, which will be discussed below.

28.3 Alternative Trial Designs

Alternative trial designs may differ from traditional RCTs in the unit of randomization (e.g., cluster RCTs or N-of-1 trials), study organization (e.g., master protocols: umbrella, basket, or platform trials), or incorporate adaptive features (e.g., adaptive trials). A brief description of each trial design, a discussion of the advantages, disadvantages, and practical challenges, with examples from nephrology, are discussed below (Table 28.1).

Table 28.1 Key features, advantages, and considerations of different alternative trial designs

Trial design	Key features	Advantages	Considerations	Examples
Cluster RCT	Randomization unit based on clusters (regions, facilities)	Greater efficiency and accuracy when contamination between groups is present (e.g., behavior-dependent interventions, such as dietary or exercise programs) Increased generalizability by group-based enrolment and allocation Ability to test the effect of decisions made at the group level (e.g., facility)	A larger sample size is often required because there is a risk of correlation between individuals within a cluster	TiME [11] MyTEMP [12] RESOLVE [13] Dial-Mag Canada [14] HiLo [15] 4Ds [16] Tackling AKI study [17] REDUCCTION [18] SWIFT [19] TEACH-PD [20]
Master protocols/ platform trial	Multiple comparison groups, both within disease subpopulations (umbrella) or between diseases with a common feature (basket), or a continuous structure in which additional interventions can be introduced (platform)	Greater operational efficiency (e.g., common screening platform) Increased efficiency in testing multiple interventions (e.g., common-control structures, new interventions amended) More efficient allocation of subgroups to distinct treatments (e.g., allocating only biomarker positive patients to target therapy)	Challenging governance and infrastructure Common-control groups and time-varying factors may introduce confounding	STAMPEDE [21]
Adaptive platform trial	Extension of platform designs allowing protocol modifications triggered by data analysis	Increased recruitment efficiency along with protocol adaptations Ability to amend multiple interventions and increase or reduce subgroup sampling, based on observed data	Considerations depend on the adaptive feature employed (e.g., type I inflation in treatment effect dependent-adaptive designs)	I-SPY2 [22] REMAP-CAP [23]

Adaptive trial	Preplanned protocol adaptations triggered by data analysis results (e.g., covariate adaptative randomization, sample size recalculation, response adaptative randomization, adaptive dose-ranging, seamless phase II/III)	Increased efficiency in the recruitment process (treatment effect-dependent designs) Efficient identification of harmed or benefited subgroups (treatment effect-dependent designs) Greater allocation in arms with higher chance of success, conditional on data analysis (treatment effect-dependent designs) Potential individualization of risk and benefits of interventions—a step to personalized medicine	Inflation of type-I error in treatment effect-dependent designs Covariate imbalance between groups at baseline in response-adaptive randomization Unwarranted correlations between treatment allocation and probability of outcomes in treatment effect-dependent designs	STOP-AKI (seamless phase 2a/2b) [24] EMPIRIKAL (dose-escalation) [25]
N-of-1 trial	Single patient randomized crossover trial Randomization of time periods, instead of participants Patient is their own control Outcomes are repeatedly measured during the study period	Conclusions drawn about treatment are specific to the individual; helps recognize effective therapies Can improves shared decision making May require fewer participants for the same level of statistical power as parallel and crossover RCTs	Not suitable to answer all research questions: ideal for chronic, stable conditions where treatment effects are reversible, and treatment has rapid onset and offset of action	StatinWISE [26] Treating hypertension in children [27]

AKI acute kidney injury, *Dial-Mag Canada* Outcomes of a Higher vs. Lower Haemodialysate Magnesium Concentration, *EMPIRIKAL* Efficacy of Mirococept for preventing ischaemic reperfusion injury in the kidney allograft, *HiLo* pragmatic trial of Higher vs. Lower serum phosphate targets in patients undergoing haemodialysis, *I-SPY 2* Investigation of Serial studies to Predict Your therapeutic response and molecular analysis 2, *MyTEMP* Major Outcomes with Personalized Dialysate TEMPerature, *STAMPEDE* Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy, *StatinWISE* Statin Web-based Investigation of Side Effects, *STOP-AKI* Safety, Tolerability, Efficacy and QoL Study of Human recAP in the Treatment of Patients with Sepsis Associated-Acute Kidney Injury, *RCT* randomized control trial, *REDUCCTION* REDUcing the burden of dialysis Catheter ComplicaTIOns: a National approach, *REMAP-CAP* A Randomized, Embedded, Multi-factorial, Adaptive Platform Trial for Community Acquired Pneumonia, *RESOLVE* Randomised Evaluation of Sodium Dialysate Levels on Vascular Events, *SWIFT* Symptom monitoring With Feedback TRIAL, *TEACH-PD* Targeted Education ApproaCH to Improve Peritoneal Dialysis, *TiME* Time to reduce Mortality in ESKD

28.3.1 Cluster RCTs

In cluster RCTs, randomization is performed for a group of patients, rather than at the individual level (Fig. 28.1) [9]. This design may be useful when individuals are randomized within the same environment as it would otherwise lead to treatment contamination [10]. Cluster RCTs are suited to complex multistage processes, such as dialysis, because the traditional randomization would require sites to deliver distinct interventions to individuals within the same environment. Delivering mutilple complex interventions is often impractical and may impose an unacceptably high burden to healthcare facilities, such as dialysis units. Cluster trials also generate evidence that is highly generalizable due to group-level recruitment and consequently has greater representativeness.

Several studies have implemented cluster designs in nephrology (Table 28.1) with the main advantage of reducing contamination in interventions implemented in dialysis centers. For instance, the HED-SMART trial [28] tested behavior interventions in hemodialysis patients. Clustering patients by dialysis shift, this study tested the effectiveness of self-management training to aid better dietary and fluid control in patients with kidney failure. This trial reported short-term improvements in intradialytic weight gain, potassium, and mineral bone markers in the intervention group, yet differences were not sustained in longer follow-up [28]. The cluster design in this trial was an interesting choice since there is a potential for treatment contamination if individual randomization was chosen. Another insightful example is the use of a cluster design to compare two interventions designed to mitigate the risk of central venous catheter-related bloodstream infections. This study implemented a comparative effectiveness trial by eliminating the risk of contamination at the service level and successfully reported that an antimicrobial barrier cap prevented bloodstream infections. These results are not only important at the patient-level, but can also help guide dialysis purchasing decisions and care policy [29].

Apart from the potential contamination, individual randomizations often lead to the enrolment of more stable, younger, and less burdened patients, who may not reflect clinical practice [8]. Cluster RCTs may help address this problem by facilitating the inclusion of a broader patient population. This is often considered a pragmatic feature of cluster RCTs, wherein inclusion criteria are less stringent. For example, the TiME trial was a cluster RCT that tested the effect of extended hemodialysis sessions ($\geq$4.25 h) as compared to regular sessions on mortality outcomes [11]. This trial was embedded into the operations of two large dialysis organizations in the US, resulting in the inclusion of a representative patient population. Unfortunately, the intervention uptake was poor across dialysis facilities, which restricted study power and jeopardized trial performance. Such an example points to a potential caveat of facility-level cluster RCTs – protocol deviations may affect a broader group of subjects as compared to individual-patient designs, which

represents implementation challenges investigators must carefully consider. In addition to TiME, other cluster RCTs currently underway or completed among hemodialysis patients include personalized temperature-reduced dialysate (MyTEMP); distinct dialysate sodium (RESOLVE) and magnesium (Dial-Mag Canada) concentrations, and phosphate-lowering strategies (HiLo, 4Ds) [12–16].

In the non-dialysis CKD setting, there are ongoing cluster RCTs addressing system-level or bundle-based interventions. These trials are focused on fluid management strategies [30], the use of electronic clinical diagnosis decision support systems in primary care to improve CKD outcomes [31], and pharmacist-driven interventions to address blood pressure control and to detect drug-related adverse events [32, 33]. There are also cluster RCTs exploring the effects of guideline-based interventions [34, 35] and the adoption of risk-based care strategies in CKD care [36]. Finally, there are ongoing trials on quality-improvement initiatives to increase kidney transplant access [37], behavior modification strategies for early CKD patients [38] and multicomponent interventions to improve outcomes at kidney failure transition [39].

A key limitation of cluster RCTs is that a larger sample size (up to 50–100%) compared to the traditional approach is often required to adequately power the study [38]. This is due to the potential collinearity between individuals within a cluster [9] and can be optimized by using a cluster crossover design (Fig. 28.2b). Another variation of the cluster trial design is the stepped-wedge cluster randomized trial (Fig. 28.2c), in which clusters are allocated to the intervention in a sequential fashion [40]. At regular time periods (steps), clusters are sequentially allocated from the control group to the intervention arm. This process is repeated until all units are exposed to the intervention [40]. Hence, this design allows both intra and intergroup comparisons, increasing trial efficiency. Stepped-wedge cluster studies are more efficient than parallel-cluster trials when the intra-cluster correlation is large enough or when the individual clusters are sizable [40]. In nephrology, a successful stepped-wedge cluster design was implemented across five hospitals in the United Kingdom, which delivered a multi-intervention program to manage and prevent acute kidney injury (AKI) [17]. This trial implemented a three-level intervention consisting of electronic detection and alarming system, a bundle for assessment, and management, and an educational program to increase AKI awareness among healthcare workers. The primary outcome of mortality assessed at 30 days was not different between study arms, albeit length of hospital stay decreased [17]. Another example of a stepped-wedge cluster randomized trial is the REDUCCTION trial which is currently underway in Australia. This study will assess whether a suite of clinical interventions at the renal service level will reduce hemodialysis catheter-related bloodstream infections [18]. Using this trial design, all participating sites serve as their own control, and the risk of "contamination" is minimized as the intervention is performed at the service level.

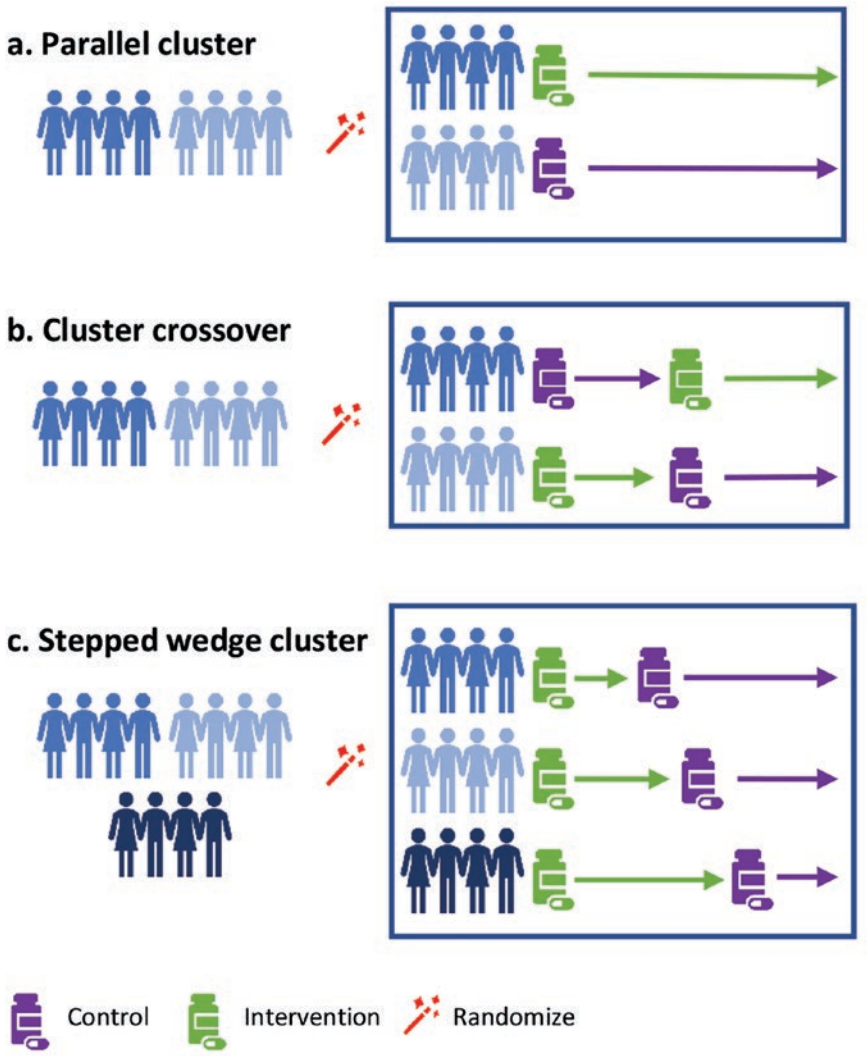

Fig. 28.2 Cluster randomized control trial designs structures: parallel (**a**), crossover (**b**), and stepped wedge (**c**)

28.3.2 Master Protocols: Umbrella, Basket, and Platform Trials

A master protocol refers to trials conducted as common substudies sharing methods and operations. The substudies often have common governance and scientific leadership. Master protocols can target single or multiple diseases and allow testing of one or several interventions [41, 42]. There are three types of master protocols: umbrella, basket, and platform trials. An umbrella trial (Fig. 28.3a) includes various

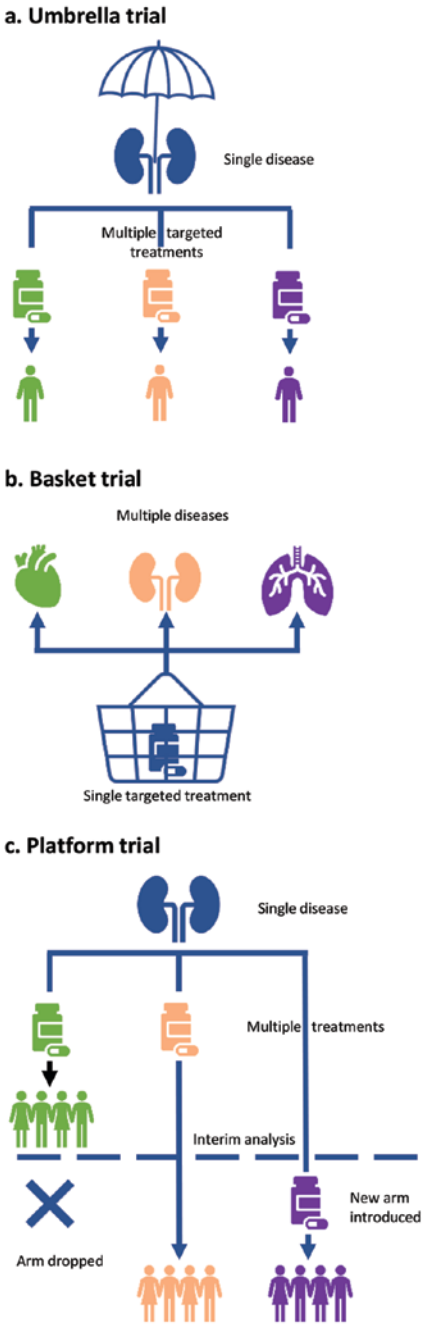

Fig. 28.3 Master trials design structures: umbrella (**a**), basket (**b**), and platform trials (**c**)

subpopulations within a single disease that is targeted by multiple interventions. A basket trial (Fig. 28.3b) defines a single intervention that is deployed among multiple diseases with a common biomarker. Each one of these subgroups are randomly allocated to either the control group or to the intervention arm. The platform trial (Fig. 28.3c), on the other hand, constitutes a continuous structure in which additional interventions can be introduced. Ineffective or harmful treatments are discontinued based on sequential analyses. This type of trial is also known as an adaptive platform trial. The platform trial shares a common set of features, such as the extensive use of sequential analyses, stopping rules, common control groups and dynamic allocation of individuals.

The use of a master protocol has several advantages, including optimization of resources due to the shared study governance, infrastructure, and methods [42]. The common screening infrastructure often allows subjects to be screened for alternative treatments. For example, in a traditional trial design, if individuals fail at the screening process, they may need to restart the full screening phase (e.g., consent, medical interview, blood or imaging samples) to be evaluated for a separate study. This frequently becomes a time-intensive procedure, reducing attractivity of clinical research and ultimately patient participation. A master protocol reduces this inefficiency by setting a common structure wherein patients can be screened for several interventions in parallel. Master protocols define a hierarchy of interventions a subject may receive, and investigators can assess eligibility criteria at the screening phase. Subjects are then enrolled in the substudy that is more likely to result in success. For example, subjects who have a specific biomarker may be randomized to certain target interventions, whereas biomarker-negative patients follow a different pathway. Over time, new groups based on biomarkers (or clusters, more generally) can be incorporated and additional interventions amended to the protocol, in a continuous flow that allows new treatments to be incorporated in the study.

In addition to a shared patient screening structure, master protocols enhance trial consistency by standardizing important definitions across operations, such as visit schedules, data management, randomization processes, outcome definitions, and quality of the oversight process. As such, a group of substudies within a master protocol are more homogeneous than trials performed individually. Importantly, this consistency may enhance comparative effectiveness of study drugs and optimize monitoring of safety across treatments. Finally, master protocols can implement a single governance structure, in which central ethical review boards, data monitoring, event adjudication, and steering committees are common to all sub studies. This unified governance model and optimized study team can drive the overall strategy across study operations, optimizing time, resources, expertise, and scientific leadership throughout.

Relatedly, an important limitation in establishing a successful platform clinical trial is the coordination between multiple, oftentimes heterogeneous stakeholders. Notably, key successful platform trials, such as the I-SPY 2 and the Lung-MAP studies [22, 43], are a result of a collaborative effort encompassing academic institutions, pharmaceutical industries, independent research organizations, diverse foundations, and public institutions. Platform trials can also have specific caveats that

challenge internal validity. Studies that employ common control groups may be affected by ecological biases if the trial arms are not parallel. Other confounders can additionally bias the results if a historical control group is selected. For instance, risk factors, diagnostic methods, and standard of care can vary over time, thus leading to bias if unbalanced between study arms. Also, platform trials are prone to type I error due to multiple comparisons at interim analyses. Despite these limitations, guidelines for platform designs have been published to ensure adequate design and reporting, particularly since they have become critical to a quick response to public health challenges, such as during the COVID-19 pandemic [44].

Master protocols have yet another pivotal role in tackling key challenges in clinical medicine: advancing the unmet need for individualized medicine. In master protocols, clinically relevant clusters can be identified according to individual markers. Individual safety and clinical response can be then predicted for specific drugs. Thus, subgroups with greater chance of net benefit are included in distinct sub studies. This is a major challenge to the use of these protocols in diseases in which clinical phenotyping by biomarkers is still an overdue goal, such as in chronic kidney disease [45]. Other than single-biomarker phenotyping, the employment of artificial intelligence methods can be used to identify subgroups of patients who may be optimally randomized in platform studies. This concept has been employed in critical care patients, such as in corticosteroids response in septic patients [46].

Master protocols have successfully been implemented in oncology, often in phase 1 and 2 RCTs [47, 48]. The I-SPY2 and the Lung-MAP trials are two key examples that contributed extensively to the discovery of innovative therapies in early stage breast cancer and advanced squamous non-small cell lung cancers, respectively [22, 43]. Both studies employ a platform that stratifies subjects following predefined biomarkers and then randomly allocated them to target interventions. Expanding the application of master protocols, the COVID-19 pandemic unleashed the potential of platform trials to effectively identify effective and safe interventions, particularly by drug repurposing [49]. The extent to which these applications will be implemented in broader clinical scenarios remains to be seen.

Although to the best of our knowledge master protocols have not yet been implemented in nephrology, the models governing the platform trials are transportable to key areas in kidney diseases. A single screening platform is highly efficient in the context of rare diseases, such as in glomerulonephritis. Both the umbrella and the basket approaches would suit trials in glomerular disorders. As an example, patients with focal segmental glomerulosclerosis could be randomized to several interventions based on clinicopathological findings in an umbrella trial [50]. Anti-complement therapies could be tested in the group of complement-mediated glomerulonephritis using a basket trial [51]. In both examples, common governance and infrastructure platforms could accelerate the rate of success in discovering new effective interventions. This could also mitigate the operational burden and costs to adequately diagnose and screen such patients to clinical trials, which could further increase diversity and democratization of clinical research across the globe.

28.3.3 Adaptive Trials

Adaptive trial designs allow for study protocol modifications while the trial ensues [52]. These adaptations must be prespecified to ensure statistical quality in the trial estimates [52]. In general, these studies define interim analyses that guide protocol modifications, such as estimates of conditional power and conditional group allocation [53].

Broadly, adaptive designs can be grouped into three categories: (1) treatment effect-independent, (2) treatment effect-dependent, and (3) other subtypes. In treatment effect-independent adaptive designs, protocol modifications are triggered by data ignoring treatment effects, i.e., outcome differences across study groups. An example of a treatment effect-independent adaptation includes predefined algorithms that ensure covariate balance in randomization by assessing the proportion of patients with specific prognostic markers in study arms. If a given prognostic factor is more prevalent in one study arm as randomization ensues, the algorithm will change allocation probabilities to achieve covariate balance. Another example of treatment effect-independent adaptation is increasing sample size given the observed rate of events in the study arms. This is different than re-estimation based on treatment effects, as discussed below. Generally, treatment effect-independent adaptations do not inflate type I error and are less likely to generate bias.

Treatment effect-dependent adaptive designs depend on observed treatment effects to prompt protocol modifications. In sample size re-estimation, observed treatment effects are used to re-estimate sample size. For example, an adaptive trial can determine, at its interim analyses, that the study would need to enroll more subjects than originally planned to meet adequate power. This dynamic enrolment can optimize trial operations by avoiding the common pitfall of reaching null conclusions due to underpower [53]. Traditionally, group sequential design RCTs were employed to decide early trial close out based on observed treatment effects. Some authors consider these studies adaptive, since these trials use interim analysis to guide study procedures (i.e., stopping rules). Group sequential designs and adaptive sample size re-estimation are alternative solutions for the same problem. Namely, specifying power often requires assumptions about treatment effects. While group sequential design starts with a large sample size and stops early if treatment effect is larger than anticipated, adaptive sample size re-estimation defines a smaller sample and increases it during the study. Theoretically, group sequential designs are more efficient [54], although in practice both the designs may be useful. Finally, another instance of treatment effect-dependent adaptation is response-adaptive randomization, in which subject allocation is conditioned on the probability of benefit or harm based on observed outcome data. For example, if the interim results suggest that the intervention may be beneficial, this modifies the allocation probabilities to offer more patients the potential effective intervention while the trial proceeds [53].

Several other adaptative trial designs also exist. In seamless phase 2–3 studies, both operations and analyses can be merged at the transition between the two phases. As such, key decisions, such as dose range selections, can be continuously

incorporated as interim analyses inform study procedures [52]. Another adaptive scheme is the prespecific transition from noninferiority to superiority endpoints. This strategy has been employed in trials in diabetic populations. In the United States, new antiglycemic agents must show noninferiority for major adverse cardiovascular events as compared to placebo at a 30% margin. Several trialists designed RCTs to test antiglycemic agents defining primary outcomes based on this regulatory requirement. Some of these studies were able to not only show noninferiority, but also claimed superiority. Indeed, although traditional RCTs can be powered to show superiority even in a noninferiority design, adaptive studies can optimize the transition from noninferiority to superiority. Interim results can inform sample size re-estimation to meet power under the new superiority endpoint, allowing a more flexible study design. In fact, current seamless phase 2–3 studies of antidiabetic drugs are following this framework [55–57].

Adaptive studies have several limitations. Protocol adaptations can come at a cost of increasing type I error due to multiple comparisons. Hence, adaptive trials employ specific statistical methodology to minimize false-positive claims. This is particularly the case for treatment effect-dependent adaptive designs, such as in conditional power estimation. In these designs, intervention effects are estimated to inform protocol decisions [58]. Other than type I error inflation, internal validity of adaptive trials may be harmed by other key potential issues. RCTs using response-adaptive randomization may be limited due to systematic errors introduced in the process. For instance, imbalance between groups may become an issue if the proportion of patients in one group increases as compared to the other due to potential benefit (or harm). This can lead to biased effect estimates, as the randomization assumption of between-group covariate balance may not hold. Moreover, this process introduces a correlation between treatment response and allocation, a condition that often requires more complex statistical modeling strategies [58]. Additionally, selection bias may result if subjects or investigators become aware of the response-adaptive randomization process and decide to enroll at a later recruitment phase to maximize the chance of individual benefit. Finally, although often claimed that novel adaptive designs are generally more efficient than traditional trials, group-sequential analysis techniques are frequently as efficient compared to adaptive sample-size estimation [54].

Adaptive designs have become key drivers of clinical trial innovations that fundamentally contributed to unleash the potential of large collaborative networks in dynamic study frameworks. Several successfully repurposed drugs and treatment strategies to manage SARS-CoV2 infections during the COVID-19 pandemic were first robustly assessed by adaptive platforms. These studies combined adaptive principles with platform designs and have notably contributed to advance evidence-based medicine despite the challenges of conducting RCTs through the pandemic [59].

In nephrology, the number of adaptive trials has increased over the years, although its proportion as compared to nonadaptive trials has decreased [60]. Most of the adaptive trials with kidney outcomes have focused on critically ill populations at risk for AKI, in which a broad range of preventive or therapeutic interventions were tested [60]. These studies mostly included group sequential analysis with stopping rules, yet more recent and innovative techniques such as sample size

re-estimation and seamless designs were implemented in some trials [61–65]. One such example was the STOP-AKI trial, using a dose-finding phase IIa/IIb design to evaluate the detoxifying enzyme human recombinant alkaline phosphatase in sepsis-associated AKI [24]. In most adaptive studies, adaptive techniques affected trial conduct, either at readjusting sample sizes or at recommending early termination [60]. In contexts where challenging factors affect recruitment, such as in critically ill populations, these novel adaptive features may play an important role in optimizing trial operations. Also, particularly in AKI, a broad range of interventions with varying levels of pre-study probabilities of success may be optimally tested in feasible trials that minimize sample size, while also allowing seamless phase transitions and dose escalation.

Other RCTs using innovative adaptive designs are underway in nephrology. For example, a RCT using dose-escalation design is underway among deceased donor kidney transplant patients [25]. In this study, Mironocept, a complement inhibitor that can be administered ex vivo to the donor kidney, is being evaluated to prevent delayed graft function compared to standard cold perfusion fluids [25]. To obtain curve-response and find minimum effective dose, this study will use dose-escalation adaptive designs, which tend to perform better than traditional methods [66]. For additional information, an innovative systematic review summarizing adaptive trials including CKD populations or kidney outcomes has been published [60].

28.3.4 N-of-1 Trials

N-of-1 ($n = 1$) trials are single-patient randomized crossover trials; that is, one patient has multiple crossovers between active treatment and control periods, with outcomes measured repeatedly over time to determine treatment response (Fig. 28.4). Trial techniques such as blinding, allocation concealment, and randomization are performed to minimize bias even though the conclusions drawn about response to treatment are specific to the individual patient. N-of-1 trials are ideal for conditions that are stable and chronic, where the treatment effects are reversible, and the treatment has a rapid onset and offset of action [67]. Moreover, N-of-1 trials may require fewer participants for the same level of statistical power as parallel and crossover RCTs [68].

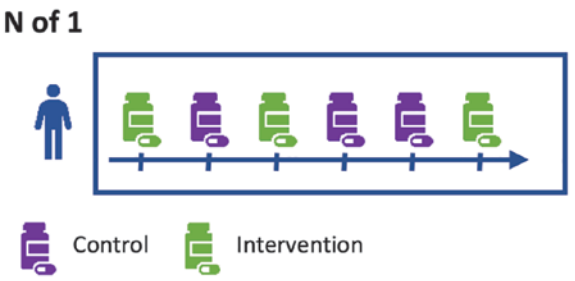

Fig. 28.4 N-of-1 trial

N-of-1 trials were initially developed in psychology but have gained momentum in multiple fields across medicine. Herrett et al. examined the side effects from taking statins in 151 patients who had previously discontinued statins because of side effects [26]. Using a double-blind N-of-1 trial they compared symptoms induced by a statin or placebo taken for 1 month in a random sequence and found that there was no difference in muscle symptoms score between statin and placebo periods. After completing the trial, two thirds of participants chose to restart long-term treatment with statins. This illustrates that N-of-1 trials can be used to assess drug effects at the group level and guide individual treatment.

This methodology has also been used in pediatric nephrology to study antihypertensive medication in children with primary hypertension [27]. Forty-two children were trialed on three drugs (amlodipine, hydrochlorothiazide, and lisinopril), rotating between the treatments in 2-week periods until the preferred therapy was identified based on blood pressure reduction and absence of side effects. Although no single drug emerged as the preferred therapy, this study design demonstrated the importance of personalized evidence-based treatment decisions in a special population group, where traditional RCTs may not be practical.

N-of-1 trials can also be used to address many unanswered questions for patients on dialysis as it is a chronic condition where the patients have repeated treatment over time. Personalized medical care could focus on treatments used in routine dialysis care which remain poorly supported by current evidence such as in anemia management, dialysate selection, and phosphate reduction strategies [69]. In fact, Rostoker et al. were able to pilot the N-of-1 trial design to assess the effects of routine colloid infusion on intradialytic hypotension in patients unresponsive to preventative measures [70].

28.4 Digitization of Trial Conduct

Trial design and conduct are closely linked. Advancement in digital technologies has created the potential to streamline trial operationalization, leveraging routinely collected health data (RCD) such as those found in clinical registries and electronic health record (EHR) systems to facilitate the conduct of clinical trials efficiently and effectively (Table 28.2). RCD can replace or supplement trial operationalization processes including identifying and recruiting eligible patients, collecting baseline variables, and outcome assessments. A randomization component can also be incorporated in some situations.

Nephrology is well suited to conduct such research because (1) kidney function is routinely collected as part of clinical care (e.g., creatinine, eGFR, urine protein: creatinine ratio, urine microalbumin: creatinine ratio); (2) kidney diseases (e.g., AKI, CKD, glomerular diseases) can be detected using valid and reproducible computer algorithms; (3) structured data such as comorbidities, medications, vital signs, procedure codes, and laboratory values can be used to collect baseline variables and perform outcome assessments; and, (4) patients with kidney disease are high users of both inpatient and outpatient services.

Table 28.2 Examples of routinely collected data: registry, EMR, and EHR

Example	Definition	Types of data collected	Potential application in a clinical trial
Registry	"An organized system that uses observational study methods to collect uniform data (clinical and other) to evaluate specified outcomes for a population defined by a particular disease, condition, or exposure, and that serves one or more predetermined scientific, clinical or policy purposes." clinical registries include disease-, health service- or product-specific registries [71]	Structured data including demographics, comorbidities, treatments, and clinical outcomes	Cohort identification via disease-specific registries Rapid consecutive enrolment Randomization built within registry portals Collection of baseline variables Outcome assessment Long-term follow-up
Electronic medical record (EMR)	EMRs are a digital version of a patient's paper medical record [72]. They contain information which is patient-specific and includes medical and clinical data which is collected as part of the medical encounter. The data is stored in an individual practice, clinic, or hospital; it does not travel outside the medical practice	Structured data including demographics, diagnosis codes, procedure codes, laboratory values, vital signs, and medication prescriptions Unstructured data including progress notes, pathology reports and radiology reports	Cohort identification via computable phenotypes Randomization built within the EHR e-Consent Collection of baseline variables Outcome assessment Long-term follow-up
Electronic health record (EHR)	EHRs are like an EMR, but the patient's health related records are linked electronically in a network of practices, clinics and/or hospitals [72]		

Nesting clinical trials with RCD from clinical registries and EHR has several advantages compared to the traditional infrastructure of RCTs. Cohort identification is an obvious advantage for disease-specific registries as they can screen very large numbers of patients for eligibility resulting in rapid consecutive enrolment substantially reducing the cost and time required in recruitment [73]. The use of clinical terminology tools such as the Systematized Nomenclature of Medicine-Clinical Terms (SNOMED-CT) and the International Classification of Disease (ICD) codes within EHR systems can also allow for rule-based computational algorithms to rapidly identify and pre-screen individuals who have specific (e.g., type 2 diabetes) as well as complex medical conditions (e.g., chronic kidney disease, glomerular diseases) [74–76]. Algorithms that combine specific diagnosis codes with one or more other structured data fields tend to improve precision more than a single code search [77]. Recruitment in EHR embedded clinical trials is also improved when physicians or research staff are alerted about a potential eligible patient at point of care or a short time thereafter [78].

The use of established infrastructures for data collection rather than building new processes specifically for the trial significantly reduces trial set up time and trial costs

[79] and therefore can potentially provide faster results. Costs are also minimized due to reduced follow-up visits and training of site staff and research coordinators as the information is often routinely collected within the databases. Both clinical registries and EHR can be used to provide long-term follow-up outcomes of participants without actively following them, thereby reducing the number of patients lost to follow-up. RCD can even be used to monitor those who are not enrolled in the study allowing full capture of the reference population generating evidence that is more representative of "real world" practice [80]. Data can also be collected using supplementary datasets in countries with unique patient identification numbers or via deterministic or probabilistic record data linkage if this is not possible.

The use of RCD in clinical trials should be used judiciously as the data collected is largely preexisting and was collected for other purposes than research. Both clinical registries and EHR may have missing data, which can introduce selection and information bias. A review of kidney failure registries globally highlighted the variability in population groups enrolled, the type and quality of data variables collected, and differences in semantics to disease definitions [81, 82]. This can be a particular consideration for inter-registry-based clinical trials, and subsequent trials will benefit from harmonization of definitions and analytic approaches. Further, numerous studies have highlighted issues with EHR data quality including errors in accuracy of billing codes [83] and fragmented data [84], meaning that central assessment and validation of data should be prioritized, and steps taken to minimize false-positive and/or false-negative findings. In order to address these concerns and provide high-quality evidence, definite clinical end points such as mortality, hospitalization for certain specific conditions, or laboratory test measurements should be used as they are less susceptible to bias. Registries and EHR may also not capture all variables specific to a study question (e.g., safety reporting, adverse events). In such cases, approaches can include alteration of the trial question, the embedding of trial-specific variables into RCD where the additional variables are consistent with the RCD purpose, or hybrid models where trial information is collected both from the RCD source and from trial-specific data collection sources.

28.4.1 Registry-Based Randomized Controlled Trials

Registries play an important role in health infrastructure planning, development of quality improvement programs, and can generate causal hypotheses based on the systematic collection of associations with outcomes [81]. The infrastructure of clinical registries, particularly the systematic collection of information, is being increasingly utilized for clinical trials over the past decade in activities such as cohort identification, data collection, and follow-up [80]. As registries move from static, paper-based formats to digital formats, the potential for modifications or additions to the standard data collection has created opportunities for the registry infrastructure to support randomized studies. This pragmatic approach is referred to as a registry-based randomized controlled trial.

Registry-based trials have been coined "the next disruptive technology in clinical research" [85]. Trial questions best suited for conduct within established registries

are those that occur within the flow of clinical practice, where clinical data and outcome measures are accurately documented, and there is minimal additional information required beyond those already collected. An illustration of a registry-based RCT is the Thrombus Aspiration during ST Segment Elevation Myocardial Infarction (TASTE) trial [86]. This trial randomized participants at the point of registration into the Swedish Coronary Angiography and angioplasty registry (SCAAR) which was at the time of coronary angiogram. This streamlined process resulted in swift recruitment of over 7200 patients within 2 years and 9 months. End points were obtained via national registries. The incremental cost for trial execution was also extremely low, approximately US $50 per patient, which is estimated to be <10% of an equivalent conventional RCT [87].

In nephrology there are many registries that have been a rich source of "real world" data for observational studies. Globally there are 79 kidney failure registries including the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA), the Swedish Renal Registry, the US Renal Data System (USRDS), and the UK Renal Registry (UKRR) [81]. There are also examples of AKI (n = 12) and non-dialysis, all-cause CKD (n = 19) registries [88]. Registries have also been established for rare kidney diseases. The Nephrotic Syndrome Study Network (NEPTUNE) registry [89] and the British Columbia Glomerulonephritis Network and Registry (BCGN Network) [90] recruit participants at the time of biopsy, while the Cure Glomerulopathy Network enrolls patients within 5 years of their first diagnostic kidney biopsy and monitors their long-term outcome [91]. In the UK, the Renal Association's National Register of Rare Kidney Diseases (RaDaR) has over 26,000 enrolments and, relatively uniquely, involves patient rather than clinician-initiated registration.

Several studies are now using routine data captured within kidney failure registries for baseline assessment and trial follow-up (Table 28.3). Examples include the UK SIMPLIFIED trial, examining whether high-dose cholecalciferol (60,000 IU fortnightly) reduces all-cause mortality in adult dialysis patients [92]. SIMPLIFIED does not require any dedicated trial visits and assesses all outcomes through the UKRR and other national registries. The major outcomes with personalized dialysate TEMPerature trial is another example which links administrative healthcare databases to capture patient characteristics and the primary composite outcome of cardiovascular-related death or cardiovascular-related hospitalization [12]. SHARP-ER uses a hybrid model wherein the conventional trial assesses the long-term outcomes of cholesterol-lowering therapy in CKD patients by linkage with dialysis registries [93]. RESOLVE which compares the effectiveness of two default dialysate sodium concentrations was the first registry-based quality assurance study within the ANZDATA registry to have developed capacity to capture baseline and outcome data [94]. Since then other randomized studies that have leveraged the ANZDATA infrastructure include the saline vs. Plasma-Lyte fluid in the peri-transplant period (BEST-Fluids) [95], and an online symptom monitoring method of patient-reported outcome measures with automated feedback to the treating clinicians (SWIFT) [96]. BEST-Fluids and SWIFT have also integrated additional data collection modules for variables not routinely captured in the registry. These studies illustrate the benefits of registry-based clinical trials including the ability to study large cohorts of patients with kidney failure in real world conditions with longitudinal follow-up.

Table 28.3 Examples of registry embedded RCTs in nephrology

Study name, reference	Setting/population	Trial characteristics	Intervention	Primary endpoint	Registry/other data sources	Registry function
SIMPLIFIED [92]	Adult dialysis patients, $n = 4200$	Pragmatic, randomized, open-label, multicenter	Cholecalciferol (60,000 IU fortnightly) vs. standard care	All-cause mortality	UKRR National Deaths Register Administrative databases	Cohort identification Baseline data collection Outcome assessment
RESOLVE [13]	Adult hemodialysis patients, 414 sites (worldwide)	Pragmatic, cluster randomized, open label, multicenter, multinational	Default dialysate sodium concentration 140 vs. 137 mmol/L	Major cardiovascular events	Multiple, including ANZDATA	Cohort identification Baseline data collection Outcome assessment
BEST-fluids [95]	Deceased donor kidney transplant recipients, $n = 800$	Pragmatic, randomized, multicenter, double blind	Plasma-Lyte 148 or 0.9% saline intravenous fluids perioperatively	Delayed graft function (need dialysis in 7 days of transplant)	ANZDATA ANZ organ donation registry Case report forms Administrative databases	Cohort identification Baseline data collection Outcome assessment
SWIFT [96]	Adult hemodialysis patients, $n = 2422$	Pragmatic, cluster randomized, open label	Symptom monitoring with feedback to treating nurses and nephrologist vs. standard of care	Response rate, completeness, and time taken to complete survey	ANZDATA	Cohort identification Baseline data collection Outcome assessment Trial-specific intervention added (i.e., PROMs added into registry) Registry coordinates intervention (i.e., feedback email to nephrologist and patient)

(continued)

Table 28.3 (continued)

Study name, reference	Setting/population	Trial characteristics	Intervention	Primary endpoint	Registry/other data sources	Registry function
PHOSPHATE [97]	Adult hemodialysis patients, $n = 3600$	Pragmatic, randomized, open-label, parallel-group, multicenter, multinational	Intensive serum phosphate target ≤1.50 nmol/L vs. liberal target (2.0–2.5 mmol/L)	Cardiovascular death or non-fatal major cardiovascular event	ANZDATA UKRR Case report forms	Cohort identification Baseline data collection Outcome assessment
MyTEMP [12]	Adult hemodialysis patients, $n = 15{,}000$	Pragmatic, cluster randomized, parallel-group, open-label, multicenter	Personalized temperature-reduced dialysate vs. standard dialysate temperature of 36.5 °C	Cardiovascular death or major cardiovascular-related hospitalization	Administrative databases	Cohort identification Baseline data collection Outcome assessment

ANZDATA Australian and New Zealand Dialysis and Transplant Registry, *BEST fluids* Better Evidence for Selecting Transplant Fluids, *MyTEMP* Major Outcomes with Personalized Dialysate TEMPerature, *PHOSPHATE* Pragmatic randomised trial of High or Standard PHosphAte Targets in End-stage kidney disease, *PROMs* patient reported outcome measures, *RCT* randomized control trial, *RESOLVE* randomised evaluation of sodium dialysate levels on vascular events, *SIMPLIFIED* Survival IMProvement with choLecalciferol in patIENts on Dialysis, *SWIFT* Symptom monitoring With Feedback TRIAL, *UKRR* UK Renal Registry

28.4.2 Electronic Health Record (EHR) Embedded Randomized Controlled Trials

EHR real-world clinical data offers longitudinal data on health and treatment outcomes for thousands of patients and can be used retrospectively, cross sectionally, and prospectively. EHR offers many new opportunities in facilitating patient identification, streamlining data collection, and even conducting large-scale pragmatic clinical trials. EHR-embedded randomized trials are well suited to rule-based algorithm assessments and interventions, and where data collection is focused and limited to critical variables that are consistently collected in routine clinical care.

The regular and frequent scheduling of in-center dialysis therapy means EHR-nested trials may be particularly suitable for trials of interventions for people receiving dialysis therapy (Table 28.4). EHR was utilized in nearly all aspects of the Time to Reduce Mortality in ESRD (TiME) trial [11]. TiME was a large pragmatic, cluster RCT that attempted to determine if a longer hemodialysis session reduced mortality and hospitalization rates. It leveraged the ability of the EHR to capture standardized dialysis-related information and outcomes such as death, hospitalization rate, predialysis blood pressure, post-dialysis hypotension, interdialytic weight gain, fluid removal rate, missed dialysis sessions, and change in quality of life. The study demonstrated that trials of dialysis interventions could be embedded into routine clinical care with over 7000 patients from 266 dialysis units enrolled in less than 3 years leading to the largest completed RCT in maintenance hemodialysis to date.

HiLo is another pragmatic, cluster randomized trial embedded into real-world practice using EHR data evaluating the effects of a "hi" phosphate target of ≥6.5 mg/dL compared to the usual standard of care, which is a targeted "lo" serum phosphate level (<5.5 mg/dL) [15]. It is estimated that HiLo will cost 5–10% of the cost of a traditional RCT as there are no onsite study staff, eConsent is obtained, methods to achieve phosphate targets are at the discretion of the clinical team, and dedicated study visits are not required as data collection and outcome assessment are extracted via the EHR [15]. In its use of RCD data, HiLo is similar to the registry embedded PHOSPHATE trial [97].

In non-dialysis CKD patients, trials are utilizing clinical diagnosis decision support systems (CDSS) within the primary care EHR system. The Kidney-Coordinated HeAlth Management Partnership (Kidney-CHAMP) is an example wherein the EHR is used to identify eligible participants (CKD patients without nephrology involvement), facilitate the intervention (nephrology recommendation based on EHR review), and assess outcomes (adoption of recommended care) [98].

Acute Kidney Injury (AKI) is a nephrological condition that may be particularly suited to study using computer-hosted algorithms. The KDIGO AKI definition is based on quantifiable changes in serum creatinine and/or urine output, parameters that are generally captured in the EHR [99]. The use of a real-time electronic alert system for worsening AKI in an ICU context increased the timeliness and number of therapeutic interventions (fluid therapy, diuretics, vasopressors) within 60 min of the alert, with improvement in short-term renal outcomes [100]. A multicenter randomized controlled trial of 6030 patients showed that a CDSS for AKI did not

Table 28.4 Examples of EHR embedded RCTs in nephrology

Study name, reference	Setting/population	Trial characteristics	Intervention	Primary endpoint	EHR/other data sources	EHR function
TiME [11]	Adult hemodialysis patients, *n* = 7035	Pragmatic, cluster randomized, parallel group, multicenter	Longer dialysis session (≥4.25 h) vs. standard of care	All-cause mortality	Institutional EHR	Cohort identification Baseline data collection Outcome assessment
HiLo [15]	Adult hemodialysis patients, *n* = 4400	Pragmatic, open-label, multicenter, cluster randomized	Less stringent control of serum phosphate to >6.5 mg/dL vs. standard of care target serum phosphate <5.5 mg/dL	All-cause mortality and all-cause hospitalization	Institutional EHRs	Cohort identification e-consent Baseline data collection Outcome assessment
Kidney-CHAMP [98]	Primary care patients with CKD in the University of Pittsburgh Medical Centre, *n* = 1650	Pragmatic, cluster randomized	Multifaceted EHR-based population health management intervention targeting evidence-based CKD care	Decline in eGFR or ESKD	Institutional EHR	Cohort identification Part of intervention delivery Baseline data collection Outcome assessment
ICD-Pieces [105]	Patients with multiple comorbid conditions (CKD, diabetes, and hypertension) in primary care clinics, *n* = 5925	Pragmatic, prospective, stratified cluster randomized	Collaborative model of primary care and subspeciality care	All-cause hospitalization	Institutional EHRs	Cohort identification Part of intervention delivery Baseline data collection Outcome assessment
SMART [106]	Five ICUs at Vanderbilt University Medical Centre, *n* = 15,802	Pragmatic, cluster randomized, multiple-crossover trial	Received 0.9% saline or balanced crystalloids	Major adverse kidney events by hospital discharge or day 30	Institutional EHR Case report forms	Cohort identification Baseline data collection Outcome assessment

Wilson et al. [101]	Inpatient units of 6 hospitals, $n = 6030$	Multicenter, parallel, randomized, double blind	Automated, electronic pop-up AKI alert with link to AKI order set and option to add AKI to problem list	Composite end point of AKI progression/ inpatient death/ inpatient dialysis within 14 days of randomization	Institutional EHRs	Cohort identification Recruitment Randomization Part of intervention delivery Baseline data collection Outcome assessment
Selby et al. [17]	Hospitalized patients in 5 UK hospitals, $n = 24{,}059$ AKI episodes	Multicenter, pragmatic, stepped wedge cluster randomized	Multifaceted intervention (AKI e-alerts, AKI care bundle, education program)	30-day mortality	Institutional EHR Linkage to UKRR	Cohort identification Part of intervention delivery Baseline data collection Outcome assessment

AKI acute kidney injury, *CKD* chronic kidney disease, *eGFR* estimated glomerular filtration rate, *EHR* electronic health record, *ESKD* end stage kidney disease), *HiLo* pragmatic trial of higher vs. lower serum phosphate targets in patients undergoing haemodialysis, *ICD-Pieces* Improving Chronic Disease Management with Parkland Intelligent e-Coordination and Evaluation System, *ICU* intensive care unit, *Kidney-CHAMP* Kidney Coordinated Health Management Partnership, *REDCap* Research Electronic Data Capture, *SMART* Isotonic Solutions with Major Adverse Renal Events Trial, *TiME* time to reduce mortality in ESKD, *UKRR* UK Renal Registry

improve the clinical composite of AKI progression, dialysis, or death despite producing more fluid prescriptions, repeated creatinine measurements, and more documentation of an AKI [101]. A third trial involving 24,000 AKI episodes found a multifaceted intervention with an AKI alert, AKI care bundle, and an education program did not reduce 30-day mortality but did reduce hospital stay [17]. The successful completion of these studies illustrate the suitability of EHR data for AKI studies and the feasibility of using EHR to evaluate and optimize health service practices.

Lastly, the role of EHR holds great promise for the study of rare diseases, including primary glomerular diseases, holds great promise. Rule-based algorithms using structured EHR data to identify different types of glomerular diseases have been developed and validated [102–104]. The ability to rapidly identify patients with glomerular diseases using diagnostic codes (either ICD-9 or SNOMED CT) and procedure codes (for kidney biopsies) which are routinely collected at point of care can therefore be utilized for cohort identification and offers opportunities to conduct large-scale clinical trials in an area of nephrology where there is a dearth of clinical evidence.

28.5 Consumer Engagement in Clinical Trials

Consumers are increasingly being involved in all stages of the research journey, joining as active collaborators on research teams (Fig. 28.5), rather than being mere trial participants. Consumer engagement improves the quality of research through

Fig. 28.5 Consumer involvement in the research journey

multiple mechanisms, including research priority setting, assessment of the study design and procedures (such as questionnaires, interview schedules, participant recruitment, and retention) from a patient-centric perspective, consumer focused analysis, and dissemination and implementation of findings [107].

Consumer involvement in nephrology research has been limited and variable until recently. Patient, researcher, and system factors have contributed to this heterogeneity including poor research infrastructure, and lack of awareness of formal mechanisms for researcher and patient involvement [108]. Also, lack of understanding of patient priorities and differences in culture and healthcare delivery across the globe have important impacts on how patient-centric a clinical trial is [108]. Increase in patient involvement across the phases of trial design, conduct, and dissemination may help improve trial performance and ultimately impact outcomes that are relevant to patients with kidney disease.

Changes in the prioritization of peak research funding bodies worldwide to involve patients in the design and conduct of clinical trials have resulted in a few exemplary examples of consumer involvement in nephrological research. CanSOLVE CKD (www.cansolveckd.ca), part of Canada's Strategy for Patient Orientated Research, engages patient partners and caregivers in priority setting, protocol review, dissemination, and governance. Projects span across basic science, clinical and population health research covering areas such as earlier diagnosis, better treatments, and innovative kidney care. The Patient-Centred Outcomes Research institute (PCORI, www.pcori.org) has been involved in the funding of patient centric research and in the development of patient engagement tools to support research [109], and the Kidney Health Initiative (KHI, https://khi.asn-online.org/) have likewise involved patients in specific activities related to research activities.

The Standardized Outcomes in Nephrology (SONG) is also a global initiative (songinitiative.org/) that has developed a set of core outcomes and outcome measures across a spectrum of kidney diseases (including peritoneal dialysis, hemodialysis, transplantation, children and adolescent, polycystic kidney disease, chronic kidney disease) in consultation with consumers, clinicians, and researchers [110–115]. This initiative has highlighted the mismatch in priorities among patients and healthcare professionals and developed assessment tools and recommendation for a list of core outcomes to be consistently included in clinical trials across the spectrum of kidney disease. These initiatives will be pivotal in ensuring that trials address meaningful outcomes to patients and those involved in their care.

28.6 Future of Nephrology Trials

The landscape for clinical trials has changed significantly over the past decade, including in the area of kidney diseases. The COVID-19 pandemic has highlighted the importance of well-designed and efficient clinical trials but also catalyzed the need to embrace technology, eliminate unnecessary paperwork, ensure efficient trial

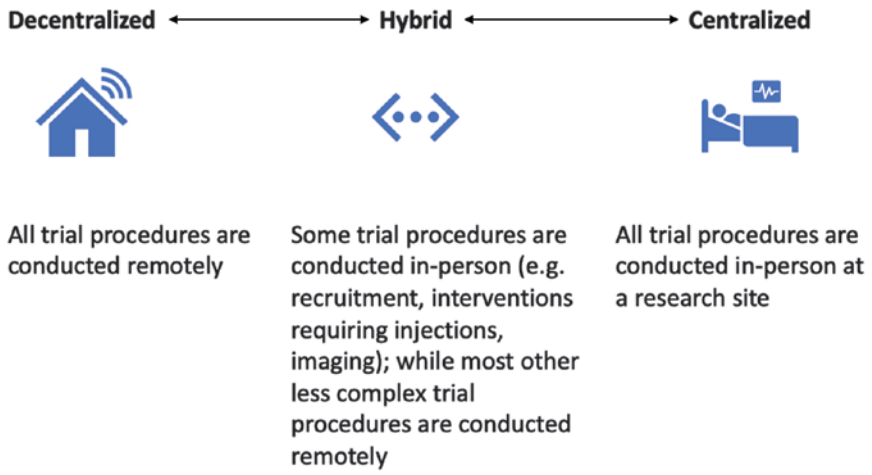

Fig. 28.6 Spectrum of clinical trials in the future

set up, and involve patients in trial design and conduct. Consideration to alternative trial design and conduct needs to be promoted and fostered to ensure that in the future people living with kidney disease have better treatment options than those that exist today.

In the near future, virtual or "decentralized" trials in which many (if not all) trial activities are conducted remotely is a very likely possibility (Fig. 28.6). Patient recruitment can occur via clinical registries or social media; informed consent via electronic means; baseline assessment and follow-up variables can be collected via telehealth, EHR extraction, and personalized digital health devices; laboratory tests can be performed locally or by home pathology services; interventions such as oral medications can be posted to patients' homes; and outcomes can be collected remotely with data linkage. The ability to answer "real world" questions by pragmatic decentralized trials, powered by robust methods, can pave the way for new and meaningful interventions for kidney diseases. Patient engagement is also a key component to foster more meaningful achievements. Welcoming innovations in clinical research will benefit patients, healthcare professionals, scientists, and stakeholders alike.

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Chapter 29
Consumer Involvement in Research and Decision-Making in Nephrology

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29.1 Introduction: Consumer Involvement in Research

Nothing about us without us [1]

The statement 'nothing about us without us' defines a movement that has empowered consumers (patients and caregivers) to be heard and included in health care, policy and research [1]. This statement was first used in healthcare in the 1990s by patient advocacy groups, particularly in the field of disability and the HIV community, that catalysed the shift from traditional roles and relationships of doctors/researchers as the experts and patients as passive participants towards inclusion and partnerships of clinicians, researchers and consumers in the research cycle from priority setting through to dissemination and implementation [2, 3].

Consumer involvement in nephrology research is an essential component to ensuring that research is relevant to the very patients it is aimed to assist. This chapter provides a summary of what consumer involvement is, what is currently

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happening in the nephrology research space and the impact of this involvement on shared decision making in clinical practice. This chapter includes a definition of consumer involvement, examples of involvement across the research cycle and summaries from consumers of their experiences being involvement in research and the impacts of this involvement. Consumer involvement in all types of research encourages innovation in design of studies and improves translation of research into improved outcomes for consumers.

29.1.1 Definitions

The terminology used to define involvement is varied, often resulting in some terms being used interchangeably but meaning different things. For example, in the United Kingdom and Australia, the term 'involvement' is used to describe the broad inclusion of consumers in research activities as more than research participants, while in Canada and the United States, the term 'engagement' may be used to describe the same concept. For this book chapter, we will follow INVOLVE, the peak body in the United Kingdom and a global leader in support of active patient and public involvement in health and social care research. According to INVOLVE, consumer involvement is defined as 'research *being carried out "with" or "by" members of the public rather than "to", "about" or "for" them*' [4] . The key terms related to consumer involvement are defined in Box 29.1.

Box 29.1: Definitions

Patient: refers to a patient with lived experience of chronic kidney disease

Caregiver: refers to an informal caregiver or family member of a patient with lived experience of chronic kidney disease (other terms may include carer, care-partner)

Consumer: refers to a patient, caregiver, family member or community member with lived experience of chronic kidney disease

Involvement: refers to sustained and meaningful contributions to the research process as more than a research subject or participant

Engagement: refers to the establishment of a relationship between patients and researchers and is the first step of involvement.

Participation: refers to consumers involved in research as subjects in trials or studies with no involvement or decision-making power in the planning, execution or translation of the study.

29.1.2 Value of Consumer Involvement

Involving consumers helps to reduce 'research waste' by improving the relevance and quality of research. Consumer participation leads to an alignment of research priorities, better patient recruitment and retention, unique insights in data analysis

and broader dissemination and translation into policy and practice [5–10]. Consumers have also reported personal benefits from being involved, including increased knowledge of their disease and the research process, greater access to information regarding their disease, developing social relationships with other consumers and health professionals outside of a clinical context and an opportunity to 'give back' to the medical community [7, 11–13].

The involvement of consumers in research is advocated for, and in some organisations, mandated at an international, national and local level. Globally, the World Health Organisation (WHO) states that 'the people have the right and duty to participate individually and collectively in the planning and implementation of their health care', including research [14]. The NIHR's INVOLVE in the United Kingdom was established in 1996 to support active public involvement across all stages of research and has developed resources to assist both researchers and consumers in doing this [4]. The Patient-Centered Outcomes Research Institute (PCORI) in the United States drives research guided by consumers to ensure they have access to relevant evidence to inform decision-making [15]. Examples in low- and middle-income countries include Chile, Columbia and Ecuador where there has been Public and Patient Involvement (PPI) in mental health service research [1, 16].

Many international academic journals require researchers to report if and how patients were involved in the research [17]. *The British Medical Journal* stipulates the inclusion of a statement in the methods section under the subheading 'patient involvement', outlining how consumers were involved in the selection of the research question and outcome measures, the design of the study, recruitment, conduct and how the results will be disseminated to study participants [17]. Consumers are being involved in academic journals in many other ways including roles as peer reviewers, writers of editorials, consumer/patient editors and authors [18–21]. *The Clinical Journal of the American Society of Nephrology (CJASN)* have appointed a Patient Voices Editor to their staff and a Consumer Editor is a part of the Cochrane Kidney and Transplant Editorial Board. It is important that all involvement in academic journals is not 'tokenistic' and those who have been involved receive training and feedback as to the impact of their contributions [21].

29.2 Consumer Involvement in Research in Nephrology

> *We do not want to be mute spectators of the research that informs our health and care. We want to be actively and equally involved in finding solutions, to address kidney disease as part of a collaborative kidney community* [19]

Consumer involvement in research in chronic kidney disease (CKD) is needed to align research efforts with the consumers' priorities and thus improve decision-making, satisfaction and clinical and quality of life outcomes [1, 22, 23]. There has been a recent shift within government, research funding bodies and professional organisations to systematically involve patients with CKD in research that is beyond tokenistic participation. The Kidney Health Initiative (KHI), a partnership between The American Society of Nephrology (ASN) and the United States Food and Drug

Administration (FDA), has established a Patient and Family Partnership Council, supported by several patient organisations, to give consumers a voice in CKD research in the United States (KHI Website). Can-SOLVE CKD (a Canadian patient-oriented kidney research network) in partnership with the Strategy for Patient Oriented Research (SPOR) at inception involved 75 patient partners across 18 research projects in Canada [24]. The Standardised Outcomes in Nephrology (SONG) Initiative has involved thousands of consumers and health professionals globally to establish consumer driven core outcome sets to inform clinical trials in kidney disease [25, 26].

The International Society of Nephrology's Advancing Clinical Trials (ISN-ACT) initiative Patient Engagement working group recently conducted a survey with 177 clinician members to understand the nature of consumer involvement in kidney trials globally and found an "absence of formal mechanisms" for involvement and did not explore the nature of involvement including research stage, activities, or consumer roles [27]. Despite these initiatives, there is scant evidence to inform or support best practice for involving consumers with CKD in research, particularly from the consumers perspective. Also, we must not lose sight of the need to include Indigenous voices and cultural diversity in the consumers, with a focus on co-design and implementation of projects with these communities and Peoples [24, 28, 29]. The International Society of Nephrology "aims to develop frameworks and tools that promote high-quality, patient-orientated clinical trials in nephrology across the globe" and to facilitate best practise, particularly in relation to clinical trials across all regions [27]. CanSOLVE CKD network based in Canada has created an Indigenous Peoples Engagement and Research Council (IPERC) which has included Indigenous ways of knowing and learning key infrastructures and has targeted specific programs of work.

There are examples of consumer led research within nephrology, noticeably in qualitative research, with topics covered including telehealth in transplant recipients, patient navigator workshops, rural and remote patient access, ability to work with CKD and transplant recipients' perspective on suspension of transplantation programs due to COVID-19 [30–33].

29.3 Consumer Involvement in Nephrology Across the Research Cycle

Reviews of patient involvement in nephrology research demonstrated that involvement is usually in relation to priority setting, study design, reviewing research and recruitment [6, 34, 35]. Below we will address each phase of the research cycle and provide examples within kidney research of how this is being implemented.

29.3.1 Priority Setting

It has been estimated that 80% of the clinical research in CKD is not aligned with the top 10% of research priorities as identified by patients and caregivers [36]. In Canada, priority setting was undertaken with patients who were on or nearing need for

dialysis, their caregivers, clinicians and researchers, to identify the most important unanswered research questions regarding the management of kidney failure [37]. The methods and framework were by the James Lind Alliance, whose aim is to develop patient centred priorities [37]. Through this process they were able to identify the top 10 research uncertainties which included communication between healthcare professionals and patients (shared decision-making), the optimal dialysis modality and management of symptoms caused by kidney failure (i.e., itchy skin) [37].

In nephrology, perhaps the largest body of work in priority setting involving consumers is the SONG initiative (https://songinitiative.org), which develops core outcomes for CKD with consumers and clinicians across broad areas including peritoneal dialysis, paediatrics, haemodialysis and transplantation [25, 38–40]. SONG aims to establish core outcomes and measures for trials ensuring they are based on shared priorities. The validated methodology underlying the SONG process is modelled on the Outcome Measure in Rheumatology (OMERACT) initiative [25, 41]. For example, the top prioritised core outcomes from SONG-HD (haemodialysis) are fatigue, cardiovascular disease, vascular access and fatigue [10].

Consumers have also been involved in the prioritisation of topics to inform the scope of clinical practice guidelines with examples including kidney biopsy, autosomal dominant polycystic kidney disease [42–44]. Workshops, surveys and consumer membership on steering committees are the most common ways for eliciting priorities within the CKD population.

29.3.2 Research Design

There are many ways that consumers can be actively involved in the design of research projects, and in nephrology, this has been primarily through participation in advisory groups convened for research projects, steering committees or through workshops [29, 43, 45]. Some examples of how consumers can contribute to research include advising on acceptability and feasibility of an intervention or research protocol, recruitment process, improved consent processes and choice of outcomes and how they will be measured [46, 47]. Consumers may also be involved in ensuring the readability of all patients facing documents including information and consent forms, and this is essential for documents aimed at culturally diverse groups to gain their feedback [17, 29].

29.3.3 Collecting Data

Roles of consumers to assist in the collection of data for research projects can include assistance with development of interview guides and surveys, facilitation of focus groups or workshops and conducting interviews [17, 29]. A strength of consumers in data collection is their ability to establish rapport with participants due to shared experiences and to identify any barriers to recruitment that may not be readily obvious to researchers [48].

29.3.4 Analysing Data

Consumers involved in data analysis to date has primarily been in relation to thematic analysis of qualitative studies, including workshops and focus groups [29, 48]. Consumers can provide nuanced insight into thematic analysis, based on their own experience with kidney disease and health systems, that may not be readily obvious to the researchers, thus together are able to provide accurate and comprehensive analysis of the information provided, particularly relating to the burden of symptoms and treatments in CKD [45, 48].

29.3.5 Dissemination

Consumers can assist with dissemination of findings through participation in conferences, production of plain language summaries, social media and being co-authors of papers, as in this chapter [2, 17, 45, 48, 49]. There are examples in nephrology of consumer involvement in topic selection and inclusion for guidelines, consumer advisory boards assisting with relaying findings to their networks and the development of educational materials for consumers [29, 43].

29.3.6 Implementation of Findings

Implementation of research findings may take the form of pilot testing, identification of barriers to interventions and providing feedback. These are important roles consumers need to play [29, 43, 47, 50]. It is critical to incorporate the perspectives of vulnerable and disadvantaged groups including ethnic minorities, people from low socioeconomic groups. Input provided by a diverse consumer advisory board can ensure the cultural safety of the intervention for the specific community [29, 51]. Examples of consumer involvement in nephrology in implementation can be seen in topic selection and outcomes for clinical guidelines, knowledge translation activities including videos and flyers and assisting with new models of care.

29.3.7 Involvement in Evaluation

Evaluation of research projects is essential for all projects, and consumers need to be a part of this through formal process evaluation, informal discussions, workshops and providing written feedback [29, 49, 50]. Consumers form a vital feedback link with participants ensuring that participants receive appropriate feedback regarding the outcomes or learnings of a project that they were a part of, which can be in the form of a video or infographic.

Patient involvement in all CKD research must be purposeful, meaningful, respectful, and transparent, and where possible across the entire research cycle.

29.4 Consumer Involvement and Shared Decision-Making

I do not want to be a passive observer in my journey with kidney disease. Involvement in research encourages active participation in our disease and furthers everyone's knowledge, that of patients, carers and health professionals [13]

As seen above, consumers desire to be equipped to make shared and informed decisions about our health and treatment decisions, with families, clinicians and treating teams. Shared decision-making in a clinical context involves an "information exchange" between the patient and the clinician, to determine the best course of action for the patient considering the evidence, clinician expertise and the priorities and preferences of the patient [52, 53]. In many ways this mirrors consumer involvement in the research setting. There is a bidirectional relationship between the nature and impact of shared decision-making on consumer involvement in research [54].

There is vast potential to engage consumers in their own clinical care and outcomes by activating them as decision makers, leading to more informed consumers with better outcomes that are more closely aligned with their individual priorities. Consumers who are living well with CKD and have positive experiences of health systems may have improved capacity and desire to become involved in research [54]. Conversely, consumers involved in research develop a better understanding of their illness, health systems and strengthen relationships with clinicians, leading to improved engagement in their care and better outcomes [8, 55].

However, implementing shared decision-making in CKD remains challenging due to the unpredictable and sometimes urgent nature of the disease and often conflicting priorities of patients and clinicians, particularly within vulnerable populations, including children [23, 56]. These populations are also typically underrepresented with regards to involvement in research [5, 7]. Thus, promoting effective communication and shared decision-making in the clinical care of people with CKD has the potential to improve their engagement and involvement in research.

More research is required to understand how shared decision-making in clinical settings can be utilised to develop relationships with researchers and allow consumers to understand their role and value as contributors to research. Development of kidney-related consumer networks can connect patients/caregivers with researchers through information sessions that provide education to patients/caregivers and opportunities for them to further be involved in research.

29.5 Consumers and Scientific Conferences

Hearing the patient voice goes a long, long way, I think it would've been a much drier session without a patient there saying 'this matters. I've learned, it's not so much about the data, it's about the heart. [57]

Apart from journal publications, one of the main ways to disseminate research findings is through participation in scientific conferences. Consumer attendance and participation at scientific conferences in all areas, but specifically in nephrology, can be beneficial for all involved especially in establishing consumer–researcher

partnerships and providing diverse opportunities for dissemination of research findings [13, 19, 49, 57, 58]. Within the nephrology research area, there are many examples of consumer attendance and participation in conferences including but not limited to the International Society of Peritoneal Dialysis (ISPD) 17th Congress, the Australian and New Zealand Society of Nephrologists ASM and the World Congress of Nephrology for the International Society of Nephrology [13, 19, 57]. In January 2020, the ISN held the first international consensus conference on consensus definitions for the outcome of kidney failure in clinical trials. This was attended by six patients, with diverse backgrounds and personal histories, who contributed in a major way to defining meaningful definitions for the purpose of clinical trials. Conferences provided an opportunity for consumers to learn and ask questions regarding their disease and treatment options, which could transfer to improved participation in their self management [58].

Consumers have had opportunities to be involved in scientific conferences in a variety of roles including attendees, co-designers of sessions, co-presenters, co-chairs and plenary speakers [13, 19, 49]. Important considerations to plan for inclusion at conferences include financial support for travel, availability of free streaming of conferences for consumers from rural and remote areas, disability access and ensuring reduced use of jargon in consumer specific sessions [49].

29.6 Challenges and Limitations to Consumer Involvement

While many of the benefits are now well established, there are challenges that prevent or promote hesitancy in researchers involving consumers in their research [7, 8, 55, 59]. Frequently cited are the resources required to effectively support meaningful involvement, including funding, time and personnel to support training and logistics [17]. With research budgets already stretched, and involvement often occurring only after grants have been awarded, it can be difficult to prioritise consumer involvement in allocating the resources needed. Other key challenges are the lack of ‘best-practice’ guidelines for how to involve consumers in research, and inconsistencies in the conducting, reporting, publishing, and synthesising of consumer involvement activities that make it difficult to learn from and improve upon what has been done in the past.

However, the unique challenges CKD consumers face may impact their ability and willingness to contribute to research. For patients, the burden of self-management responsibilities (e.g.., peritoneal dialysis) and time-consuming, invasive life-sustaining therapy (e.g., dialysis) and prolonged periods of illness, including ongoing symptoms of fatigue and impaired cognitive function, might make involvement exceptionally challenging [27]. For caregivers, the burden of caring responsibilities can become all-consuming and may lead to burnout, limiting their ability to be involved. There are also language and cultural barriers and geographic barriers for rural and remote patient who may want to be involved [27]. Barriers to consumer involvement vary enormously throughout the world, particularly due to different health systems, resource availability and demographics [27].

One issue that is raised frequently is that of representation, where it is questioned whether consumers who are actively involved in research are representative of the wider consumer group [60]. This can reduce the legitimacy of the consumer roles in the research team and places an expectation on them that is not equitable with those of others on a research team. We do not expect clinicians and researchers to be representative of their peers [60]. Rather, diversity and relevant lived experience and perspective are important to recruitment of consumers for involvement, to ensure maximum value and impact.

To date, most of the examples of consumer involvement in nephrology have been seen in high-income countries such as Australia, Canada, United Kingdom and the United States [27].

29.7 Evaluation and Reporting of Patient/Consumer Involvement in Nephrology Research

Reporting and publishing of consumer involvement practices in CKD remains scarce, limiting transparency around consumer contributions and impacts, and the vast majority of published papers do not report using the GRIPP/GRIPP2 checklists which have been designed for this specific purpose [61, 62]. The *BMJ* and *BMJ Open* require authors to include a 'Patient and Public Involvement statement' as a part of the methods section in all papers submitted to the journal. It is essential that clearer, more concise reporting of consumer involvement occurs in nephrology, so we can measure and understand the impact of involvement on nephrology research and where and how it needs to be improved [7]. It is important that all consumer contributions to research are recognised and acknowledged on an equal footing with all the researchers involved.

Another reporting checklist to use when reporting research involving Indigenous Peoples is the CONSIDER statement [63]. This involved the collaboration and inclusion of guidelines for research for Indigenous Peoples form the following nations: Australia (Aboriginal and Torres Strait Islanders), Canada (First Nations Peoples, Métis), Hawaii (Native Hawaiian), New Zealand (Māori), Taiwan (Taiwan Indigenous Tribes), United States of America (First Nations Peoples) and Northern Scandinavian countries (Sami) [63].

29.8 Strategies for Involving Patients in Research in CKD

> *I believe in the power that we have as consumers to change what's important to us, and make sure that we understand what's going on in the research community* [11]

There have been large steps forward in ensuring consumer involvement in nephrology research, but as identified above there are many barriers to successfully and sustainably implementing it across all areas and stages of research. This is essential, particularly in CKD due to the long-term nature of the disease, the

diminished quality of life experienced and the increased risk of mortality [36, 64, 65]. Nephrology is taking steps to improve the quantity and quality of consumer involvement, but there is still a way to go in some areas and barriers that require resolution to enable long-term sustainable consumer involvement [54]. It is essential that there is increased reporting and evaluation of all consumer involvement in CKD research to further understand the consumers perspective and the impact this has on the research [34].

National workshops were conducted in Australia with patients, caregivers, clinicians and researchers to discuss how to improve consumer involvement in CKD research [54]. The findings from these workshops are included in Table 29.1 which summarises the considerations and suggestions needed to be in place to develop and strengthen consumer involvement going forward and are consistent with existing frameworks including PCORI and INVOVLE [4, 15, 54].

Table 29.1 Suggestions for effective engagement and involvement (Gutman et al. [54])

Domain	Considerations and suggestions for patient/caregiver involvement
Engagement and selection	• Plan and budget for involvement at the earliest stage of the research project • Clearly define roles, required commitment, and expected impact with 'terms of reference' document, allow time for reflection and questions • Roles may vary by project and/or research stage and can range from consultation to partnership • Consider the potential benefits for the patient/caregiver and clearly explain these • Consider the cross-section of patients/caregivers involved and structure projects to enable broad participation • Select patients/caregivers for whom your project is most relevant, and whose expertise is best suited (e.g. target peritoneal dialysis patients for projects/interventions designed for peritoneal dialysis patients) • Use engagement methods preferred by target population • Work with physicians and nurses to engage patients/caregivers • Patient mentors to induct new patients into research projects
Training, support and education	• Provide education for patient/caregiver partners • Provide training in research methods • Consider psychological, mental and physical demands of involvement: – Ensure environment is accessible and can accommodate for patient needs (e.g., place to do PD) – Use simple language to reduce cognitive burden of involvement on patients – Have referrals available in case of distress (e.g., social worker) – Offer flexible meeting options (e.g., videoconferencing, regular breaks, time for questions) – Where possible, embed opportunities into routine care (e.g., transplant clinics) • Financial reimbursement/aid may assist patients/caregiver to become involved: – Consider financial burden/sacrifice of involvement on patients/caregivers, and at a minimum, cover costs incurred (e.g., travel expenses, time off work) ∘ Consider implementing a system to reimburse patients/caregivers

Table 29.1 (continued)

Domain	Considerations and suggestions for patient/caregiver involvement
Empowering the patient voice	• Appoint more than one patient representative on a steering committee/working group • Consider forming a diverse patient advisory group to oversee research activities • Produce induction packets with relevant materials (e.g., glossary of terms/acronyms, background reading, helpful resources, videos) • Use lay language where possible and explain technical/medical terminology when necessary • Allow time before or after meetings for patients/caregivers to consider their response/opinion • Involve across all stages of the research, however early involvement, before grant stage, allows patient/caregiver contributions to be more easily integrated • Partner patient/caregiver with experienced research 'buddy'
Connection and community	• Establish and expand a database to contact patients • Consider building a patient dedicated research showcase portal/hub with lay language summaries of research and impacts, and opportunities for involvement to connect patients with researchers with potential for patients/caregivers to customise their profiles • Keep communications open with regular updates via newsletters, emails, texts, and/or social media, even if no formal results are available • Collaborate with patient/caregiver and community organisations to raise awareness of kidney • Disease, encourage knowledge exchange and advertise opportunities for involvement • Establish relationships with other research organisations to pool resources for patient/caregiver engagement to mitigate competing priorities, leverage collective goals and streamline communications to patients/caregivers • Disseminate research findings in plain language and informal settings to patients/caregivers to educate them about the outcomes and impact of research (including the impact of their involvement), build trust with the research community and encourage future involvement

29.9 Stories of Involvement in Research by Consumers

29.9.1 Chandana Guha (Australia)

The effect of chronic kidney disease is pervasive with the management of the disease involving multiple care settings and interventions to protect and prevent disease progression. Traversing through each stage of the disease until kidney failure, my experiences as a caregiver were not limited to the treatment and management of the disease and recovery, but encompassed shared understanding of resulting changes in lifestyle, shifts in psychosocial statuses, relationships, employment and concepts of self that resulted from prolonged periods of illness or caregiving.

My involvement in research has been at various levels across a variety of roles and responsibilities that ranged from engagement in the work of generating research findings, to the development of the evidence bases that inform best care practice. What was common across these engagements as a consumer was the ability to draw from multifaceted lived experiences. Mapping my own needs and priorities to those of consumers whose stories intersected with mine was an elementary process [66]. The contribution of my lived experience played a role in determining research priorities that were at the core of what patients most wanted, in the conduct of patient friendly research, in improving research processes, dissemination of findings and implementation into policy and practice.

The need for consumer engagement in research is recognised, but the quality of consumer engagement is still insignificant in many sectors of medical research. Integrated models of care that are underpinned by patient, provider and researcher partnerships needs to be central to patient- and family-centred care and evidence-based practice [67].

29.9.2 Daniel Gallego (Spain)

The improvement of the access to consumers in research is crucial. Nowadays, there is not a user-friendly form to engage patients in research, terminology and wording of forms are discouraging patients to be involved. We usually are filling the forms by our own, bureaucracy and time consumption are important barriers to engage consumers in research.

However, new tools of PRO (Patient Reported Outcomes) are being designed incorporating patients in research from the early beginning of the clinical trials. Until today, the tools to measure Health related Quality of Life (HrQoL) had been designed for professionals, so we need to design new tools to incorporate the patient journey and patient experience, to address the humanistic burden of kidney disease.

The humanistic burden of kidney disease, which means HrQoL, Daily Life Activities, Life Participation and Caregivers HrQoL, should be addressed by research in the incoming years.

Another important issues, as sexuality, physical appearance, ability to exercise or ability to travel are now taken into consideration for the researchers as crucial aspects, that really matter to the consumers.

In research, as customers we would like to address three important aspects that should fill the gap, bridging the knowledge gap to better kidney care:

1. Voice
2. Co-responsibility
3. Co-production

As costumers, we would like to have voice, being present in every single decision that involves us, as patients or citizens, as an equal in all the strategics, plans, approaches, or decisions, these means that patients should be incorporated in all the

breakthrough process, including research, to share and enable a real shared decision-making.

The concept of co-responsibility in research means that we would like to manage kidney disease as users, costumers, patients, and citizens, without the purpose of replace the health professionals, being the actors of our own life.

Finally, we would love to have co-production as an author's/user in research and clinical trials, offering our knowledge and expertise in kidney disease, behind numerous years learning to manage kidney disease.

We are only kidney patients during the appointments or treatments, the rest of the time we are persons trying to have a fulfilling life, with hobbies, dreams and daily life activities. The perception of life is that really matters to the customers, so this matter should be addressed by the research incorporating the way of living of people with kidney disease.

29.9.3 Kelly Malheiros [3]

Make life worth living!

Hemodialysis is not the end.

Hemodialysis is the beginning.

"- Have you ever heard about hemodialysis?"—asked the doctor.

"- Yes!"—I answered.

"- You need to start immediately. We will implant the catheter. Initially we need to take 10 kg of liquid from your body."

"- And then?"—I asked.

"- Then we talk about the rest."

It was 26th September 2020 when I had the diagnosis. I did not know that this treatment would radically change my way of thinking and live life.

I was completely disoriented, was not even able to use my cell phone and my legs did not have strength to make me get out of bed. Nobody has told me what a catheter was, how the machine worked and what were the reactions that I could have. And what would be the "rest"? On the day of hospital discharge, I realized: I would have to get hemodialysis 3 times a week, 4 h a day. A shock for me, a person who always worked a lot, traveling a lot, making talks all around Brazil. Urea had gone down; I was recovering my weight and memory and I decided that I would face this treatment. On the beginning I was very cold and wrapped me up like it was going to snow: hat, socks, gloves and blankets. Over time, I gave up all these accessories. After 15 days on dialysis, I have received the Psychologist visit:

"-Now is not the time to plan anything. Live one day at a time."

How could I? I had lived 49 years of my life planning and now I have to live "today"? Then I realized that hemodialysis is a place of untold histories, of untold pains, of screaming silences. People do not know nothing about you. The journey is lonely, and nobody tell us: during the 4 h you are your only company.

It has been 1 year since I am on hemodialysis and I have found that is there where I find strength and life to live in the world outside.

The hemodialysis has turned into “love dialysis” for a number of events during this time of new routines. One of the things I have learned after listening to the stories of people going through this challenge along with me is:

Every time you have a problem, and you try to run away with it, it will follow you: no matter where you try to hide.

We love to postpone!

To kick the can down the road!

To hide!

To make it work.

But problems, most of the times, are only solved if we bring them to the sundeck. It is hard to work it out hidden in the ship’s hold: with no light and with a lack of oxygen.

Solving a problem may require giving up comfort or pleasure. You may have to confront your vulnerabilities to work things out. Sorting things out may mean “losing”.

I will solve… and then?

When we have a different understanding about the time we live, urging makes part of our agenda.

Choosing what makes you happy momentarily may be quite different from what you want for your whole life.

Choose to solve!

We do not have all the time in the world.

Make life worth living.

29.10 Conclusions and Future Directions

Optimising consumer involvement in nephrology research is still in the developmental stage, and there remain many barriers limiting consistent involvement of patients/caregivers across all the research cycle in alignment of research priorities, enhancing shared decision-making and producing research that is of importance to all stakeholders. The burden of CKD, including its predilection for those from lower socioeconomic status, and poorly educated, are only some of the existing barriers to involvement. Language, cognition and time commitments which respect individual need will have to be addressed by the research community. Through education of consumers and their families, by providing opportunities and recognition of consumers skills, and ongoing support over time, the research community can facilitate the development of kidney related consumer networks. As a community, there is ongoing recognition of the need to connect patients/caregivers with researchers, inclusive of Indigenous and culturally diverse consumer groups. There is a need for more discussion and exploration of consumers ability or practicality of involvement in basic science research as there is little information around this area. Transparency

in reporting and rigorous measurement of impact of consumer involvement in all nephrology research is needed to enable and sustain the development of these important networks.

29.11 Consumer Involvement in This Chapter

Co-Authors Nicole Scholes-Robertson, Chandana Guha, Daniel Gallego and Kelly Malheiros have lived experience as patients with CKD or caregiving responsibilities. They have been involved in drafting the chapter, reviewing, and providing feedback and prioritising the contents of this chapter's contents. All have provided a case study of their involvement in nephrology research and their experiences as CKD patients.

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Chapter 30
Social Media and Interaction Between Healthcare Professionals and the Kidney Patient

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30.1 Introduction

In recent years, physicians to educate patients on a disease process increasingly utilize social media; patients also have increased the use of social media to find a hospital, physicians, and physician networks most capable of treating their condition.

Motives and expectations from social media use can be diverse. Health-related social media use by patients is usually due to the need for increasing the knowledge on their disease, expressing their emotions, sharing their experience on their disease and its treatment, being in touch with doctors, finding answers for additional and forgotten questions, getting advice, receiving education, and checking their progress and goals. Patients can tell their story and exchange ideas and feelings so that they themselves create a real community on a specific topic.

Social media communication can collaborate in a faster way the most important findings, new diagnostic tools, and treatments of a disease reducing knowledge transmission to other health care professionals and patients; however, during the pandemic we saw that social media become a potential threat due to the ability of different social media platforms to quickly disseminate false information and fake

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news. That is why it is extremely important for every society to have educated scientists and health care professionals to be leaders in delivering fact based information to the public. It is also important to focus on the quality of the content of the multiple online platforms for patients with health-related information on the Internet is a key point that needs to be address and communicate with patients [1]. As health misinformation, "anti-vaxxer" movements and non-evidence based "alternative" therapeutics bloom on the Internet, clinicians and educators should fill in the void and provide proper and reliable medical knowledge to dilute the information pollution and increase the health literacy of our population [2].

Social media use especially Twitter platform, has increased exponentially in the last decade, and is having a profound impact on the Nephrology world. The use of these platforms is contributing to continuous educational and professional development by exposing nephrologists to new research, allowing them to connect with experts, to exchange experiences, or to engage in scientific debates. Its power to communicate openly, and faster than ever before, makes it the ultimate "medical lounge." On social media, anyone can become a teacher or learner: lifelong learning now goes beyond textbooks, lectures, and journal articles and is available anywhere [3].

The use of other social media platforms such as Facebook, Instagram, and Snapchat (among others) has robust audiences and is ripe for further development and expansion to the general public.

30.2 Social Media Origins

For more than 400 years, scientific publications and conferences have remained the primary means of disseminating and sharing scientific results among colleagues. In 1684, the first English medical journal, Curious Medicine, was published, giving rise to the ability to connect and share scientific findings on a level never thought of before [4].

In 1812, the New England Journal of Medicine (NEJM) was published. Years later in 1823 the British surgeon Thomas Wakely published The Lancet. Soon after, medical societies began to establish Conferences with research presentations and discussions, giving scientists the ability to share and discuss their work with their peers on a more personal basis [5].

Index Medicus is a curated subset of MEDLINE, comprising a comprehensive bibliographic index of scientific journal articles whose first publication began in 1879 by John Shaw Billings, chief of the Library of the US Army Office of the Surgeon General. This library later became the United States National Library of Medicine (NLM) [6].

The last issue of the Index Medicus was published in December 2004 (Volume 45). The stated reason for suspending print publication was that online resources had supplanted it, notably PubMed, which continues to include the Index as a subset of the journals it covers.

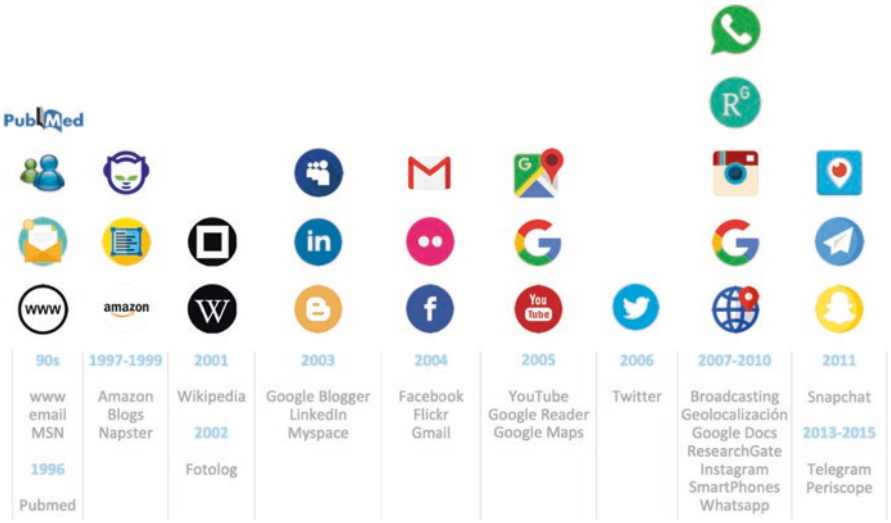

Fig. 30.1 Shows the evolution of different social media platforms

Healthcare professionals and patients increasingly turn to the Internet, including social media platforms, to search for medical information and peer support [7]. But what is social media? "**Social media**" generally refers to Internet-based tools that allow individuals and communities to gather and communicate; to share information, ideas, personal messages, images, and other content; and, in some cases, to collaborate with other users in real time. The most popular social networks are (Fig. 30.1):

Facebook: Social network connecting friends and family, *but also unknown people with common interests,* by allowing shared status updates, photos, videos, and instant messages. It is a channel that ***networks people***. The use of this platform makes easier to establish a closer link with patients. It has 2.9 billion users.

Twitter: Social network that allows share information (text sharing up to 280 characters per tweet). You can include images, videos, text, and links. Is a channel that ***networks ideas***. Easier to connect with your audience (colleagues and organizations) and those you follow. It has 206 million users.

LinkedIn: Social network specifically designed for business and professionals. LinkedIn can share ideas and contents. It is a "Virtual CV." It is a channel that ***networks professionals***. It has 645 million users.

Research Gate: Social network that connects scientists and researchers. It is a channel that allows you to share documents, ask and answer questions, and find collaborators. It is a channel that connects researchers. It has 15 million users.

Instagram: Social network that connects brands and people. It is a channel that allows you to share photos and videos. It is a channel that connects people. It has 1.39 billion users.

Social network sites are increasingly attracting the attention of academic and industry researchers intrigued by their affordances and reach. Most sites support the maintenance of pre-existing social networks, but others help strangers connect based on shared interests, political views, or activities. Some sites cater to diverse audiences, while others attract people based on common language or shared racial, sexual, religious, or nationality-based identities. Sites also vary in the extent to which they incorporate new information and communication tools, such as mobile connectivity, blogging, and photo/video-sharing [8]. For example, in the medical field the social network that attracts more healthcare professionals in the field of nephrology is Twitter.

Social media for patients can be classified into two generic categories: general-purpose online social networks and virtual health communities. General-purpose online social networks are Facebook, Twitter, Instagram, and YouTube. They are the most used social media platforms for health information. Virtual health communities are social media platforms that are designed for individuals to facilitate online interaction around specific health topics. Online community groups, like www.Inspire.com and ask-a-doctor websites, such as www.MDTalks.com, mostly use virtual health communities [9].

30.3 Patient Centered e-Health

"E-health is an emerging field in the intersection of medical informatics, public health, and business, referring to health services and information delivered or enhanced through the Internet and related technologies. In a broader sense, the term characterizes not only a technical development, but also a state-of-mind, a way of thinking, an attitude, and a commitment for networked, global thinking, to improve healthcare locally, regionally, and worldwide by using information and communication technology." Such is the definition of e-health by Gunther Eysenbach, editor of Journal of Medical Internet Research. According to him, the "e" in e-health does not only stand for "electronic," but implies a number of other "e's," namely: efficiency, enhancing quality of care, evidence-based, empowerment of consumers and patients, encouragement of a new relationship (between the patient and health professional), education of physicians through online sources (continuing medical education) and consumers, enabling information exchange and communication in a standardized way between healthcare and establishments, extending the scope of health care beyond its conventional boundaries, ethics, and equity [10].

The World Health Organization (WHO) defines it as "the use of information and communications technology in support of health and health-related fields." This includes a variety of utilities, from mobile health (m-health) to telehealth.

It is undeniable that the COVID-19 pandemic significantly accelerated this transformation of healthcare (traditionally face-to-face contact) to being more digital (allowing for remote networking). The digital landscape of e-health is transforming the practice of medicine, and how we approach patients from a diagnostic and therapeutic perspective.

Electronic health records (EHRs) are a fundamental aspect of e-health. Different EHRs offer various levels of functionality and allow for exchanging communications among healthcare providers and also allow for electronic prescribing.

m-Health is the use of mobile technologies (smartphones, tablets, etc.) for health.

Bluetooth-enabled "smart" devices allow sensors to connect to mobile devices to track data and provide trends. One example of this is the novel glucose-sensing technology that allows continuous monitoring of blood glucose (CMBG). By providing this information, the user (patient) is able to obtain real-time readings that provide guidance in adjusting medication dosages, thereby limiting episodes of hypoglycemia.

Wearable devices that monitor physical activity and heart rate are used as fitness trackers. Some devices have irregular pulse detection algorithms and EKG recording capabilities.

With the COVID-19 pandemic and the need to practice social distancing, there has been a significant upsurge in the use of telehealth, which is "the use of telecommunications and virtual technology to deliver healthcare outside of traditional settings." While there are perceived advantages to this set up such as decreased waiting times and costs of travel on the part of the patient; there are also disadvantages: the lack of face-to-face component removes the personal touch that is inherent in physician–patient encounters. For trainees in particular, there is the lost art of physical examination [11].

All e-health domains have been evolving rapidly and continue to do so. Remote teleconsultation is now routine in some locales where reimbursement issues have been addressed. In low-middle income countries however, due to suboptimal communications infrastructure (unreliable Internet connectivity), e-health practices may be significantly limited.

There are a limited number of studies that look at the e-health technologies for person-centered health care. Further research should be conducted to understand the applications of e-health to improve the quality of health care and patient outcomes and evaluate its cost-effectiveness.

30.4 Social Media as a Tool for Telehealth

Today we know that using social networks for the dissemination of scientific information of an article is seven times more likely to be cited compared to not using these networks [12]. This is one of the reasons that has increased the scientific information released by the nephrological community on social networks, where the entire population, not only health professionals have access; with this, it has been recurrent to request advice, opinions, or even medical consultations through these channels [13]. This practice, unimaginable and even demonized just a decade ago, has revolutionized the probability of obtaining the opinion of world experts in nephrology immediately for some patients, even despite being thousands of kilometers away, thus facilitating, not only their education and understanding of their disease, but also decisions about approaches and therapeutic options.

The use of social media as a tool for telehealth in nephrology has been particularly important in the context of COVID-19, where social distancing and security measures led to the cancellation and delay of face-to-face consultations, it should be noted that our patients have a greater susceptibility to infection by SARS-CoV-2 due to their pathologies and treatments, using social networks was an interesting option in these cases [14, 15]. A good example of the use of social media as a telehealth tool is the development of applications for smartphones and profiles on social networks like Instagram (Renal Health) that was created by Bezerra da Silva Junior et al. aiming to educate patients to self-monitor and cope with their CKD and to increase adherence to treatment. In the subsequent study performed by this group, they have analyzed spontaneous patients' feedbacks and comments posted on the Renal Health Instagram in order to know which information was needed the most and by which types of patients. During the first 15 months since the release of this profile, there were 3380 followers, a total of 449 posts, with 36,079 "likes." Most of the followers were patients and parents; they gave spontaneous testimonials of their experiences with having kidney disease and were thankful for the information provided and for the response we gave to their questions. From the analysis of the comments, the authors found that information on nutrition, physical activities, and kidney transplant were the most discussed and valued [16].

Another example of the use of social media as a Telehealth tool for advocacy, educating the public about the need, process, and outcomes of live kidney donors was shown in a survey. The study authors' inquired patients who had received a transplant and those awaiting a transplant about current social media use, sites visited, frequency and duration of social media use, and willingness to use social media to share the need for living kidney donors. Approximately half of all kidney transplant patients surveyed used social media (104/199, 52.3%), and approximately one-third (66/199, 33.2%) had more than 100 friends in their social media network. Facebook was the most popular site, and 51% (102/199) reported that they would be willing to post information about living kidney donation on their social networks. More than a quarter of the sample (75/199, 37.7%) had posted about their health status in the past. This study showed that social media holds great promise for health-related education and awareness and that transplant programs can help increase the number of living donors by providing guidance to kidney transplant patients on how to use social media, to be advocates, and to provide information about living kidney donation to their social network [17].

A systematic review of 98 original articles analyzed the uses, benefits, and/or limitations of social networks for health communication among patients and health professionals. It concluded that the use of social networks for health communication has several benefits, but it is necessary to control the quality and reliability of the information exchanged [18].

Social networks have democratized medical education and communication between patients and health professionals, with this the flow of information between both channels is an additional tool that nephrologists must take advantage to provide higher quality care (Fig. 30.2), emphasizing the characteristics of our dialysis

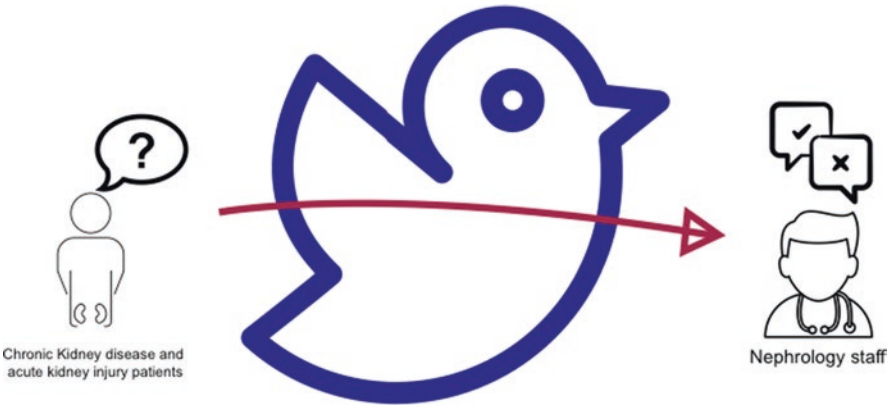

Fig. 30.2 Information flow between patients and nephrologists

patients, which inherent in the process of their disease, refer asthenia, weakness, fatigue, difficulties and risks of falls, therefore social networks to establish effective communication with them is particularly interesting; in another scenario, such as patients who are discharged after an acute kidney injury episode, where it has been shown that it is difficult to follow up and return to the hospital, in them too, using social networks may play a very important role [19].

30.5 Social Media Education for Patients

As we have previously discussed patients are increasingly active online, and they are frequent social media users for health purposes. In 2015, it has been estimated that 62% of entire adult population of the USA uses Internet and 72% of adult Internet users use Facebook social media platform; in a survey, 72% of Internet users say they looked online for health information within the past year [10]. Different social media attracts different age groups, and for example, blogging has become less popular among teens and young adults since 2006 and more popular within older adults. Similarly, there are differences in social media use depending on gender, race/ethnicity, education, and income characteristics [11].

Online health communities are mostly used in the communication between physician and consumer. Through these sites, physicians and health care organizations can communicate with individuals helping them to learn more about their health-related problem and make better future decision on their health and health care. Online health communities can also be used to enhance the communication among patients around a specific medical topic. So patients can share their opinions and experiences in order to empower themselves and play an active role in their health care processes and education [20].

Virtual health communities are mostly used in the communication between consumers. These social media platforms are typically built upon mass collaboration on health-related topics, favoring social interactions and social support among patients. In Canada, a secure, members-only, patient-centric online self-management virtual health community was developed that empowers patients with chronic kidney disease to be better informed, connected, and engaged in their treatment and care by providing them with information on how to manage their condition (www.ForaHealthyMe.com) [21]. The portal in Canada enables patients to enter data related to their condition. The collected data allows healthcare providers to monitor the patients' condition and will enable researchers to build a decision support tool for healthcare system administrators and physicians. The virtual healthcare communities should have three major components: a patient education module, communications and information sharing, and a disease specific interactive chart to track and record important medical events.

Health discussion boards and forums are the most used platform in the patient-to-patient collaboration. The discussion boards and forum are typically topic-oriented platforms to discuss about a specific disease or health-related topic. Usually, patients can initiate discussion threads on a topic, asking a question or seeking support from others on the platform, and in response to the thread initiator, other patients can post their comments and provide their experience, information, sympathy, and thoughts about the thread topic [21].

Surprisingly, clinicians tend to overlook patient access to and use of information and communication technologies to manage their health [10]. One study reported that less than 25% of chronic kidney disease patients obtained information about renal health care from the Internet. The information and communication technologies most preferred by their renal health care teams were telephone (56.5%), Internet (50%), email (48.3%), and text messages (46%) [10].

Health care systems and patients are increasingly turning to the Internet—including websites as well as social media platforms—for health-related information and support. The US-based National Kidney Foundation, for example, designed a comprehensive and user-friendly digital ecosystem that contains content relevant to each audience and helps promote prompt interactions between CKD patients and their health care providers. The ecosystem received high satisfaction scores (88%) on the ForeSee survey, a customer satisfaction survey administered on the US National Institute of Diabetes and Digestive and Kidney Diseases websites. Results from a paper by Gee et al. showed that chronic care needs to reform to incorporate information and communication tools. They concluded that (1) e-Health (electronic health) education has a critical role in self-care; (2) e-Health support should be put into the community, and patients should be empowered with the benefits of the e-community (electronic community) or virtual communities; and (3) productive technology-based interactions ensure feedback loop between the patient and the provider [22].

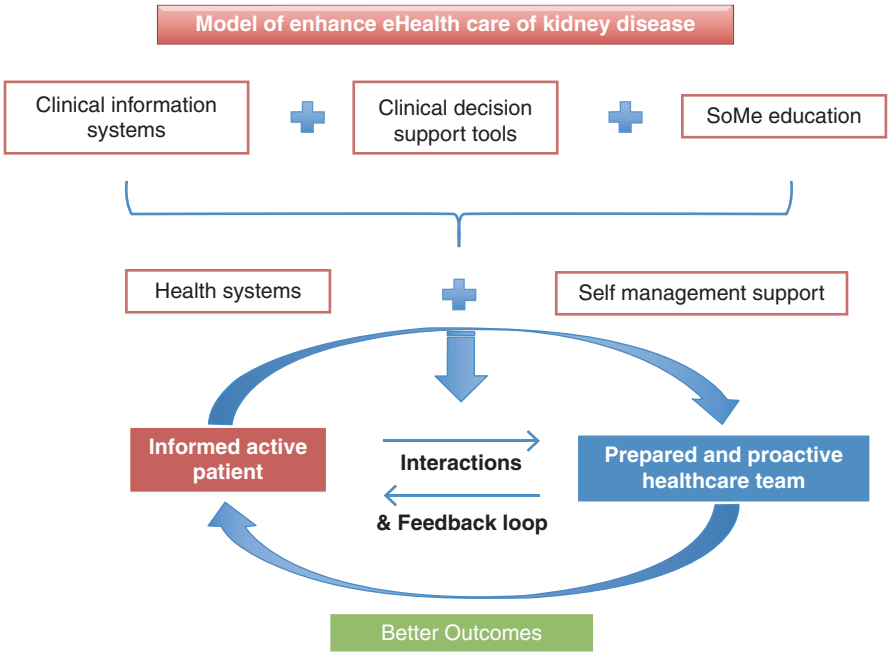

Fig. 30.3 Model of enhanced e-Health care

The use of social media tools-education couple with improved clinical information systems, clinical decision support tools, self-management support could enhance the e-Health care model and improve patients and healthcare professional interactions, increasing feedback and improving outcomes of kidney diseases (Fig. 30.3).

30.6 Conclusions

Social media platforms offer the possibility for patients to communicate among them and with physicians. These platforms can help patients to be better informed about their condition and more involved in their treatment. Social media platforms like virtual communities empower patients with chronic kidney disease to be better informed, connected, and engaged in their treatment and care by providing information on how to manage their condition. In this way, patients are not passive consumers of health information but can play an active role in the delivery of health services through an online environment. Social media platforms could also help capture health informatics data that could help to inform healthcare systems decision-making and policy research.

Nevertheless, it is difficult to control or regulate the sources and their quality, and bad or misleading information can be detrimental for patients as well as influence their confidence on physicians and their mutual relationship.

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Chapter 31
Innovations in Nephrology Education

Augusto Cesar Soares dos Santos Junior and Daniel Costa Chalabi Calazans

31.1 Introduction

Triggered by the recent revolution posed by the digital era, medical education has evolved enormously over the last decade [1–6]. The use of multiple digital tools like webinars, podcasts, and visual abstracts, made available through communication channels like Twitter, YouTube, Facebook, and Instagram, not only boosted the participation of nephrologists in continuing medical education programs but also promoted a wider global interaction within the nephrology community [7, 8].

It is a current belief that by neglecting access to science, the cycle of poor education, poverty, and unequal distribution of wealth gets engraved in societies. Continuing medical education has, therefore, always been challenged by socioeconomic inequalities, especially in low- and middle-income countries (LMICs) [9]. More recently, the COVID-19 pandemic further reinforced the concept that achieving improvements in healthcare globally requires building and sustaining early and mid-career researchers aiming at closing the gaps in both critical appraisal and research capacity [9, 10]. Additionally, while the COVID-19 pandemic shocked economies and healthcare systems across the globe, access to reliable medical information became a recurrent theme in all segments of societies [1, 3–6, 11, 12]. Therefore, common citizens have become even more interested in understanding

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the late advances in medicine, attracting a new public to medical education programs [10].

Traditionally the dissemination of medical education was often unidirectional and much dependent on face-to-face meetings [13]. With the advent of the Internet and the development of Social Media tools, a whole new scenario came into place [14–16]. This traditional educational model was confronted by the exponential increase in the amount of information generated and by an unplanned growing capacity of dissemination. Hence, the simple incorporation of the digital social media tools to the traditional model not only increased our capacity to broadcast information but also potentialized the dissemination of misinformation contributing to an era of infodemic [17]. In 1994, Doug Altman published an editorial entitled "The scandal of poor medical research" [18], acknowledging the "need for less research, better research, and research done for the right reasons." The digital era not only facilitated access to education but has also amplified the magnitude of fake news, bad science, and distorted facts. Consequently, presenting information accurately, concisely, and effectively in today's scientific environment has become more difficult than it was at any time in the past [4, 8, 12, 18–20].

In the field of nephrology, as in other medical fields, the massive incoming amount of information had, therefore, to be counterbalanced by initiatives enabling an environment capable of encouraging the critical appraisal of the available evidence [21–30]. Subsequently, continuing medical education in clinical nephrology and kidney transplantation has also experienced several improvements resulting in a swift shift from the traditional unidirectional model to a multidirectional and scientific atmosphere fostering the scientific evidence-based approach [31]. In this regard, Burton Rose pioneered nearly 45 years ago. As a young nephrology fellow, in 1971, he began a meticulous task of summarizing complex concepts in nephrology and making them available to a broader audience. At that time, long before the era of the commercially available Internet, he and many other educators engaged in a mission of transforming education by using tools that could increase not only accessibility but also ease the interpretation and applicability of the incoming discoveries [8]. Since then, we directed our efforts to implement strategies encouraging broad education programs that could at the same time reduce the distance to good practices and fight poor medical practices and misguided believes [8].

Much of this phenomenon was triggered by the surge of evidence-based medicine and the development of a group of leaders interested in promoting the practice of good scientific standards. The evidence-based medicine (EBM) movement started in 1981 when a group of clinical epidemiologists at McMaster University, Canada, led by David Sackett, published the first of a series of articles advising physicians how to appraise the medical literature [32]. The term EBM, first coined by Gordon Guyatt, one of Sackett's mentees [19], merges three major components for medical decision making, a concept which became one of the most important milestones to shape modern medicine: the best scientific evidence, the individual practitioner's clinical expertise, and the patients' preference [32].

To date, many healthcare professionals still lack the core competencies of critical appraisal, question formulation, study design, and the skills to translate evidence into practice. Encouraging the diversity of ideas and at the same time fostering critical thinking, building capacity, and filling the gaps in education are essential ingredients to help us innovate in healthcare and overcome these hurdles. The new digital age aims at implementing broad educational programs while improving our capacity to discuss the sustainability of healthcare systems and address issues like access and budget priorities. Taken together, these actions can lead us to a more balanced scenario, allowing better policies with less room for poor science, reducing the distance to good practices, and increasing bench-to-bedside research. In this context, merging the EBM principles with modern digital educational tools remains an inspirational challenge pursued by the architects and designers of continuing medical education programs [10, 33].

31.2 The Transition from In-Person to Virtual Congresses

Since the World Health Organization (WHO) announced the emergence of several cases of pneumonia in Wuhan City, Hubei Province, China, medical education changed dramatically [14]. One of the early consequences of the wider local community transmission of COVID-19 cases was the establishment of extensive policies encouraging the adoption of social distancing, quarantines, and lockdowns measures [34]. As a consequence, conferences, symposiums, and congresses had to be completely redesigned. Planned events had to be canceled and were replaced by virtual meetings which become the default method of dissemination of medical education [1–6, 11, 12]. Since then, medical societies across the globe lead nephrologists to a totally new educational experience. However, this transformation was already taking place even before the COVID-19 crisis.

The International Society of Nephrology (ISN), as a global nephrology society, initiated a trial of social media-based dissemination of nephrology content at the ISN World Congress of Nephrology (WCN) in March 2015, Cape Town, South Africa. In 2015, complete coverage of the conference was accomplished using the Twitter handle @isnkidneycare and the hashtag #ISNWCN2015 [7]. Strengthened by the success of this experience, the ISN envisioned the ISN social media task force initiative in 2016. As a result, the coverage of the ISN WCN 2017, held in Mexico City, included clear policies encouraging the sharing of the content presented through social media platforms culminating in the establishment of an official social media education team, the @ISNeducation. Since 2015, the @ISNeducation team has developed and incorporated several social media education tools, such as visual abstracts, Twitter quizzes, and poster interviews. At the ISN WCN 2019, on-site preselected physical posters were converted to "poster talks," a video clip of approximately 140 s duration shared on social media during the conference. Additionally, at the ISN WCN 2021 (@ISNWCN; #ISNWCN) poster interviews, speaker interviews, online quizzes, were also uploaded to the ISN YouTube

account and shared on Twitter, Facebook, Instagram, LinkedIn, and select WhatsApp groups. The ISN WCN 2021 was attended by roughly 3900 participants, being the first fully virtual World Congress of Nephrology.

The European Renal Association (ERA) and the American Society of Nephrology (ASN) also constructed similar initiatives. Both the societies transitioned their congresses to a new standard, allowing the European Congress of Nephrology and the Kidney Week, respectfully, to occur as virtual meetings. National societies, as the Sociedade Brasileira de Nefrologia, also pioneered as examples of successful initiatives to bring congresses to a virtual platform. Like the ISN, all these societies build an extensive agenda and a comprehensive strategy to cover medical conferences and promote nephrology education across the global nephrology community encouraging wider participation, inclusiveness, and community engagement. As we see some locations gradually lifting the adaption of social distancing, quarantines, and lockdowns measures, much debate is being raised on how to plan in-person congresses without denying the advances experienced by the surge of the virtual meetings [35, 36]. Possibly, merged virtual and in-person formats will become the new standard, allowing education to profit from the best of these two strategies [22, 24, 30, 37].

31.3 Massive Open Online Courses (MOOC)

MOOCs are online courses, mostly created by universities and institutions from around the globe, which are often accessible without any costs or prerequisites. Medical MOOCs offer health care professionals across different disciplines the opportunity to study the latest developments in their field in a place and time-independent way [31, 38, 39].

The term MOOC was first used in 2008 to describe an open online course designed by George Siemens (formerly of Athabasca University, Canada) and Stephen Downes (National Research Council of Canada) [40]. Over the years the MOOC course design has evolved into many different formats ranging from instructor-provided content to discussion groups. Regardless of the differences between the current formats, MOOCs have become an attractive tool allowing students worldwide to access powerful educational features and content [20, 41].

MOOC can offer learners in under-resourced countries a unique possibility to access information otherwise unreachable. One of the most important features of MOOCs is their capacity to provide access to materials outside of their university's curriculum without having to travel abroad. Additionally, for institutions hosting their courses, once a MOOC has been created, it can be used multiple times without extra effort or costs [31]. The course Principles and Practice of Clinical Research by the T H Chan Harvard School of Public Health is an example on how students can create international communities, building an integrated network with shared interests, actively engaging with a large group of intrinsically motivated peers, as well as instructors, involved in the online discussions and assignments [13]. In this course, learners can study at a convenient place and time by watching short video lectures,

taking interactive quizzes, and completing games and assessments at their own pace depending on preexisting knowledge and experience [15].

Although MOOCs originally have been designed for learners outside of the university walls, nowadays more and more institutions explore ways to integrate online courses into medical school curricula, in continuing professional development programs, and interprofessional education. One great example, in the field of nephrology, is the course "Clinical Kidney, Pancreas and Islet Transplantation," developed by transplant professionals at Leiden University Medical Center (LUMC) together with the Centre for Innovation of Leiden University in the Netherlands.[1] The course is taught by a multidisciplinary team of transplant professionals aimed at providing state-of-the-art updates on clinical kidney, pancreas, and islet transplantation. The course includes four modules: (1) before transplantation, (2) the challenged patient and the procedure, (3) early challenges, and (4) late challenges after transplantation. The offered modules include lectures, interactive patient cases, quizzes, interviews with well-known experts in the field, and games. To increase the understanding of the presented concepts, 3D animations illustrating the kidney, pancreas, and islet transplantation were developed. Additionally, 3D animations were also developed to illustrate the different aspects of the immune system including immune suppression and cell therapy. At its launch, the course was endorsed by various professional organizations, including the European Society of Organ Transplantation, the Transplantation Society, the ISN and the European Society of Pathology, and the European Federation for immunogenetics (EFI). In 2018 the MOOC was accredited for Continuing Medical Education (CME-credit) for healthcare professionals. Besides informal use in graduate, resident, and CME at LUMC, the MOOC has also been offered in the formal curricula of the second year of medical school, the Leiden University Honours program, and the international Leiden Oxford Transplantation Summer School (LOTS). For LOTS the MOOC is a mandatory preparation activity and submitting the course certificate as proof of completion is a requirement to be admitted to the on-campus part of the program. This annual summer school is designed for biomedical and medical students where 20 international students interested in transplantation and clinical research are admitted after selection. Starting January 2016 until mid-Nov 2021, approximately 16,500 students enrolled in the course, coming from over 90 different countries, representing all continents of the globe [31].

31.4 Other Digital Resources

The rapid evolution of technology over the last few decades has radically transformed the way people communicate, seek information, and process knowledge. CME has also been through this transformation. The growing use of Visual Abstracts

[1] https://www.coursera.org/learn/clinical-kidney-transplantation/home/welcome

(VA), infographics, and quizzes[2] to illustrate scientific publications is one good example of how digital media is breaking barriers in medical education. VA is a single concise, pictorial summary providing a snapshot of the central content of a scientific publication. The interactive online tweeter quizzes are untimed, multiple-choice questions covering topics in nephrology. Webinars[3] became one of the most used tools to provide state-of-the-art discussion on emerging themes during the COVID-19 pandemic. Much of this content was made available in multiple idioms and kept open access to increase visibility and reach. Large databases were also developed, as exemplified below, allowing online access to updates in the field of nephrology.

Currently, the ISN offers its members access to the ISN Academy,[4] its official eLearning portal, allowing access to more than 8000 educational and interactive resources by topic, speaker, event, and content type, including video presentations, cases, articles, webinars, guidelines and more. Similarly, the European Renal Association (ERA) also built the Nephrology Educational Portal (NEP),[5] a totally redesigned gateway with access to a diverse range of educational material from recorded lectures to courses.

To improve the global nephrology community's participation in clinical trial research, the ISN launched the ISN-Advancing Clinical Trials (ISN-ACT).[6] The ISN-ACT calls on investigators and interested individuals to join the initiative to generate more high-quality clinical trials and studies in nephrology. This initiative is aimed at growing the capacity of the global nephrology community to lead and participate in clinical trials, by contributing, collaborating, and sharing experiences. Several complementary tools were designed because of this initiative [42]. The ISN-ACT Global Trials Focus[7] is a monthly open access list identifying and summarizing recent interesting trials relevant to kidney disease. The ISN-ACT Toolkit[8] is an open online resource guiding anyone wishing to start a clinical trial or to participate as a trial site, regardless of their level of clinical trial experience and their local resources. The ISN-ACT Narrative-based Clinical Trials[9] was created to translate technical information to patients or healthcare personnel without previous knowledge in clinical research. More recently, all these initiatives were complemented by the creation of the Global Kidney Patient Trials Network (GKPTN) aimed to accelerate the development of new treatments, developing a network of

[2] https://www.theisn.org/initiatives/covid-19/knowledge-sharing-tools-infographics-quizzes/

[3] https://www.theisn.org/initiatives/covid-19/webinars/

[4] https://academy.theisn.org/isn

[5] https://www.era-online.org/en/nep/

[6] https://www.theisn.org/in-action/research/clinical-trials-isn-act/

[7] https://www.theisn.org/in-action/research/clinical-trials-isn-act/global-trials-focus-oct-21/

[8] https://www.theisn.org/in-action/research/clinical-trials-isn-act/isn-act-toolkit/

[9] https://www.theisn.org/in-action/research/clinical-trials-isn-act/isn-act-toolkit/narratives-on-isn-act-clinical-trials-toolkit/

patients with kidney disease willing to participate in clinical research projects, accessible through a single global chronic kidney disease platform.

The Glomerular Disease Study and Trial Consortium (GlomCon)[10] is a new project, supported by the European Renal Association (ERA), intending to create platforms for clinicians and scientists to exchange ideas, participate in online conferences, and collaborate on basic science and clinical research projects.

The NephJC[11] is a nephrology journal club that uses Twitter to generate discussion and review the current literature related to nephrology. Its mission is to increase free open access to medical education on nephrology, hypertension, and transplantation. The NephJC live chat occurs in three time zones, two times a month: every other Tuesday at 9 p.m. Eastern Time for the Americas, and the day after, at 9 p.m. IST and 9 p.m. GMT on every other Wednesday for the Asian and European communities. It is an open opportunity for nephrologists, residents, fellows, cardiologists, internists, urologists, radiologists, pharmacologists, and patients to contribute to the discussion. In 2019 NephJC started its podcast, Freely Filtered. This is a round-table discussion about the most recent NephJC articles. NephTrials is a shared initiative, supported by the ISN, aimed at increasing awareness about ongoing trials in nephrology, as well as to teaching the wider nephrology community what it takes to design and conduct clinical trials, especially concerning different trial designs.

31.5 Discussion and Conclusions

Providing nephrology education is not only a way to deliver continuing medical education but also part of a strategic plan to offer integrated care to patients with kidney diseases [43]. In this regard, it is critical to develop flexible curriculums allowing nephrology education to continue beyond the context of the pandemic, merging both virtual and in-person activities [24, 44].

Online continuing medical education has been growing rapidly, however, the success of future innovations in nephrology education depends much on the proposed format. While it is a current belief that sole transfer of content is not sufficient, many initiatives are being designed aimed at fostering critical thinking skills [45]. The underlying interface of these initiatives should be easy to use, have a good visual layout to stimulate participation, and offer technical support. In this scenario, computer literacy has become one fundamental prerequisite.

Given the complexity of modern health problems, multidisciplinary teamwork in healthcare is important in improving patient outcomes [9]. Therefore, designing tools to promote interactions broadly allowing multidisciplinary teamwork should

[10] https://glomcon.org

[11] http://www.nephjc.com

be part of the plan. Online education should also focus on the inclusion of minority groups, further addressing the effect of language and cultural barriers. Language proficiency strongly impacts learning, and non-native English speakers might be less likely to succeed. Therefore, providing education in different languages is an important measure to be developed. Cultural, ethnic, and age-related factors should be considered as well as other individual factors as motivation and time availability.

In conclusion, acknowledging the impact of socioeconomic disparities not only at the helm of healthcare assistance but also in the educational process, respecting the diversity of ideas and, allowing people from different backgrounds to work together, are great ways to transform hurdles into incentives to innovation. Quality control should always be carefully monitored avoiding lower-quality initiatives to thrive. Fighting poor science and misinformation will require the development of an Evidence-Based Medicine (EBM) background "curriculum" based on independence, collaboration, and critical thinking. Innovations centered on ethics and collaboration are more likely to create the perfect environment needed to construct strong networks in the scientific community capable of building capacity globally and filling the gaps in nephrology education.

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