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# Functional Renal Imaging: New Trends in Radiology and Nuclear Medicine

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**The objective of this work is to compare the characteristics of various techniques for functional renal imaging, with a focus on nuclear medicine and magnetic resonance imaging. Even with low spatial resolution and rather poor signal-to-noise ratio, classical nuclear medicine has the advantage of linearity and good sensitivity. It remains the gold standard technique for renal relative functional assessment. Technetium-99m ($^{99m}$Tc)-labeled diethylenetriamine penta-acetate remains the reference glomerular tracer. Tubular tracers have been improved: $^{123}$I- or $^{131}$I-hippuran, $^{99m}$Tc-MAG3 and, recently, $^{99m}$Tc-nitrilotriacetic acid. However, advancement in molecular imaging has not produced a groundbreaking tracer. Renal magnetic resonance imaging with classical gadolinated tracers probably has potential in this domain but has a lack of linearity and, therefore, its value still needs evaluation. Moreover, the advent of nephrogenic systemic fibrosis has delayed its expansion. Other developments, such as diffusion or blood oxygen level-dependent imaging, may have a role in the future. The other modalities have a limited role in clinical practice for functional renal imaging.**
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The main function of the kidneys is to maintain the homeostasis of extracellular content. Imaging techniques can indirectly explore this function by showing processes that are used for this purpose: perfusion, filtration, secretion, concentration, and drainage. Unlike anatomic imaging, functional imaging aims at showing dynamic processes, ie, evolution over time. Some functional processes can be visualized by their motion (eg, the heart beating), but most are stationary, which makes them more difficult to visualize: for example, every water molecule leaving the organ is replaced by another water molecule. Major functional processes are occurring at a microscopic scale, but macroscopic parameters do not change over time. In these conditions, visualizing functional processes requires some kind of labeling to interfere with the steady state. Most of the time, this labeling is performed by the injection of a diagnostic agent, but physical labeling, such as tagging or arterial spin labeling in magnetic resonance imaging (MRI), also may be used. Therefore, when comparing different techniques for functional imaging, 2 aspects must be addressed: general image characteristics linked to the imaging technique and tracer characteristics.

## Imaging

Medical imaging encompasses 4 main techniques: radiology (including computed tomography [CT]), nuclear medicine (NM), including positron emission tomography (PET), ultrasound (US), and MRI. In addition, optical imaging (OI) can be considered for small animal imaging. In practice, renal functional imaging needs close follow-up over time with sequential imaging. Because of limitations attributable to radiation burden, this makes radiological techniques hardly suited to functional renal imaging. Contrast media (CM) suited to US techniques are mostly vascular, which makes them inappropriate for renal functional imaging beyond mere perfusion studies. Therefore, this review focuses on NM and MRI. A specific article in this issue of the journal[1] addresses renal PET; therefore, the present article will focus on gamma-emitters.

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## Physical Principles

MR shows the signal that hydrogen nuclei emit after proper excitation when they are set in a strong magnetic field. This signal comes from the magnetic moment of hydrogen nuclei. Images can thus be acquired even without any diagnostic agent: diagnostic agents act as contrast agents (CAs) (they modify the image). NM uses injected tracers that emit gamma-rays (positrons for PET), which are localized to make an image. Therefore, injection of a diagnostic agent is mandatory to obtain any image. An advantage of classical NM over PET is that it is possible to use radionuclides that emit photons with different energy levels. Thus, several biomarkers can potentially be distinguished at the same time, as opposed to PET radionuclides, which all emit the same energy photons (511 keV).

## Spatial Resolution

Imaging is localizing information. The localizing technique depends on the imaging modality. Electromagnetic waves can be localized, at best, with a precision of one-half their wavelength. For high-energy physics (gamma photons), wavelengths are a few picometers. For MR, radiofrequency waves are used, with wavelength of several meters. This would suggest a much better resolution for NM. In fact, it is the opposite: spatial localization in MRI is achieved through magnetic field gradients, which theoretically enable localization at a micrometer scale, although signal-to-noise ratio usually limits it to a millimeter scale. In contrast, gamma rays cannot be focused with lenses, so lead collimators are used and effective resolving power is approximately 1 cm in humans (a slightly better resolution can be obtained with dedicated small animal imaging).

In practice, in dynamic renal imaging, MRI typically provides multiple slices of approximately 1-cm slice thickness and 2-mm in-plane resolution, whereas NM provides 2-dimensional (2D) projections with approximately a 10-mm in-plane resolution (Table 1). It may be sensible to look into the requirements in resolution for renal functional imaging. The first aim of spatial resolution is to separate right from left kidney: both NM and MRI perform well in this respect. Another point is to separate upper from lower parts of the kidney for duplicate systems; this can be done easily in MRI but it is more tricky for NM, both because the 2 are not necessarily well-separated on 2D projection images. Separation between kidneys and other organs is easily attained in MRI and only indirectly in NM because 2D projection yields superimposition of other structures over kidneys; however, this superimposition is addressed with signal processing and background removal (or more sophisticated techniques, such as Patlak-Rutland technique[2,3]).

Distinction between the parenchyma and cavities can be done with MRI but not in NM on 2D-projections because of parenchyma and cavity superimposition. Distinction between cortex and medulla can be done in MRI, provided the region of interest is drawn with caution, but not in NM. Of course, microstructures, such as nephrons or small vessels, are not distinguishable when any of these techniques are used. Finally, delimitation of large vascular structures, such as aorta, to obtain arterial input function is quite easily achieved with MRI but is more difficult with NM.

To summarize, the main issue in functional imaging is the separation between right and left kidney, which both modalities perform well. Better arterial input functions and separation in duplicate kidneys are an advantage for MRI. The projection image for NM seems to be a drawback but it has also its advantage: the whole kidney is seen in a single image (MR usually samples function on a few slices) and the time taken to draw regions of interest is much less for NM.

Kidneys are located under the diaphragm and experience movements caused by ventilation motion. Usually, MRI uses registration techniques to compensate the motion, which takes time. NM techniques usually skip this step, and motion blurring is not even considered in NM because one does not usually make it out from poor spatial resolution. This is a strong limitation for cortex versus medulla separation. Gated imaging is possible in NM, but it lengthens the time for data acquisition.

## Temporal Resolution

Temporal resolution is not an intrinsic physical limitation: in theory, images could be obtained in a fraction of a second for all techniques. It is rather a matter of trade-off between signal-to-noise ratio, image quality, and spatial resolution. Both NM and MRI can reach image cadence of a few seconds per image, which is enough for perfusion (vascular transit time is approximately 5 s), renal uptake (peak usually obtained in a few minutes), drainage, and even to correct for breathing artifacts. Cardiac motion is not really an issue but could even be corrected for with electrocardiogram gating. To summarize, temporal resolution is not an issue. Synchronization

**Table 1 Typical Imaging Parameters for Human Dynamic Renal Imaging (These Values Vary Greatly According to Imager Performances and Settings)**

| | MRI | NM | PET | CT | US |
|---|---|---|---|---|---|
| Spatial resolution | | | | | |
| Type | Multiple 2D slices | 2D projections | 3D | Multiple 2D slices | Single 2D slices |
| Slice thickness | 10 mm | N/A | 5 mm | 1 mm | 5-10 mm |
| In-plane | 2 mm | 10 mm | 5 mm | 1 mm | 1 mm |
| Temporal resolution | 3 s | 1-20 s | 10 s* | <1 s† | <1 s |
| Tracer sensitivity | μmol-mmol | pmol | pmol | 100 mmol | NA |

*For list mode.

†Cannot be repeated as fast because of radiation burden.

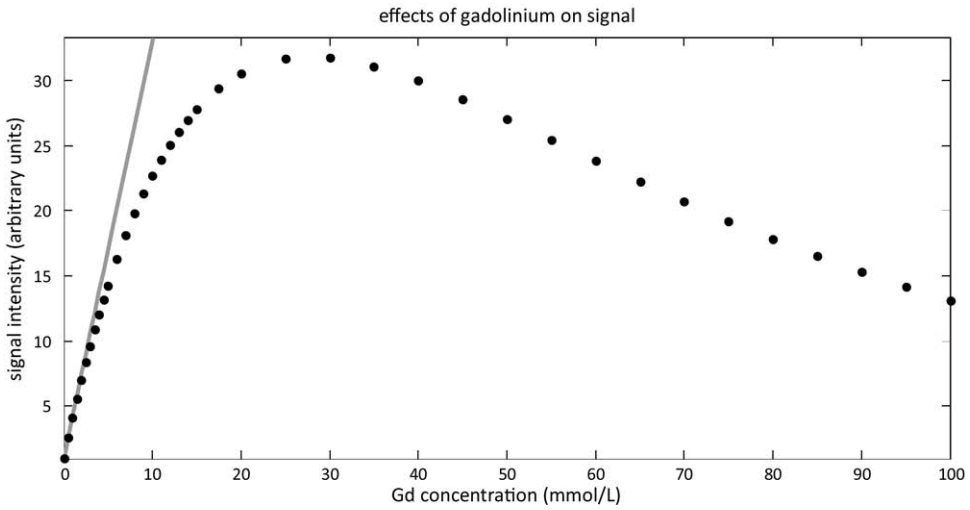


**Figure 1** Example of relationship between CM concentration and signal; the first part of the curve is reasonably linear after correction for baseline. The first part of the curve is mostly attributable to longitudinal relaxation (signal increase); the second part is mostly attributable to transversal relaxation (signal decay). This curve is a simulation with TR = 17 ms, TE = 4 ms, flip angle 90°, $T_1$ = 1100 ms, $T_2$ = 76 ms, $r_1 = 3\,L \cdot mmol^{-1} \cdot s^{-1}$ and $r_2 = 4L \times mmol^{-1} \cdot s^{-1}$. Plasma concentration at peak should range around 2 $mmol \cdot L^{-1}$ in the linearity range, but much greater concentrations will be found in distal tubules.

with ventilation is usually not implemented in NM but would be needed if a separation between cortex and medulla were sought.

## Noise

In NM, noise mostly comes from Poissonian statistics (radioactivity emission is random, with a random direction and its detection is also a random process). Noise is therefore proportional to the square root of signal intensity and it is quantitatively noticeable. In MRI, noise comes from electromagnetic fluctuations in patient and it is usually less visible in image.

However, in time-activity curves, the MRI signal comes from a difference between baseline and contrast images so signal-to-noise ratios appear to be comparable in NM and MRI. Noise becomes a problem in infants but it is the case for both techniques.

## Radiation Burden

The dose taken from an NM renal procedure is approximately 1 mSv,[4] which is about one-third of the natural radiation burden during 1 year. This is considered as "negligible" to "minimal" in irradiation classifications. A CT scanner brings a greater dose (approximately 8 mSv for a single volume; it may be less if a "low-dose" procedure is used but would yield unacceptable data for dynamic imaging).

NM uses injected tracers that emit gamma-rays (positrons for PET), which are localized to make an image. Therefore, injection of a diagnostic agent is mandatory to obtain any image. An advantage of classical NM over PET is that it is possible to use radionuclides that emit photons with different energy levels; thus, several biomarkers can potentially be distinguished at the same time, as opposed to PET radionuclides, which all emit the same energy photons (511 keV).

# Tracers and Contrast Media

### Relationship Between Tracer and Signal

In NM, the image is the projection of tracer amount in each pixel, with a very good linearity between tracer activity and signal intensity. Radioactivity itself is independent from anything: no influence from chemical environment, pH, temperature, or any other physical parameter. This makes it very robust for quantification. A few corrections however must be made for proper quantification (gamma-ray attenuation, scattering, partial volume effect . . .) but in modern gamma-cameras, these techniques are readily available with possible acquisition of transmission maps with dedicated CT attached to the camera.

In MRI, the signal comes from hydrogen nuclei. Therefore, even without injection of CA, there is a baseline signal. The relationship between signal and tracer amount is thus not linear but it depends on the imaging sequence used; in most cases, it is given by the following relationship[5]:

$$S(C) = S_0 \cdot \frac{1 - \exp\left(-TR\left(\frac{1}{T_{1,0}} + r_1 \times C\right)\right)}{1 - \exp\left(-\frac{TR}{T_{1,0}}\right)} \times \exp(-TE \times r_2 \times C) \times \frac{1 - \exp\left(\frac{1}{T_{1,0}} + r_1 \times C\right) \cdot \cos\alpha}{1 - \exp\left(-\frac{TR}{T_{1,0}}\right) \cdot \cos\alpha}$$

Where $S_0$ is the baseline signal, $TE$ the echo time, $TR$ the repetition time, $\alpha$ the flip angle, $T_{1,0}$ the baseline longitudinal

relaxation time, $C$ the CA concentration, and $r_1$ and $r_2$ its longitudinal and transversal relaxivities, respectively. This relationship is clearly nonlinear.

However, as seen in Figure 1, the relationship may be made linear for a limited range of concentration. This is not straightforward for renal imaging, though, because urine concentration along nephron involves variation of approximately 100-fold in concentration.

Alternatively, instead of using the classical MRI signal, it is possible to assess directly the magnetization from a signal phase map, which is not straightforward but is linear. This technique was suggested only recently[6] and has not been used for renal imaging yet.

Linearity is decisive for quantification because adding the signal from small volumes inside a bigger voxel will make sense only if the signal is linear. Also, one should remain within the ascending part of the curve to be able to infer concentration from signal.

### Sensitivity to Diagnostic Agents

Typical amounts of tracer that are injected in NM range approximately 100 MBq. Considering a period of a few hours, the amount of radioactive injected activity is approximately 10 pmol. PET tracers show even more sensitivity. In contrast, typical amounts of gadolinated CM that are injected in MRI range approximately 10 mmol. The factor of $10^9$ explains the lack of toxicity for NM tracers. Even if MRI tracers are safe in most cases, the discovery of nephrogenic systemic fibrosis (NSF; see the subheading "Gadolinium-Based Contrast Agents") has become quite a concern in renal imaging for patients with severe renal failure or under dialysis. As summarized by Blankenberg and Strauss,[7] "MRI requires concentrations of paramagnetic substances of about $10^{-6}$ molar to change relaxivity, while iodinated CAs require about $10^{-2}$ molar to achieve opacification with CT . . . radiotracers are useful to evaluate high affinity, low abundance systems . . . will not cause a *pharmacological effect* . . . biological systems that are particularly sensitive to small quantities of material, such as many receptor systems and intra-cellular processes, can be readily evaluated with radiotracers."

## Toxicity of Contrast Agents

Radiological CAs are widely used in daily practice, providing radiological images with fundamental information about vascular lumen, perfusion of tissues, and renal function. However, they have various levels of toxicity, which have to be taken into account before administration.

### US Contrast Agents

***Agents.*** US CAs used in clinical practice are based on microbubbles between 2 and 6 μm in diameter that are filled with a high-molecular-weight gas (as perfluorocarbon or sulfur hexafluoride) with low solubility in the blood.[8] There are several types of shells with different rigidity levels (eg, denaturated albumin, phospholipids, surfactant, cyanoacrylate). These microbubbles do not diffuse into the interstitium and have a pure intravascular distribution. The gas is exhaled via the lungs 10 to 15 minutes after injection whereas the components of the shell are metabolized or filtered by the kidney and eliminated by the liver.[9]

***Toxicity.*** The incidence of adverse reactions in humans is very low, much lower than with iodine-based contrast agents. They are not nephrotoxic. They may be responsible for hypersensitivity reactions with an incidence around 0.001%.[10] Reactions are usually transient, and of mild intensity. However, hypotensive reactions have been observed and rare deaths have been reported in cardiac patients with severe ischemic cardiac disease.[11]

### Iodine Contrast Agents

Iodine CAs are the most widely used in the world, the greatest proportion being injected intravenously during CT scan examinations. The second application concerns intraarterial injections during arteriography and angiocoronarography.

***Agents.*** Iodine agents are classified according to their physicochemical properties: osmolality, viscosity, and ionicity. The osmolality depends on the ratio of the number of iodine atoms and the number of particles per molecule. Ionic monomers have the greatest osmolality (high osmolality CM) with 3 atoms of iodine for 2 particles (ratio = 1.5). Nonionic monomers and ionic dimers have a lower osmolality (low osmolality contrast media [LOCM]) but still superior to plasma (ratio = 3). Only nonionic dimers show an iso-osmolality with plasma (ratio = 6). However, the viscosity of iso-osmolar CAs is much greater than LOCM.

***Toxicity.*** The general toxicity of iodine CAs is related either to allergic-like accidents, such as cutaneous, respiratory, or anaphylactoid reactions, or to direct toxic effects, as renal, cardiac, or neurological reactions. All these effects are described in numerous reviews and books. In this article, we focus on the nephrotoxicity of these agents, the so-called contrast induced nephropathy (CIN).

CIN is defined as a sudden rapid deterioration of renal function that results from parenteral administration of contrast media (CM). This renal function deterioration has been variably defined as a minimum increase from baseline serum creatinine (SCr) values of 25% to 50% or an absolute increase in SCr of 0.5 to 1.0 mg/dL (44 μmol/L) within 3 days after injection.

The physiopathology of CIN is complex and associated with several mechanisms, including hypoperfusion of the medulla, direct tubular cell injury, and tubular obstruction by casts.[12] Osmolality and viscosity of agents are responsible for most of these effects.

Although CIN is exceptional in patients with normal renal function, it occurs in patients with impaired renal function and more frequently when diabetes mellitus is associated. However, its incidence has been reported to range from less than 1% to more than 30% in patients with impaired renal function. This wide variation is the result of several factors, including a lack of consensus in definitions, assessments based on SCr levels rather than measures of kidney function, differing patient populations (such as inpatients vs outpatients), wide variability in CM doses, variation in timing of

patient follow-up, variation in the patient's hydration state and, finally, the different routes of administration (intraarterial vs intravenous).[13]

In a recent review of the literature, Rao and Newhouse[14] reported that only 40 among more than 3081 publications analyzed renal toxicity after intravenous injection of CM. In only 2 of these[15,16] was the incidence of postcontrast renal dysfunction compared with the incidence of renal dysfunction in a matched control group of patients who did not receive contrast material. This is of primary importance, taking into account the fact that creatinine level increases in patients who are not receiving contrast material, as often as it does in published series of patients who are receiving contrast material.[17] Therefore, the incidence of CIN may have been largely overestimated when the intravenous route is used. This discrepancy was recently highlighted in a recent review,[18] in which the authors stated that "[t]Thresholds of creatinine above which *CM* are withheld for *CT* should be increased to improve the accuracy of *CT* examinations."

High osmolality CM have been abandoned for intravascular injection because of their greater nephrotoxicity, demonstrated by several studies.[19] They are still used only for local administration. The benefits of using iso-osmolar contrast media (IOCM) versus LOCM remain controversial. Authors of the Nephrotoxic Effects in High-Risk Patients Undergoing Angiography (NEPHRIC) study suggested that IOCM induced a lower incidence of CIN than LOCM in diabetic patients with moderate chronic kidney disease after intra-arterial administration.[20] These results were not confirmed by other studies.[21-24] Conversely, it is clear there is no advantage with IOCM for intravenous studies.[25] The absence of significant difference between these 2 classes is probably attributable to the greater viscosity of IOCM, which is more than 5-fold greater than plasma viscosity. Viscosity may be responsible for altered rheological properties, perturbation of renal hemodynamics, and increased tubular resistance.[26,27]

Prevention of CIN is now well advertised in many recommendations and web sites, such as the European Society of Urogenital Radiology site (http://www.esur.org). Even if these guidelines differ slightly regarding advice on the prophylactic use of drugs and the isoosmolar dimer to reduce the incidence of CIN, consistency was found in relation to the importance of hydration, cessation of intake of nephrotoxic drugs, and administration of the lowest-possible dose of contrast medium.[28]

### Gadolinium-Based Contrast Agents

***Agents.*** Regular low-molecular-weight gadolinium (GD) chelates (a chelate is molecule multiply bounded to a single central metal ion) are the only MRI agents used in clinical imaging. They are molecules of less than 1 kg/mol and can be considered as glomerular tracers, because, like iodine contrast agents used for X-rays and technetium-99m-labeled diethylenetriamine penta-acetate ($^{99m}$Tc-DTPA) or $^{51}$Cr-ethylene diamine tetraacetic acid used in NM, these chelates are freely filtered at the first pass by the glomeruli without any tubular secretion or reabsorption. They are nonspecific agents with unrestricted interstitial diffusion, resulting in a large volume of distribution (distribution volume of 269 ml/kg for gadolinium-tetraazacyclododecanetetraacetic acid [GD-DOTA]), including plasmatic and interstitial volume, and a rapid plasma clearance (5 mL/min/kg) implying a relatively short blood half-life (<1 h). The elimination half-life is prolonged in patients with renal insufficiency and may exceed 30 hours.

Free gadolinium (Gd) is classically recognized as being toxic, which is why Gd is linked with a ligand. Two structurally distinct categories of ligands are used: (1) "'macrocyclic"" chelates (GD-DOTA, Gd–HP-DO3A, or Gd-BT-DO3A) where Gd is "'caged"" in the preorganized cavity of the ligand, and (2) open chain, "'linear"" chelates (ie, Gd- diethylenetriamine penta-acetate [DTPA], Gd-BOPTA, Gd-DTPA-BMEA, Gd-EOB-DTPA, or Gd-DTPA-BMA).[29] The differences in the stability between the commercially available Gd-CA have not shown important differences in the safety of these agents. Nevertheless, the recently observed strong association between Gd-chelates and nephrogenic systemic fibrosis NSF suggests that it is probable the stability of the chelate is clinically relevant in patients with marked renal impairment.[30]

## Toxicity

GD-based contrast agents can induce CIN, which is identical to the CIN from iodinated contrast material.[31] Although patients in renal failure are at risk for developing CIN with double dose of GD-based agents, they are probably not at risk with lower dosage.

NSF was first described in 1997 in patients with end-stage renal disease. It is characterized by scleroderma-like skin changes that mainly affect the limbs and trunk. The induration of the skin can progress to cause flexion contracture of joints. The fibrotic changes also may affect other organs, such as muscles, heart, liver, and lungs. The disease can be aggressive in some patients, leading to serious physical disability or even death.[32] Most of cases were encountered with administration of nonionic linear chelates and, to a lesser degree, with ionic linear ones.

Patients with renal insufficiency, including those with chronic renal insufficiency in whom the glomerular filtration rate (GFR) is less than 30 mL/min/1.73 m$^2$, those with acute renal insufficiency, and those under dialysis, are at risk of developing NSF.[33,34] The exact pathophysiology of NSF remains unknown. A possible etiologic factor involved in the development of NSF is the dissociation of the Gd ion from its ligand by a process of transmetallation, which interferes with the regulatory action of signals that inhibit the differentiation of monocytes to fibrocytes.[35]

It has been suggested that different Gd-based CAs may have different frequencies of association with the development NSF, depending on their thermodynamic stabilities and dissociation constants.[29,30] The other risk factors thought to be associated with renal impairment for NSF development include edema, metabolic acidosis, thrombotic events, high-dose erythropoietin, systemic inflammation, and recent surgery.[36] Gadodiamide (Omnipaque; GE Healthcare, Madison, WI), which has been the agent most commonly reported to

be associated with the development of NSF, has one of the lowest thermodynamic stability constants and one of the greatest dissociation rates compared with other agents.[37] Therefore, Omnipaque, Optimark, and Magnevist have been contraindicated in Europe in high-risk patients. In these patients, it is recommended to use the most stable agents with macrocyclic ligands and the lowest possible dose.[38] Determination of SCr is recommended in patients at risk of renal impairment only. Patients with a GFR lower than 30 mL/min but not undergoing chronic hemodialysis are at risk of both CIN and NSF. An alternative study, such as US or NM, is preferred if possible. If not, the risk-benefit of each technique must be weighted.

# Available Parameters

Considering the various techniques and many available tracers, several parameters can be assessed with functional renal imaging.

## Relative GFR

It is now validated that NM dynamic renal scanning provides reliable relative GFR (percentage of right/left function). Selective ureteral catheterization is no longer performed for this indication. In recent years, several techniques have been proposed to provide the same parameters with MRI with the use of dynamic contrast-enhanced techniques and either "surface" techniques or Patlak-Rutland techniques or deconvolution analysis (usually referred to as "compartmental" analysis).[39-41] These techniques provide encouraging results but they are hampered by the lack of linearity. Further validation is needed though.

## Absolute GFR

Many techniques have been proposed in NM to obtain an absolute value for GFR. Among these techniques, the Gates' method[42] is probably the most famous. However, the effective precision is even less than that obtained with creatinine-based formulas.[43] Attempts have also been made with MRI without any convincing validation.[44]

## Perfusion

Perfusion studies have been carried out with MRI with either contrast agent labeling or magnetic labeling: either transversal (phase-contrast measurements, in fact measuring renal blood flow)[45] or longitudinal (arterial spin labeling, measuring true perfusion).[46] Semiquantitative techniques have been published in NM for perfusion, the most physiological of which by Peters et al.[47] However, no validation has been published and both the accuracy and precision of this technique remain doubtful. US studies have been used with microbubbles contrast.[48]

## Drainage

Many drainage indexes have been proposed in the last 50 years in NM,[49] with only a few of them validated in some clinical situations. Similar indexes probably could be proposed in MRI and better resolution could improve the separation between cortex and medulla. However, in most MR studies, the term "transit time" only refers to the delay in contrast appearance in the pelvis.

## Oxygen Imaging

Functional MRI has been used for decades to image brain activity with a technique called BOLD (blood oxygen level-dependent). A greater brain activity induces an increase blood flow with a paradoxical decrease in deoxyhemoglobin concentration. Because the deoxyhemoglobin has a paramagnetic effect, brain activation increases MRI signal. In kidney, Prasad et al[50] showed that the same technique can image the effects of furosemide on medulla (decreased oxygen consumption) as an increased signal in T2*-weighted images.

## MRI Diffusion Imaging

Diffusion MRI has the ability to show the diffusion of water molecules in tissues at a microscopic scale, much beyond the spatial resolution. It has mostly been used in brain imaging, especially for early stroke imaging and tractography. However, it also has been proposed for renal imaging[51,52] and can yield information on microstructure (vessels, tubules, and swelling) in fibrosis, hydronephrosis, or renal masses. Nevertheless, this technique shows microanatomy rather than true function and diffusion does not directly show any physiological parameter, though correlation between diffusion coefficient and renal function has been shown.[53]

## MRI Sodium Imaging

MRI has the ability to detect other nuclei than hydrogen, including $^{23}$Na. Maril et al[54] showed that MRI sodium imaging was able to show the corticopapillary osmotic gradient (higher sodium concentration in the medulla). They also showed that the gradient increased after water deprivation. This technique, although rather preliminary, has the potential to show, in a noninvasive way, without any CA injection, the insight of one of the most important parts of renal physiology.

# New Imaging Agents

Molecular imaging is now clearly established as a cornerstone novel in science with a development driven by diverse modalities ranging from classical NM, PET, and MRI to OI techniques. Key points to be considered for development of new agents include the molecular imaging agent (probe or tracer), the localization of the target, which can be intracellular or cell surface proteins, and the performances of the detection system.

Current evaluation of radiopharmaceuticals requires a first step of assessment in animal models. The variety provided by novel chemistry and the availability of isotopes with compelling physical properties, are fully in line with PET imaging requirements. Since 1970, refined analytical techniques have been developed that allow chemists to understand the actual structure of many materials at atomic and molecular scale. Soft chemistry processes are well suited for the construction of novel materials with original structures and shapes. Currently, chemical processes enable the controlled design of

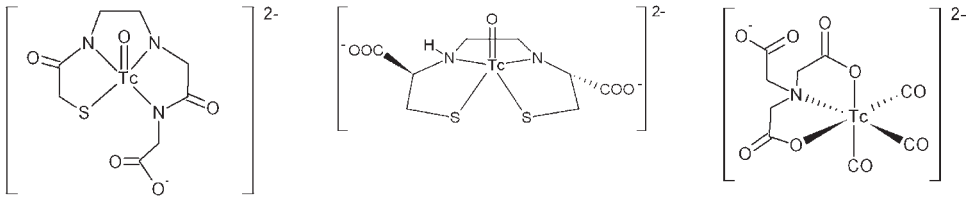


**Figure 2** Chemical formulae for MAG3, EC and NTA.

new materials for applications in nonclinical and clinical imaging, such as nanotubes of carbons, inorganic quantum dots (QDs) or organic nanoparticles.

There is an increasing demand for imaging tools to perform noninvasive experiments in living systems for biomedical research and medicine. During the last decade, imaging methods have increasingly served for biodistribution studies, thus allowing a significant reduction in the number of required animals and significantly more experimental data to be harvested for each animal in longitudinal studies. However, the high cost of several of these techniques and several technological barriers limit their widespread use. In view of the soaring costs of clinical drug development, the optimization of early stage trial design is mandatory to maximize the collection of evidence for efficacy and proof of mechanism, end points that have, in several examples, already been provided by molecular imaging.

We shall briefly review recent development in evaluation of renal function. As for any drug agents, it is difficult to find animal models that mimic human conditions. Imaging of biological pathways and measurements of physiological functions are based on specific molecular probes that allow quantification of specific processes. We must keep in mind that the variety of interspecies changes and our limited knowledge of pathophysiological pathways explain a great part of the failures when translating nonclinical results to human beings. We shall not dwell upon the extensive ongoing research in PET imaging because another article in the same issue addresses this point.[1] In the last section, we shall review 2 general approaches to optimize production of novel molecules.

## Radiopharmaceutical Diagnostic Products for Evaluation of the Renal System

The targets of molecular imaging proteins can be membrane receptors or proteins involved in cellular metabolism or in cell functions or RNA (antisense imaging). On renal tubular cells, cell surface proteins antigens and receptors have been identified as potential targets. Most recent studies of new PET radiopharmaceuticals have focused on angiotensin receptors. The angiotensin II subtype 1 receptor (AT1R) has been linked to the development and progression of renovascular hypertension. AT1R PET measurements have been published with the radioligand ATR1 $C^{11}$-KR 31173,[55] renin receptors, and vascular endothelial growth factor receptors. The cell surface CAIX (carbonic anhydrase type IX) is also an excellent candidate as it is overexpressed in clear cell renal carcinoma. Targeting this enzyme is currently investigated in both diagnostic imaging and radioimmunotherapy.

Regarding the evaluation of renal function, the most used diagnostic agents have the same class of molecular targets, ie, membrane transporters OAT and OCT (organic anion/cation transporter). These active carriers are well known and described as in their mechanism of action for their affinities for different radiopharmaceuticals (para-aminohippuric acid ortho-iodohippurate (OIH), and $^{99m}$Tc-mercaptoacetyltriglycine) or inhibitors (probenicid). The goal of many teams during the last decade was to find a renal tracer capable to measure effective renal plasma flow (ERPF).

## Established Tracers in NM

As a glomerular tracer, $^{99m}$Tc-DTPA could hardly be improved (freely filtered, not reabsorbed). In fact, is has no molecular renal specificity (DTPA is used in MRI as a general CA). Most of the tracer research has thus concentrated on tubular tracers, which are more specific to kidneys.

### Development History of Radiopharmaceuticals Designed for the Assessment of Renal Function

Search for the ideal radiopharmaceutical to measure ERPF has been underway since the early 1960s.[56] Para-aminohippuric acid is considered the gold standard for measurement of ERPF, but it is not used in routine clinical practice because of the lengthy chemical analysis procedure. A simpler, yet very accurate method was introduced with OIH labeled with iodine-131, which offered the advantage of speed and simplicity. Its major drawback is related to the fact that the permissible maximum activity administered is so low that it precludes precise evaluation of the angiogram phase of the renogram suboptimal imaging characteristics of the 364-keV photon of $^{131}$I and the delivery of relatively high radiation doses to kidney and thyroid in patients with impaired renal function. Labeling with iodine-123 resulted in better imaging properties but, due to restricted availability and high costs, it did not gain widespread use. Nonetheless, OIH remains the method of reference.

Efforts then focused on the development of $^{99m}$Tc agents because of their favorable physical properties (monochromatic energy emission, short physical half-life, low cost, and great availability). Progress in $^{99m}$Tc radiopharmaceuticals relies principally on 3 factors: development of highly specific tracer agents, better imaging quality, and convenience of use (easy labeling and storage). $^{99m}$Tc-mercaptoacetyltriglycine ($^{99m}$Tc-MAG3) has a [$^{99m}$Tc(V)O] core (see Fig. 2) and was

introduced in 1986 in Europe to compensate the limitations of $^{131}$I–OIH. It became the radiopharmaceutical of choice in the evaluation of transplant kidney, diagnosis of tubular necrosis, and scintigraphic study of tubular function. The image quality of the $^{99m}$Tc-MAG3 images is superior to that of $^{131}$I–OIH. Moreover, the extraction fraction of $^{99m}$Tc-MAG3 is substantially greater than the 20% extraction fraction of $^{99m}$Tc-DTPA. The greater extraction of $^{99m}$Tc-MAG3 led to its superior performance compared with $^{99m}$Tc-DTPA in adult and pediatric patients with suspected obstruction. However, it is not the perfect replacement for OIH as high and variable plasma protein binding (75%-90%) and clearances that are only 50% to 60% of the OIH clearance make accurate measurements of ERPF difficult. A small part of $^{99m}$Tc-MAG3 activity is eliminated via the hepatobiliary pathway and this percentage increases in patients with reduced renal function; the resulting activity in the gallbladder has been mistaken for activity in the kidney.[57] Another drawback is related to the preparation and formulation of the compound, which requires with some kits to be heated at 100°C for 10 minutes and to be stored in the dark at 4°C after reformulation to prevent oxidation.

Nevertheless, despite improved image quality and diagnostic superiority over $^{99m}$Tc-diethyltriaminepentaacetic acid, $^{99m}$Tc-MAG3 still has its limitations. A larger issue is the fact that the clearance of $^{99m}$Tc-MAG3 is only 50% to 60% of the clearance of $^{131}$I-OIH;[58-60] the fact that $^{99m}$Tc-MAG3 does not provide a direct measurement of ERPF led Jafri et al[61] to conclude that $^{99m}$Tc-MAG3 is not suitable as a replacement for $^{131}$I-OIH for the measurement of ERPF. Another issue reported in the literature is the reproducibility of $^{99m}$Tc-MAG3 clearance based on plasma sample measurements.

The reproducibility of the plasma sample $^{99m}$Tc-MAG3 clearance was evaluated by Kotzerke et al,[62] and they concluded that it was not precise enough to evaluate a change in kidney function after surgery or chemotherapy.

### $^{99m}$Tc-N,N′-Ethylene-(L, L)-Dicysteine ($^{99m}$Tc-L, L-EC)

This molecule has also a [$^{99m}$Tc(V) O] core. The kit for the preparation of $^{99m}$Tc-N,N′-ethylene-(L,L)-dicysteine ($^{99m}$Tc-L,L-EC) was developed by IZOTOP, a pharmaceutical firm based in Budapest, Hungary, and granted a marketing authorization in 1992, based on prevailing Hungarian national regulatory standards.

EC belongs to the tubular tracer class,[59] which includes OIH, para-aminohippuric acid, and MAG3. These agents are secreted by an active transport system on which plasma protein binding has limited impact in contrast to GFR. This renal transport system for organic anions can be saturated and the subject of drug interactions. Numerous drugs, including probenecid and beta lactamines, have been described as substrates for this transport system. The first publication highlighting the potential value of $^{99m}$Tc-L,L-EC as an alternative for MAG3 was published in 1990.[58] The structures of MAG3 and L,L-EC contain an oxotechnetiumglycine sequence. One can assume that this structural moiety mimics the carbonyl glycine sequence in the side-chain of hippuran, which is generally believed to be essential for an efficient fit with the tubular receptor proteins.

$^{99m}$Tc-EC is a metabolite of ECD (ethylene cysteine dimer), which is rapidly excreted by the kidney. ECD is a radiopharmaceutical for evaluation of the cerebral perfusion marketed as Neurolite. From a chemical standpoint, EC corresponds to *N*,*N*′-ethylene-L, L-dicysteine, one of the 4 isomers that can be produced during a cysteine-based synthesis. The L,L isomer is obtained by stereo-specific synthesis based on L-cysteine. The quality of the final product after labeling can be confirmed by high-performance liquid chromatography. The choice of the L,L isomer is based on the results of preclinical studies performed mainly on murine species. The reference product, MAG3 has a lower clearance than hippuran, related to higher plasma protein binding. The research by Eshima et al[63] clearly demonstrated that the 3 EC isomers, (D,D), (D, L), and (L, L) had higher EC/OIH extraction fraction ratios (0.88-0.99) than that of $^{99m}$Tc-MAG3 (0.71) at an albumin concentration of 2.5 g/dL.

The renal excretion characteristics of $^{99m}$Tc-L,L-EC are almost identical to those of $^{99m}$Tc-MAG3 and OIH. The quality of dynamic images and the shape of the typical 3-phase renogram also have demonstrated very similar scintigraphic properties to $^{99m}$Tc-MAG3. However, a major feature of $^{99m}$Tc-L,L-EC over $^{99m}$Tc-MAG3 is its negligible hepatobiliary localization and its high kidney-to-background ratio, which improve the renal delineation[64-66] and provide better image quality even in patients with severe renal failure.

### $^{99m}$Tc(CO)$_3$-Nitrilotriacetic Acid (NTA)

This new molecule has a different core, a tricarbonyl core, which has a high chemical stability. Studies in rats showed that the pharmacokinetics of the tricarbonyl core radiopharmaceutical $^{99m}$Tc(CO)$_3$-nitrilotriacetic acid, $^{99m}$Tc(CO)$_3$(NTA), were identical to those of $^{131}$I *ortho*-iodohippuran. Taylor et al[67] reported the pharmacokinetics of these 2 tracers in healthy volunteers in 2010. The aminopolycarboxylate ligand, $^{99m}$Tc(CO)$_3$(NTA), is characterized by kinetic stability, small size of the complex, and high hydrophilic properties favoring its secretion by tubular transport systems. It was prepared with commercially available NTA and a commercially available kit and isolated by reversed-phase high-performance liquid chromatography. Both drugs were simultaneously injected with, respectively an activity of 74 MBq for $^{99m}$Tc(CO)$_3$(NTA) and 9.25 MBq for $^{131}$I-OIH in 9 healthy volunteers. Acquisition of images of each renal tracer was performed for 24 minute. Plasma clearances were determined from 8 blood samples obtained 3-90 minutes after injection by the use of the single-injection, 2-compartment model. There was no significant difference in the plasma clearances of $^{99m}$Tc(CO)$_3$(NTA) 475 ± 105 mL/min and $^{131}$I-OIH, versus 472 ± 108 mL/min. The plasma protein binding of $^{99m}$Tc(CO)$_3$(NTA) was 43% ± 5%, which was significantly lower ($P < 0.001$) than the plasma protein binding (75% ± 3%) of $^{131}$I-OIH. Image quality with $^{99m}$Tc(CO)$_3$-nitrilotriacetic acid provided renal images of excellent quality, and the parameters estimated from the analysis of renograms were similar to those obtained with $^{131}$I-OIH. These preliminary results in young healthy volunteers suggest that $^{99m}$Tc(CO)$_3$(NTA) could take an important place in the armamentarium of renal diagnostic agents.

# New Directions

Beyond these tubular tracers, other modern techniques can be used to target new CAs. Although no renal tracer obtained with these techniques is currently available, it may interest the reader to have an overview of what could provide us with new renal tracers tomorrow.

## Optical Imaging

OI approaches are attractive low cost alternatives to NM imaging. Development of new and more sensitive optical sensors have benefited from semiconductor nanocrystals, fluorescent proteins, or near-infrared fluorescent molecules.[68] These techniques have very high sensitivity levels ($10^{-15}$-$10^{-17}$ mol/L for bioluminescence, $10^{-9}$-$10^{-12}$ mol/L for fluorescence). They enable real time imaging of small animal models. Their major disadvantage when using visible light is poor tissue penetration (1 cm) due to scatter and light absorption. Fluorescent probes are used but still present numerous disadvantages, including autofluorescence from tissue organic components due to constant probe illumination during signal acquisition. This autofluorescence often results in poor signal-to-noise ratio and major difficulties for deep tissue imaging caused by intrinsic tissue signal attenuation. The probe's emission has thus to be tuned in the tissue transparency window (wavelength from 650 nm to the infrared), in which light attenuation is largely due to scattering rather than to absorption.

Bioluminescence has some advantages over fluorescence because the signal is produced in the presence of substrate (no excitation light source is needed) and there is no background autofluorescence. In all cases, tomographic imaging remains a challenge. It is also possible to perform in vivo imaging of tumors with protease-activated near-infrared fluorescent probes.

New technologies to produce long luminescent nanoparticles emitting in the red to near-infrared range are emerging. The probes can be optically excited before in vivo local or systemic injection (see Fig. 3). The long-lasting afterglow (also called persistent luminescence) can reach several hours and permits the removal of the background. As noise is originating from in situ excitation, the significant signal-to-noise ratio improvement allows detection in rather deep organs and real-time biodistribution monitoring of active elements hours after injection.[69]

Semiconductor QDs are also useful compounds for an effective approach to fluorescent nanotools. These inorganic nanoparticles are characterized by large 1- and two-photon absorption cross-sections, good fluorescence quantum yields, broad excitation but narrow emission bands, and high photostability. So they have a particular interest for in vitro and in vivo imaging with an emphasis on specific labeling of cells and tissues. Because their emission spectra can be tuned by playing on their size and composition, they can be used for multicolor imaging. QDs are superior to traditional organic dyes: It has been estimated that QDs are 20 times brighter and 100 times more stable than traditional fluorescent reporters (much less photobleaching). For single-particle tracking, the irregular blinking of QDs is a minor drawback.

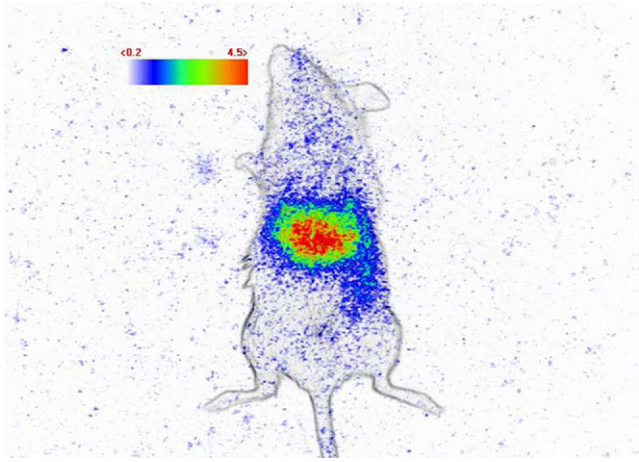


**Figure 3** Image of persistent luminescent nanoparticles developed at the Unité de Pharmacologie Chimique et Génétique et d'Imagerie (UPCGI, CNRS UMR8151, Inserm U1022, ENSCP, University Paris Descartes) injected into a mouse and observed for 15 minutes under an ICCD camera. (Courtesy of Dr. C. Richard.)

The drawbacks of inorganic QDs are the risks of biological toxicity, due in particular to the presence of heavy metals, such as cadmium and blinking. Their surface functionalization is possible but demanding. These inorganic nano-objects also raise several questions with respect to environmental issues. It explains the recent development of organic dots by only a few teams.[70]

## Monoclonal Antibodies

New models for the optimization of imaging agents have been developed. As an example, we shall review recent data relating to antibody based vectors. Monoclonal antibodies (mAbs) are very attractive candidates in NM both for diagnosis and targeted therapy as these large molecules have several advantages. mAbs are high-affinity molecules that recognize cell surface molecules with a high specificity, thus providing high detection signal. In current practice, clinical development of mAbs has been slow and often disappointing mainly due to short half-life, the existence of human antimouse antibody responses, and weak performance of rodent mAbs in human beings. Progress in antibody engineering technologies now enables industrial scale synthesis of fully human or humanized mAbs. These novel antibodies do not trigger immunogenic reactions, but very few human mAbs have been successful as diagnostics agents. A major drawback of using intact antibodies as imaging probes is that they circulate in the blood for several days, making them unsuitable as imaging probes (clearance rate of Fab or Fab' > F[ab'] 2 > immunoglobulin G).

Research is underway to improve antibody pharmacokinetics and retain specificity. Protein engineering gave birth to a series of antibody variants that differ in antigen binding sites and size. They are being evaluated as potential imaging probes. It is easy to produce Fab fragments by proteolysis; the main disadvantage of Fab is that it has only 1 antigen binding site per molecule, thus reducing the overall functional affinity

of the antibody. The first idea was to work with single-chain variable fragment scFv composed of variable light and heavy domains joined by a flexible peptide linker. However, limitations of this approach are the fact that we have fast clearing fragments (scFv; 25 kg/mol), with 1 antigen-binding site (monovalent) and consequently a low accumulation in tumors because of the low exposure time to the target. Reflecting on the strategy regarding the size of the fragments is essential because larger fragments exhibit usually better tumor penetration, and excellent tumor to blood ratios.

Once the optimal antibody-based vector has been designed and generated, several modalities of labeling can be used to label the candidate vectors. Optical probes can be used in nonclinical studies. The authors of several nonclinical studies have used antibody fragments conjugated to fluorescence (near infrared dyes), bioluminescence (luciferases), or QDs. They can be tagged with radionuclides (PET, single-photon emission computed tomography), or magnetic nanoparticles (MRI). As an example, the recent work evaluating Nanoprobes of iron oxide Nanoprobes conjugated to Herceptin may make Ab fragments attractive MRI agents.

## Aptamers

A new model of production of imaging agents is provided by aptamers. An aptamer is a synthetic oligonucleotide, usually an RNA which can bind a specific ligand and sometimes catalyzes a chemical reaction on the ligand. Most often, aptamers are synthetic compounds, isolated in vitro from combinatorial libraries of many compounds by a random sequence selection iterative method called "systematic evolution of ligands by exponential enrichment" (SELEX). The SELEX method is based on a library of oligonucleotides whose sequence to the 5' end and 3' end is known and whose core sequence is random. This collection of oligonucleotides is prepared according to the same principle of synthetic oligonucleotides. During steps of the elongation of nucleotides of the central region, the 4 bases are used to react instead of only put a single base as for synthesis of a specified nucleotide. Thus, this method provides a collection of oligonucleotides with different central sequences. The SELEX method selects the oligonucleotides that bind with greater affinity with a target thanks to affinity chromatography is used to select these candidate molecules. Then, the oligonucleotides are separated from the target molecules and are amplified by cloning or polymerase chain reaction. The stage of selection of oligonucleotides with high affinity for the target molecule and the amplification step are repeated several times to obtain oligonucleotides with the highest affinity for the target. The oligonucleotides selected are called aptamers.[60]

The concept is the screening of libraries in vivo allowing a direct approach in living animals. It requires parallel chemical synthesis and radiolabeling of the bank ($^{11}C$ or $^{18}F$). Pharmacokinetic studies by PET imaging in rats give information on metabolic pathways in vivo and on the transfer through the brain-blood barrier. Aptamers are biochemical tools that can be used in diagnostic or therapeutic applications depending on the target against which they are directed. As regards to their selectivity and binding properties to ligands, aptamers are often compared with antibodies. The molecular weight of aptamers is approximately 9 to 15 kg/mol compared with 150 kg/mol for immunoglobulin G and their potency to discriminate for example theophylline and caffeine is excellent. Constants of affinity range from 5 to 100 nmol/L.

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