

Inulin and Other Renal Function Biomarkers: Current Standards, Practical Alternatives, And Emerging Approaches

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ABSTRACT

Accurate assessment of glomerular filtration rate is essential for diagnosing and staging chronic kidney disease, evaluating potential kidney donors, adjusting drug doses, and monitoring renal function in critically ill patients. Inulin clearance remains the physiologic reference method for measured glomerular filtration rate because inulin is freely filtered by the glomerulus and is neither reabsorbed, secreted, nor metabolized by the kidney. However, its requirement for continuous infusion, timed blood and urine collection, and technically demanding laboratory procedures limits routine clinical use. Iohexol plasma clearance has emerged as the most practical and extensively validated exogenous alternative, offering acceptable accuracy, low toxicity, and simpler sampling protocols. Endogenous biomarkers, particularly serum creatinine and cystatin C, remain the basis of routine estimated glomerular filtration rate equations. Creatinine is inexpensive and widely available but is affected by muscle mass, diet, age, sex, and tubular secretion. Cystatin C is less dependent on muscle mass but may be influenced by inflammation, corticosteroid exposure, obesity, smoking, and thyroid dysfunction. Combined creatinine–cystatin C equations generally provide greater accuracy than either biomarker alone. Emerging biomarkers, including D-serine, D-asparagine, and proenkephalin, may improve kidney function assessment in selected populations but remain investigational. This review summarizes the physiologic basis, clinical utility, limitations, and future role of inulin and other markers used to measure or estimate renal function.

KEYWORDS: inulin clearance; glomerular filtration rate; iohexol; creatinine; cystatin C; D-serine; proenkephalin; renal biomarkers.

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INTRODUCTION

Glomerular filtration rate is widely considered the best overall index of kidney filtration function. It is used to diagnose and classify chronic kidney disease, guide drug dosing, assess living kidney donors, evaluate patients with atypical body composition, and monitor changes in kidney function over time.

Glomerular filtration rate can be evaluated in three principal ways:

Direct measurement using an exogenous filtration marker.

Estimation from endogenous serum biomarkers.

Use of emerging biomarkers designed to improve accuracy in specific populations.

Inulin clearance remains the traditional reference method for measured glomerular filtration rate. However, its complexity has limited its clinical availability, prompting the use of alternative exogenous tracers such as iohexol and routine estimated glomerular filtration rate equations based on creatinine and cystatin C [1–3].

No single biomarker is ideal in every patient. The appropriate method depends on the clinical question, expected level of kidney function, body composition, comorbidities, feasibility, and required precision.

METHODS

This narrative review was developed from the uploaded literature synthesis on inulin and other renal function

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biomarkers. The review focused on measured glomerular filtration rate methods, endogenous estimating markers, practical clinical limitations, special populations, and emerging biomarkers. References are numbered according to their first appearance in the manuscript.

Physiologic Requirements of an Ideal Filtration Marker

An ideal glomerular filtration marker should:

Be freely filtered by the glomerulus.

Not bind significantly to plasma proteins.

Not be reabsorbed by renal tubules.

Not be secreted by renal tubules.

Not be synthesized or metabolized by the kidney.

Be physiologically inert and nontoxic.

Remain stable during measurement.

Be inexpensive and easy to quantify.

Inulin closely approximates these characteristics, which explains its historical role as the reference marker for measured glomerular filtration rate [2,4].

Inulin Clearance

Physiologic Basis

Inulin is an exogenous fructose polysaccharide that is freely filtered across the glomerular capillary membrane. Under standard conditions, it is not meaningfully reabsorbed, secreted, synthesized, or metabolized by the renal tubules.

Measurement Protocol

Traditional inulin clearance requires:

Intravenous administration of inulin.

A loading dose followed by continuous infusion.

Achievement of stable plasma concentration.

Timed urine collections.

Repeated blood sampling.

Accurate urine volume measurement.

In children or patients unable to void reliably, urinary catheterization may be necessary. Errors in urine collection can significantly affect the final result [2,4].

Advantages

The main advantages of inulin include:

Strong physiologic validity.

Minimal influence from muscle mass or diet.

Applicability in adults and children.

Utility as a comparator for validating other filtration markers.

Limitations

The method is rarely used in routine practice because it is:

Labor intensive.

Time consuming.

Expensive.

Dependent on accurate timed urine collection.

Technically difficult in small children and frail patients.

Not widely standardized across laboratories.

For these reasons, inulin is best viewed as a reference research method rather than a practical everyday clinical test [1,2].

Iohexol Plasma Clearance

Iohexol is a nonionic contrast agent that has become one of the most widely supported alternatives for measured glomerular filtration rate. It is administered intravenously,

and its disappearance from plasma is assessed using timed blood samples.

Advantages

Iohexol offers several practical benefits:

No timed urine collection is required in plasma-clearance protocols.

It has low toxicity at the small doses used for GFR measurement.

It has minimal clinically relevant protein binding.

Laboratory quantification is reproducible.

It is suitable for adults and children.

It can be used in outpatient settings.

Systematic review evidence suggests that iohexol plasma clearance provides acceptable accuracy compared with renal inulin clearance and is among the strongest candidates for a standardized common measured-GFR method [1,5].

Limitations

Potential limitations include:

Need for intravenous administration.

Dependence on accurate sampling times.

Longer sampling periods in patients with very low GFR.

Interlaboratory variation in assay methods.

Incomplete standardization of abbreviated sampling protocols.

In patients with advanced kidney dysfunction, delayed blood sampling is particularly important because early samples may overestimate the elimination rate and therefore overestimate GFR [5].

Other Exogenous Filtration Markers

Other measured-GFR tracers include:

Iothalamate.

Chromium-51 ethylenediaminetetraacetic acid.

Technetium-99m diethylenetriaminepentaacetic acid.

Other radiolabeled or nonradiolabeled compounds.

These methods may be accurate but are limited by radiation exposure, availability, assay complexity, regulatory requirements, or regional practice variation.

The choice of tracer often depends more on institutional resources than on a universally accepted hierarchy. Nevertheless, iohexol has gained increasing support because it avoids radiation and can be assessed using plasma clearance alone [1,5,6].

Serum Creatinine

Serum creatinine is the most commonly used endogenous marker of kidney function.

Physiologic Basis

Creatinine is generated from creatine metabolism, predominantly in skeletal muscle. It is filtered by the glomerulus but is also secreted by renal tubules. Consequently, creatinine clearance tends to overestimate true GFR, particularly when kidney function is reduced [4,7].

Advantages

Creatinine is:

Inexpensive.

Widely available.

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Rapidly measured.

Familiar to clinicians.

Incorporated into validated eGFR equations.

Non-GFR Determinants

Creatinine concentration is influenced by:

Muscle mass.

Age.

Sex.

Diet and meat intake.

Body size.

Amputation.

Cachexia.

Neuromuscular disease.

Critical illness.

Medications affecting tubular secretion.

A patient with very low muscle mass may have a deceptively low creatinine despite clinically important kidney dysfunction. Conversely, a muscular individual may have an elevated creatinine despite preserved filtration.

Creatinine-Based eGFR

Modern equations estimate GFR using serum creatinine together with demographic variables. These equations are generally more useful than isolated creatinine values but remain estimates rather than direct measurements.

Creatinine-based eGFR may be unreliable when:

Kidney function is changing rapidly.

Muscle mass is markedly abnormal.

The patient is critically ill.

Extreme body size is present.

Precise GFR measurement is clinically necessary.

Although it incorporates urinary excretion, creatinine clearance usually overestimates GFR because of tubular secretion. It is also highly vulnerable to incomplete or inaccurate urine collection.

For most stable patients, validated serum-based eGFR equations are preferred over routine 24-hour creatinine clearance. Measured creatinine clearance may still be useful in selected cases, but results should be interpreted cautiously [4,7].

Cystatin C

Cystatin C is a low-molecular-weight protein produced by most nucleated cells. It is freely filtered by the glomerulus and subsequently reabsorbed and metabolized in proximal tubular cells.

Advantages

Compared with creatinine, cystatin C is:

Less dependent on skeletal muscle mass.

Less affected by dietary meat intake.

Potentially more accurate in frailty, amputation, cachexia, or low muscle mass.

Useful as a confirmatory marker when creatinine-based estimates may be misleading.

Cystatin C has demonstrated stronger correlation with inulin-measured GFR than creatinine in some populations [8].

Limitations

Cystatin C is not exclusively determined by GFR. It may be influenced by:

Systemic inflammation.

Corticosteroid therapy.

Thyroid dysfunction.

Obesity.

Smoking.

Malignancy.

Critical illness.

Its performance may also be less reliable at very low levels of kidney function, and assay cost and availability remain barriers in some settings [8,9].

Combined Creatinine–Cystatin C Equations

Because creatinine and cystatin C are influenced by different non-GFR factors, combining both markers improves precision.

Combined equations generally:

Reduce error compared with either marker alone.

Improve classification near chronic kidney disease thresholds.

Help resolve discordant creatinine- and cystatin-based estimates.

Improve assessment in patients with atypical muscle mass.

Evidence suggests that combined creatinine–cystatin C equations may improve accuracy by approximately 10% compared with either biomarker used alone [6,9].

Combined estimation is especially useful when clinical decisions depend on a narrow GFR threshold, such as:

Drug dosing.

Kidney donation.

Chemotherapy eligibility.

Chronic kidney disease staging.

Referral for advanced renal care.

When Measured GFR Is Clinically Indicated

Directly measured GFR may be appropriate when estimated GFR is likely to be inaccurate or when a highly precise value is required.

Potential indications include:

Evaluation of living kidney donors.

Assessment before nephrotoxic chemotherapy.

Extreme obesity or very low body weight.

Severe malnutrition or cachexia.

Major limb amputation.

Neuromuscular disease.

Liver cirrhosis.

Unusual dietary intake.

Discordant creatinine and cystatin C results.

Research studies.

Selected pediatric cases.

Precise dosing of drugs with narrow therapeutic indices.

In these settings, iohexol plasma clearance is often a more practical option than inulin clearance [5,6].

Renal Biomarkers in Critical Illness

Assessment of kidney function in critically ill patients is particularly difficult because serum creatinine may lag behind

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acute changes in filtration. Fluid accumulation, muscle catabolism, reduced creatinine production, and rapidly changing renal function may produce misleading values.

Creatinine-based equations were primarily developed for steady-state conditions and should be interpreted cautiously in acute kidney injury.

Cystatin C may detect filtration changes earlier in some settings, but inflammation and corticosteroid exposure can also alter its concentration.

Measured GFR is rarely feasible during unstable critical illness, which has encouraged investigation of alternative biomarkers such as proenkephalin [10].

Proenkephalin

Proenkephalin is an endogenous peptide proposed as a marker of real-time kidney filtration.

In septic shock and critical illness, proenkephalin has demonstrated a stronger relationship with iothexol-measured GFR than creatinine-based estimates in some studies. Its potential advantages include:

Rapid response to changes in kidney function.

Less dependence on muscle mass.

Possible utility in non-steady-state conditions.

However, current evidence remains limited, and standardized thresholds, assay availability, and outcome-based validation are still needed before routine clinical implementation [10].

D-Serine

D-serine is an endogenous amino acid that has emerged as a potential filtration marker. In studies of living kidney donors and transplant recipients, D-serine clearance showed lower proportional bias than creatinine clearance when compared with inulin-measured GFR [11].

A combined D-serine–creatinine equation demonstrated high validation accuracy in a transplant-focused population.

Proposed advantages include:

Lower dependence on muscle mass.

Strong correlation with inulin clearance.

Potential improvement over creatinine alone.

Nevertheless, the available evidence remains concentrated in limited populations, particularly kidney donors and transplant recipients. Broad validation in chronic kidney disease, critical illness, pediatrics, and general populations is required [11,12].

D-Asparagine and Other Emerging Markers

D-asparagine is being evaluated alongside D-serine as another possible endogenous marker of filtration. Preliminary theoretical and clinical work suggests that these D-amino acids may reflect GFR while being less affected by muscle mass.

Additional emerging approaches include:

Biomarker panels rather than single markers.

Metabolomic profiles.

Proteomic signatures.

Real-time biosensors.

Machine-learning equations integrating clinical and laboratory variables.

No emerging endogenous biomarker currently matches the validation and standardization of measured exogenous GFR methods [12,13].

Biomarkers of Kidney Damage Versus Kidney Function

It is important to distinguish filtration markers from biomarkers of structural kidney injury.

Markers such as:

Urinary albumin.

Neutrophil gelatinase-associated lipocalin.

Kidney injury molecule-1.

Tissue inhibitor of metalloproteinases-2.

Insulin-like growth factor-binding protein 7.

may indicate kidney damage or tubular stress but do not directly measure GFR.

Kidney function and kidney injury are related but distinct concepts. A patient may have structural kidney injury before GFR falls, or reduced GFR without active tubular injury. Clinical interpretation should therefore combine filtration assessment with urinary protein, sediment findings, imaging, and other markers when appropriate.

Pediatric Considerations

Renal function assessment in children presents additional challenges because body size, growth, muscle mass, and developmental stage influence biomarker interpretation.

Inulin clearance remains a valid reference method but is particularly difficult in small children because of:

Continuous infusion requirements.

Timed urine collection.

Potential need for catheterization.

Blood-volume limitations.

Iothexol plasma clearance is often preferred when measured GFR is required because it can be performed using limited blood sampling. Creatinine-based pediatric equations remain useful in routine practice, while cystatin C may be helpful in children with atypical muscle mass [2].

Kidney Transplantation and Living Donation

Precise kidney function assessment is important before and after transplantation.

In living donors, measured GFR may be required because small errors can affect donor eligibility and long-term risk assessment. Iothexol and inulin remain appropriate reference methods, while combined endogenous estimates may assist screening.

In transplant recipients, creatinine may not accurately reflect early changes in graft function because of variable muscle mass, medications, and tubular secretion. Cystatin C, measured GFR, and emerging markers such as D-serine may offer complementary information [11].

Clinical Interpretation of Discordant Biomarkers

Discordance between creatinine-based and cystatin C–based eGFR is common.

A lower cystatin C–based estimate may reflect:

Low muscle mass causing creatinine to overestimate kidney function.

Inflammation or corticosteroid effects increasing cystatin C.

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Greater cardiovascular or systemic illness burden.
A lower creatinine-based estimate may reflect:
High muscle mass.
High meat intake.
Creatine supplementation.
Medications reducing tubular creatinine secretion.
When results are clinically important, the recommended approach is to:
Review non-GFR determinants of both markers.
Calculate a combined creatinine–cystatin C estimate.
Consider direct measured GFR if uncertainty remains.
Proposed Clinical Algorithm
A practical approach to renal filtration assessment includes:
Begin with serum creatinine and creatinine-based eGFR in most stable patients.
Assess whether creatinine is likely to be misleading, considering muscle mass, diet, body size, and medications.
Measure cystatin C when muscle mass is atypical or greater precision is required.
Use a combined creatinine–cystatin C equation when the two markers are discordant or a clinical threshold is important.
Perform measured GFR when estimates remain unreliable or precise measurement is necessary.
Prefer iothexol plasma clearance for practical measured GFR in many clinical settings.
Use inulin clearance primarily as a reference or research method when maximal physiologic accuracy is required.
Interpret acute kidney injury cautiously, because steady-state eGFR equations may be invalid.
Do not confuse filtration markers with injury biomarkers.
Reserve emerging biomarkers such as D-serine or proenkephalin for research or specialized use until further validation.

DISCUSSION

The major challenge in renal function assessment is the tension between physiologic accuracy and clinical practicality. Inulin remains the conceptual reference standard because its renal handling most closely reflects pure glomerular filtration. However, its logistical burden makes it unsuitable for routine care.
Iothexol plasma clearance provides the strongest practical alternative among measured-GFR methods. It avoids timed urine collection and can be performed using standardized plasma sampling, although protocol harmonization remains incomplete.
Creatinine remains indispensable because of its availability, cost, and integration into widely used eGFR equations. Nevertheless, it should not be interpreted without considering muscle mass, diet, tubular secretion, and clinical context.
Cystatin C provides valuable complementary information, particularly in patients with low or abnormal muscle mass. However, it is not free from confounding. The most accurate routine strategy is often the combined use of creatinine and cystatin C rather than replacement of one marker by the other.

Emerging biomarkers such as D-serine and proenkephalin are promising, but current evidence is insufficient for widespread clinical adoption. Future progress will likely involve combinations of biomarkers, standardized measured-GFR protocols, and individualized interpretation supported by computational models.

CONCLUSION

Inulin clearance remains the physiologic reference method for measured glomerular filtration rate, but its technical complexity limits routine use. Iothexol plasma clearance is the best-established practical alternative for direct GFR measurement. Serum creatinine remains the dominant routine biomarker, although its interpretation is limited by muscle mass, diet, and tubular secretion. Cystatin C is less dependent on muscle mass and improves assessment in selected patients, but it is affected by several non-GFR factors. Combined creatinine–cystatin C equations generally provide the most accurate routine estimate. Emerging biomarkers, including D-serine, D-asparagine, and proenkephalin, may improve future renal function assessment but remain investigational. The optimal strategy should match the required precision to the patient's clinical characteristics and the consequences of measurement error.

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