Utility of Morning and Midday Spot Urine Protein–Creatinine Ratios in Predicting 24-Hour Proteinuria: A Clinical Review

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ABSTRACT

Background: Proteinuria is a key biomarker in the diagnosis, staging, and monitoring of kidney diseases, reflecting both glomerular integrity and tubular reabsorptive capacity. While the 24-hour urine protein measurement is traditionally regarded as the gold standard for quantifying proteinuria, it is inconvenient, prone to collection errors, and challenging for patient compliance. The spot urine protein-to-creatinine ratio (UPCR) has emerged as a reliable surrogate, offering simplicity, speed, and reproducibility. However, the timing of spot urine sampling particularly morning versus midday can influence protein excretion due to diurnal variations in renal hemodynamics, posture, dietary intake, and physical activity. This variability has implications for diagnostic accuracy, disease monitoring, and clinical decision-making. This review critically examines the comparative utility of morning and midday spot UPCR measurements in predicting 24-hour proteinuria. We explore the underlying physiological mechanisms governing diurnal protein excretion, assess existing evidence from comparative studies, evaluate clinical relevance in specific kidney disorders, and discuss analytical and practical considerations for implementation in routine practice.

Conclusion: Evidence suggests that while both morning and midday UPCR correlate strongly with 24-hour proteinuria, their relative performance varies with clinical context. Morning samples may be more reliable in conditions with orthostatic proteinuria or where nocturnal excretion is minimal, whereas midday samples may better reflect cumulative daytime protein excretion and dynamic renal hemodynamics. In diseases such as lupus nephritis, diabetic nephropathy, preeclampsia, and myeloma kidney, spot UPCR offers a practical, cost-effective alternative to 24-hour collections, though variability and context-specific interpretation remain critical. Understanding these nuances can guide optimal timing of UPCR measurement, improving diagnostic precision and patient care.

Keywords: *Morning ,Midday, Spot Urine Protein–Creatinine Ratios*

INTRODUCTION

Proteinuria represents a pivotal diagnostic and prognostic parameter in nephrology, serving as a hallmark of both glomerular and tubular disorders. It reflects an imbalance between the filtration of plasma proteins across the glomerular barrier and their reabsorption in the proximal tubule. Persistent proteinuria is a recognized risk factor for progression to chronic kidney disease (CKD) and cardiovascular morbidity.

Historically, the 24-hour urine protein measurement has been the gold standard, yet it is cumbersome for patients, susceptible to incomplete collection, and resource-intensive for laboratories. The spot UPCR provides a practical alternative, correlating well with 24-hour excretion in diverse populations. However, diurnal variations in proteinuria raise questions about the optimal timing for sample collection. Factors such as posture, dietary intake, circadian rhythms, and renal hemodynamics can cause significant intra-day fluctuations in urinary protein concentration.

The primary aim of this review is to evaluate the comparative performance of morning and midday spot UPCR in predicting 24-hour proteinuria. We address the underlying physiology of protein filtration, pathophysiology in disease states, analytical methods, threshold values, and evidence from clinical studies. Furthermore, we discuss disease-specific applications, limitations, and future research directions to refine the use of UPCR in clinical practice.

Protein Filtration and Reabsorption

The kidney's role in protein handling is primarily determined by the structure and function of the glomerular filtration barrier and the reabsorptive capacity of the proximal tubule. The glomerular barrier consists of three distinct layers: the fenestrated endothelium, the glomerular basement membrane (GBM), and the podocyte slit diaphragm. Together, they form a size- and charge-selective filter that restricts the passage of most plasma proteins, particularly high-molecular-weight and negatively charged proteins such as albumin [1]. Under physiological conditions, only small amounts of low-molecular-weight proteins (e.g., β2-microglobulin, retinol-binding protein) and trace quantities of albumin pass into the filtrate.

Once filtered, proteins are largely reabsorbed by the proximal tubular epithelial cells via receptor-mediated endocytosis, involving the multiligand receptors megalin and cubilin [2]. This endocytic process directs filtered proteins into lysosomes for degradation into amino acids, which are subsequently returned to the circulation. The efficiency of tubular reabsorption is such that normal urinary protein excretion in adults is typically less than 150 mg/day, with albumin accounting for <30 mg/day [3]. The balance between glomerular filtration and tubular reabsorption ensures minimal protein loss under healthy conditions.

Disruption in this equilibrium can occur through structural damage to any layer of the glomerular barrier or through impaired tubular reabsorption. Glomerular injury increases permeability, leading to higher quantities of albumin and other large proteins in the filtrate, while tubular dysfunction impairs reclamation of filtered low-molecular-weight proteins [4]. Both mechanisms can coexist in mixed renal pathologies, altering the quantity and composition of urinary proteins. These physiological principles underpin the interpretation of proteinuria measurements and explain why timing of urine sampling may influence results — changes in posture, activity, and renal blood flow can transiently alter filtration and reabsorption dynamics, producing measurable differences between morning and midday protein excretion [5].

Determinants of the Transglomerular Passage of Plasma Proteins

The passage of plasma proteins across the glomerular filtration barrier is regulated by an interplay of size, charge, shape, and hemodynamic forces. Under physiological conditions, the barrier efficiently restricts macromolecules such as albumin despite their relatively small molecular radius (~3.6 nm), owing largely to the strong negative charge of the glomerular basement membrane (GBM) and podocyte slit diaphragm [6]. Larger proteins like immunoglobulins (>150 kDa) are almost completely excluded, while smaller neutral or positively charged proteins may cross more readily [7].

Charge selectivity is a key determinant in this process. The GBM contains heparan sulfate proteoglycans that impart a fixed negative charge, creating electrostatic repulsion for anionic proteins [8]. Experimental models have shown that enzymatic removal of these charges, as in heparanase overexpression, results in increased albumin permeability without major structural changes, underscoring the functional role of electrostatic barriers [9].

Hemodynamic factors also exert a profound influence. The **glomerular filtration rate (GFR)**, intraglomerular pressure, and plasma oncotic pressure determine the net filtration forces described by Starling's equation. Elevated intraglomerular pressure, as seen in hypertension or early diabetic nephropathy, can increase protein flux even in the absence of overt structural barrier damage [10]. Conversely, reductions in renal plasma flow or GFR can limit filtration despite barrier injury, sometimes masking proteinuria severity.

Furthermore, protein passage is not purely a passive process; the endothelial glycocalyx plays an active role in modulating permeability. Damage to the glycocalyx, from inflammatory cytokines or oxidative stress, increases sieving of proteins [11]. This is particularly relevant in systemic diseases such as sepsis, lupus, and preeclampsia, where endothelial injury coincides with proteinuric states.

These determinants collectively explain why the amount and composition of urinary protein can vary over the course of the day. Postural changes, salt intake, and physical activity alter renal blood flow and intraglomerular pressure, thereby modifying protein flux. This physiological variability is central to understanding differences between morning and midday spot urine protein–creatinine ratios [12].

Pathophysiology of Abnormal Urinary Protein Excretion in Glomerular Diseases

Abnormal protein excretion in glomerular diseases results from structural and functional disruptions in the glomerular filtration barrier. This barrier — composed of the fenestrated endothelium, GBM, and podocyte slit diaphragm — normally provides both **size selectivity** and **charge selectivity**. In disease states such as minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and membranous nephropathy, injury to podocytes or alterations in GBM composition can markedly increase protein permeability [13]. The loss or effacement of podocyte foot processes, a hallmark of many proteinuric diseases, eliminates critical components of the slit diaphragm including nephrin and podocin, leading to increased albumin filtration [14].

Inflammatory and immune-mediated processes further contribute to proteinuria by damaging endothelial cells and disrupting GBM integrity. In lupus nephritis, deposition of immune complexes within the glomerulus triggers complement activation, inflammatory cytokine release, and endothelial swelling, all of which promote protein leakage [15]. Similarly, in post-infectious glomerulonephritis, immune deposits in the subepithelial space alter GBM charge and structure, compromising its barrier function.

In addition to structural injury, **hemodynamic changes** in glomerular diseases can exacerbate proteinuria. For example, hyperfiltration in early diabetic nephropathy increases intraglomerular pressure, stretching the filtration barrier and forcing more proteins across despite intact barrier components [16]. This mechanical strain may initiate a cycle of injury, as filtered proteins can activate tubular cells, provoke inflammatory responses, and cause further nephron damage.

Tubulointerstitial changes also play a role in sustaining proteinuria in glomerular diseases. Filtered proteins that escape reabsorption by proximal tubular cells can trigger inflammatory and fibrotic pathways, leading to progressive tubulointerstitial injury [17]. This secondary injury not only worsens kidney function but also reduces tubular reabsorptive capacity, amplifying urinary protein losses.

Importantly, the magnitude and type of proteinuria often reflect the underlying glomerular pathology. Selective proteinuria, dominated by albumin, is typical of MCD, while non-selective proteinuria, containing larger proteins such as immunoglobulins, occurs in advanced glomerular injury [18]. Recognizing these patterns is essential for diagnosis and for interpreting the relationship between spot UPCR and 24-hour proteinuria, especially when comparing morning versus midday measurements, where variability may differ by disease type.

Hemodynamic Factors and Proteinuria

Hemodynamic alterations within the kidney are central to both the initiation and progression of proteinuria. The glomerular capillary hydrostatic pressure is a major determinant of filtration, and increases in this pressure can significantly enhance protein flux across the filtration barrier even in the absence of gross structural damage [19]. This is well illustrated in early diabetic nephropathy, where afferent arteriolar vasodilation and efferent arteriolar constriction produce intraglomerular hypertension. The heightened transcapillary pressure gradient stretches the GBM and widens slit diaphragms, allowing more proteins to escape into the filtrate [20].

Systemic hypertension exerts similar effects on the glomerulus. Chronic elevation of blood pressure leads to adaptive — and ultimately maladaptive — structural changes in the renal microvasculature, including hyaline arteriolosclerosis and intimal thickening. These changes impair autoregulation of renal blood flow, causing fluctuations in glomerular pressure and increased protein leak during peaks of systemic pressure [21]. Furthermore, hypertensive injury can trigger podocyte detachment and microaneurysm formation, compounding the permeability defect.

The renin–angiotensin–aldosterone system (RAAS) plays a pivotal role in these hemodynamic processes. Angiotensin II increases efferent arteriolar tone, which helps maintain GFR in low-perfusion states but also raises glomerular capillary pressure, contributing to proteinuria [22]. In addition, angiotensin II has direct effects on podocytes and mesangial cells, promoting oxidative stress, cytoskeletal disruption, and matrix expansion, all of which impair the filtration barrier [23]. Pharmacologic blockade of RAAS with ACE inhibitors or ARBs reduces intraglomerular pressure and proteinuria, highlighting the causal role of hemodynamics in protein excretion.

Hemodynamic changes can also have a diurnal component, influencing differences between morning and midday spot urine protein–creatinine ratios. Physical activity, postural shifts, and variations in salt and water intake alter systemic and intraglomerular pressures throughout the day [24]. Orthostatic proteinuria, predominantly seen in adolescents and young adults, exemplifies this phenomenon — protein excretion is

minimal in early morning supine samples but increases significantly in midday upright samples [25]. Understanding these variations is essential when interpreting spot UPCR results, as timing of collection may capture different hemodynamic states.

Evaluation of Proteinuria

The evaluation of proteinuria begins with distinguishing between transient, orthostatic, and persistent forms, as this classification has important prognostic and therapeutic implications [26]. **Transient proteinuria** can occur in association with fever, vigorous exercise, dehydration, or acute illness and is typically benign. **Orthostatic proteinuria** is characterized by normal protein excretion in the early morning after overnight recumbency, with increased protein excretion during the day in the upright position, most often seen in adolescents and young adults [27]. **Persistent proteinuria**, by contrast, often indicates underlying renal pathology and warrants thorough investigation, particularly if exceeding 500 mg/day or associated with hematuria or reduced renal function.

Initial screening for proteinuria is commonly performed using urine dipstick testing, which provides a semi-quantitative assessment based primarily on albumin concentration [28]. While rapid and inexpensive, dipsticks have limitations: they are less sensitive to non-albumin proteins, can yield false positives in highly concentrated urine, and false negatives in dilute urine or in the presence of non-albumin proteins such as those found in multiple myeloma [29]. Therefore, positive dipstick results require confirmation and quantification using more reliable methods such as the urine protein-to-creatinine ratio (UPCR) or albumin-to-creatinine ratio (ACR).

Quantitative assessment can be performed using either timed urine collections (e.g., 24-hour protein measurement) or spot urine testing. The 24-hour collection is considered the reference standard, offering a direct measure of total protein excretion. However, it is cumbersome, prone to incomplete collection, and subject to pre-analytical errors [30]. Spot urine testing, in contrast, estimates daily protein excretion by normalizing protein concentration to creatinine concentration, which compensates for variations in urine dilution. This method is practical for outpatient settings, facilitates repeated measurements, and has been validated in diverse patient populations [31].

In interpreting proteinuria measurements, timing of sample collection is a critical factor. Diurnal variations in protein excretion can produce different results from morning versus midday samples, especially in conditions like orthostatic proteinuria, diabetic nephropathy, or hypertensive nephrosclerosis [32]. Recognizing these patterns can help clinicians select the most appropriate sampling strategy, improving diagnostic accuracy and reducing unnecessary repeat testing. In the context of this review, comparing morning and midday UPCR values is essential for understanding their correlation with 24-hour protein excretion and determining their optimal use in specific clinical scenarios.

Urine Composition

Urine composition reflects the kidney's integrated role in filtration, reabsorption, secretion, and excretion, and it provides valuable insight into systemic and renal health. In healthy adults, urine is approximately **95% water** and **5% solutes**. The principal solutes include urea — the end product of protein metabolism — as well as creatinine, sodium, potassium, chloride, phosphate, sulfate, and small amounts of organic acids [33]. Trace quantities of proteins, peptides, and hormones are also present under normal conditions, with total daily protein

excretion generally <150 mg [34]. The relative concentrations of these components vary based on diet, hydration status, circadian rhythms, and metabolic activity.

In addition to electrolytes and metabolic waste products, urine contains low-molecular-weight organic compounds, such as uric acid, amino acids, and glucose in trace amounts. Larger molecules like albumin are minimally present in normal urine due to the selective permeability of the glomerular barrier and efficient proximal tubular reabsorption [35]. Any significant increase in the concentration of proteins, glucose, ketones, or abnormal pigments in urine often signals underlying pathology. For instance, sustained proteinuria may indicate glomerular disease, while glycosuria in the presence of normal blood glucose levels suggests tubular dysfunction, as in Fanconi syndrome [36].

Urine composition can be influenced by both physiological and pathological factors. Dietary protein intake increases urea and acid excretion, while high salt intake raises sodium and chloride excretion, potentially affecting urine osmolarity and volume [37]. Physical activity and posture also alter urinary constituents; for example, upright posture and physical exertion can increase protein excretion in individuals with orthostatic proteinuria [38]. Hydration status has a direct effect on solute concentration, and dilute urine can mask the presence of protein on dipstick testing, whereas concentrated urine may exaggerate protein readings.

From a diagnostic perspective, urine composition offers a non-invasive window into renal and systemic health. Quantifying specific solutes, such as albumin or creatinine, enables standardized ratios like the protein-to-creatinine ratio (P/C ratio), which normalize for urine concentration variability [39]. Understanding the baseline composition of urine and the factors that modify it is critical for interpreting both spot and timed urine tests, particularly when comparing morning and midday collections for protein quantification.

Urine as a Diagnostic Tool

Urine has been used as a diagnostic medium since ancient times, with early physicians recognizing changes in color, odor, and clarity as indicators of disease. Modern medicine has refined these observations into structured laboratory analyses, making urinalysis a cornerstone of diagnostic evaluation in nephrology and general practice. The composition of urine provides a dynamic reflection of renal filtration, tubular function, and systemic metabolic processes [40]. It is a non-invasive sample that can be obtained easily and repeatedly, allowing for serial monitoring of disease progression and response to therapy.

The diagnostic utility of urine spans qualitative, semi-quantitative, and quantitative assessments. Qualitative tests, such as dipstick urinalysis, detect the presence of proteins, glucose, ketones, blood, bilirubin, and leukocyte esterase, providing rapid point-of-care results [41]. Microscopic examination can identify red blood cells, white blood cells, casts, and crystals, offering clues to glomerular versus non-glomerular sources of pathology. Quantitative assessments, including the protein-to-creatinine ratio (P/C ratio) and albumin-to-creatinine ratio (ACR), standardize protein measurements by correcting for urine concentration, facilitating comparison between individuals and over time [42].

Recent advances have expanded the diagnostic potential of urine beyond traditional parameters. Proteomic and metabolomic profiling of urine has identified novel biomarkers for early detection of kidney injury, such as neutrophil gelatinase–associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) [43]. In glomerular diseases, specific urinary peptide patterns have been linked to disease activity, prognosis, and therapeutic

response. The ease of obtaining urine samples without invasive procedures has also facilitated large-scale screening programs for chronic kidney disease (CKD) and pregnancy-related complications like preeclampsia. Despite these advantages, urine-based diagnostics have limitations. Pre-analytical factors, including sample timing, storage conditions, and contamination, can affect results [44]. Diurnal variation in certain solutes, notably protein, can lead to discrepancies between morning and midday samples, potentially complicating interpretation if the timing is not standardized. Furthermore, while certain biomarkers are promising, their clinical application requires validation in diverse populations and standardization across laboratories. Understanding both the potential and limitations of urine as a diagnostic tool is essential for accurate application in clinical practice and for interpreting studies comparing morning and midday P/C ratios against 24-hour proteinuria.

Clinical Urine Tests

Clinical urine tests are a cornerstone of nephrological assessment, offering a wide range of qualitative and quantitative evaluations that can guide diagnosis, monitor disease progression, and assess treatment response. The simplest and most widely used method is the **urine dipstick test**, which can rapidly screen for protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrites, and leukocyte esterase [45]. Dipstick testing is inexpensive, easy to perform, and provides immediate results at the point of care. However, it is semi-quantitative at best and primarily detects albumin; it is less sensitive to non-albumin proteins such as immunoglobulin light chains, which can be missed in conditions like multiple myeloma [46].

Microscopic urinalysis remains a vital follow-up to dipstick screening. Examination of urine sediment can reveal red blood cells (RBCs), white blood cells (WBCs), epithelial cells, casts, and crystals. The morphology of RBCs can help differentiate glomerular from non-glomerular bleeding, with dysmorphic RBCs and acanthocytes indicating a glomerular source [47]. Casts, particularly red cell casts, are pathognomonic for glomerulonephritis, while fatty casts may suggest nephrotic syndrome. Crystals can point toward metabolic disorders or urinary tract stone disease.

Quantitative tests allow for more precise evaluation of urinary protein excretion. The **protein-to-creatinine ratio (P/C ratio)** and **albumin-to-creatinine ratio (ACR)** are now widely used to estimate 24-hour protein or albumin excretion from a single voided sample. These ratios account for urine concentration variability, enabling clinicians to assess proteinuria without requiring cumbersome timed collections [48]. Timed urine collections, including 24-hour samples, remain the reference standard for precise measurement, but their use is increasingly reserved for research settings, complex cases, or when spot test results are equivocal.

Specialized urine tests expand diagnostic capabilities in certain conditions. For example, **Bence Jones protein testing** detects free light chains in multiple myeloma, while urine immunofixation electrophoresis can identify specific monoclonal proteins [49]. In pregnancy, spot P/C ratio measurements are often used to help diagnose preeclampsia, with threshold values varying by guideline. Importantly, the accuracy of these tests can be influenced by the timing of sample collection. Morning urine samples tend to be more concentrated and less affected by recent activity or diet, whereas midday samples may reflect higher protein excretion due to postural and hemodynamic effects. Understanding these timing differences is essential for interpreting spot P/C results, particularly when comparing them to 24-hour measurements.

Protein to Creatinine Ratio (P/C Ratio)

The **protein-to-creatinine ratio (P/C ratio)** is a standardized method for estimating daily protein excretion from a single spot urine sample. It is calculated by dividing the urine protein concentration (mg/dL) by the urine creatinine concentration (mg/dL), yielding a dimensionless value that correlates with grams of protein excreted per day [50]. This normalization accounts for variations in urine concentration caused by differences in hydration status, diuresis, and urine osmolality. Creatinine is used as the denominator because it is produced at a relatively constant rate by muscle metabolism and excreted steadily throughout the day in healthy individuals [51].

The principal advantage of the P/C ratio is its ability to provide an accurate estimate of 24-hour proteinuria without requiring cumbersome timed urine collections. Numerous studies have demonstrated strong correlations between P/C ratio values and 24-hour protein excretion in both adult and pediatric populations [52]. This method is particularly useful in outpatient settings, in patients with poor compliance for timed collections, and in clinical situations requiring rapid decision-making, such as pregnancy-related hypertensive disorders or acute glomerular diseases.

The P/C ratio also facilitates longitudinal monitoring of proteinuria, enabling clinicians to track therapeutic responses and disease progression. Because it is less susceptible to day-to-day variability than 24-hour collections, repeated P/C ratio measurements provide a more consistent picture of proteinuria trends [53]. Furthermore, it allows for stratification of disease severity: values above certain thresholds correlate with nephrotic-range proteinuria, while smaller elevations may indicate subclinical glomerular injury or early-stage chronic kidney disease.

However, several limitations must be considered. Creatinine excretion is influenced by muscle mass, age, sex, and certain chronic illnesses, potentially affecting the accuracy of the ratio in individuals with low muscle mass (e.g., elderly, malnourished, or chronically ill patients) [54]. Additionally, diurnal variation in protein excretion means that the timing of spot sample collection can alter the P/C ratio. Morning samples may be less affected by recent activity and dietary protein intake, whereas midday samples may reflect higher protein excretion in conditions such as orthostatic proteinuria [55]. Understanding these nuances is essential for interpreting P/C ratio results and for optimizing its use as a surrogate for 24-hour urine protein measurements.

Proteinuria Evaluation

The evaluation of proteinuria serves multiple clinical purposes: it aids in diagnosing renal disease, assessing disease severity, monitoring progression, and gauging response to therapy. Proteinuria is not only a marker of glomerular or tubular injury but also an independent risk factor for the progression of chronic kidney disease (CKD) and cardiovascular morbidity [56]. Therefore, accurate quantification and characterization of urinary protein are essential for guiding clinical management.

Initial evaluation typically begins with a **screening test** such as a urine dipstick, which is widely available, inexpensive, and easy to use. However, because the dipstick primarily detects albumin, it may miss non-albumin proteinuria and is prone to false positives and negatives depending on urine concentration, pH, and the presence of interfering substances [57]. A positive dipstick finding warrants confirmation through quantitative methods,

such as the protein-to-creatinine ratio (P/C ratio) or albumin-to-creatinine ratio (ACR), or by performing a timed collection, most often the 24-hour urine protein measurement.

Quantitative assessment allows clinicians to differentiate between sub-nephrotic and nephrotic-range proteinuria. In adults, nephrotic-range proteinuria is defined as protein excretion exceeding 3.5 g/day, usually associated with hypoalbuminemia, hyperlipidemia, and edema [58]. Recognizing this threshold is critical, as nephrotic-range proteinuria often reflects significant glomerular injury, such as in minimal change disease, focal segmental glomerulosclerosis (FSGS), or membranous nephropathy. In contrast, lower levels of proteinuria may be seen in early diabetic nephropathy, hypertensive nephrosclerosis, or as part of transient or orthostatic patterns.

The choice between **spot urine testing and timed collections** depends on clinical context. Spot P/C or ACR measurements are convenient, reduce patient burden, and have been validated in many patient populations [59]. However, these spot values can be influenced by diurnal variation in protein excretion, meaning morning and midday collections may yield different results. For instance, morning samples tend to be more concentrated and less influenced by posture or recent dietary protein intake, while midday samples may show higher protein levels in conditions like orthostatic proteinuria or during periods of high physical activity [60].

A comprehensive evaluation also considers the **type of protein excreted**. Electrophoresis and immunofixation can differentiate selective albuminuria from non-selective patterns that include immunoglobulins, as in advanced glomerular damage, or light chains, as in multiple myeloma. This distinction is important not only for diagnosis but also for interpreting quantitative results and understanding potential discrepancies between spot and 24-hour protein measurements. Ultimately, accurate proteinuria evaluation hinges on selecting the appropriate test, interpreting results within the clinical context, and accounting for timing-related variability when comparing morning versus midday spot measurements.

Threshold Value for Spot Urine P/C Ratio

Establishing accurate threshold values for the **spot urine protein-to-creatinine ratio (P/C ratio)** is essential for its use as a surrogate for 24-hour protein excretion. In general, a P/C ratio of **0.2 g/g** (200 mg/g) is considered the upper limit of normal for adults, corresponding to approximately 200 mg of protein excretion per day [61]. Values above this threshold indicate abnormal proteinuria, while a P/C ratio of **3.5 g/g** or greater typically corresponds to nephrotic-range proteinuria, paralleling the conventional 3.5 g/day definition from 24-hour collections [62]. These cutoffs facilitate clinical decision-making, enabling rapid assessment and triage without the delays and inconveniences of timed urine sampling.

Thresholds may vary between populations and clinical contexts. In children, the normal cutoff for P/C ratio is lower — typically <0.2 g/g in older children and <0.5 g/g in infants — due to differences in creatinine excretion relative to body size and muscle mass [63]. In pregnancy, particularly when screening for preeclampsia, cutoffs for abnormal proteinuria often range between 0.3 and 0.35 g/g, depending on the guideline followed [64]. Disease-specific studies also suggest that optimal cutoffs may differ in certain conditions, such as lupus nephritis or diabetic nephropathy, to maximize sensitivity and specificity for detecting clinically meaningful proteinuria.

Timing of sample collection can influence whether results cross a diagnostic threshold. Morning P/C ratios are often slightly lower due to overnight supine posture, reduced renal perfusion, and lack of recent dietary or physical activity influences [65]. Midday P/C ratios may be higher, especially in patients with orthostatic

proteinuria or those experiencing activity-induced increases in protein excretion. This variability can potentially lead to reclassification of a patient's proteinuria status depending on when the sample is collected. Such fluctuations highlight the need for standardizing collection timing in both clinical practice and research protocols.

In research comparing P/C ratio with 24-hour proteinuria, studies have found that applying disease- and timing-specific thresholds improves agreement between the two methods [66]. For instance, in hypertensive pregnancy, a midday cutoff of 0.35 g/g may align better with the 24-hour gold standard than a morning sample at the same threshold. Similarly, in diabetic nephropathy, consistent use of first morning voids may reduce false-positive diagnoses. Ultimately, understanding and applying context-appropriate thresholds is essential for accurate diagnosis, prognostication, and monitoring in patients with proteinuria.

Methods of Protein and Creatinine Evaluation

Accurate determination of urinary protein concentration is critical for calculating the protein-to-creatinine (P/C) ratio and for assessing proteinuria severity. Several analytical methods are available, each with its own strengths and limitations. The most widely used techniques for total protein measurement in urine include **colorimetric methods**, **turbidimetric assays**, and **immunoassays** [67]. Colorimetric assays, such as the pyrogallol red–molybdate method, rely on the binding of dye to protein molecules, producing a measurable color change. Turbidimetric methods, including sulfosalicylic acid precipitation, detect protein by measuring the turbidity generated when proteins are denatured and aggregated. Immunoassays, which use antibodies directed against specific proteins like albumin, offer greater specificity but can be more costly and may not detect non-albumin proteins unless tailored for them.

For creatinine measurement, the **Jaffe reaction** remains the most commonly used method, in which creatinine reacts with alkaline picrate to form a colored complex measurable by spectrophotometry [68]. While inexpensive and widely available, the Jaffe method is susceptible to interference from other chromogens such as glucose, ketones, and ascorbic acid. Enzymatic creatinine assays, which utilize creatininase or creatinine deaminase, provide greater specificity and are less prone to interference, though they are generally more expensive [69].

Standardization of methods across laboratories is essential to ensure comparability of results. Variability in assay selection, calibration, and reporting units can lead to discrepancies in P/C ratio values, particularly in multicenter studies or when monitoring patients longitudinally across different healthcare facilities [70]. For example, differences in protein assays' sensitivity to certain proteins may produce systematically higher or lower results, affecting threshold-based clinical decisions.

Pre-analytical factors also significantly influence measurement accuracy. Urine sample handling — including timing of collection, storage temperature, and delays in analysis — can alter protein stability. Prolonged storage at room temperature can lead to bacterial overgrowth and proteolysis, reducing measured protein concentration [71]. Creatinine concentration is generally stable but can still be affected by dilution from excessive fluid intake or contamination from other sources. Thus, consistent collection protocols, particularly regarding morning versus midday timing, are vital for minimizing variability in P/C ratio results.

Finally, it is important to recognize that proteinuria often includes a mixture of protein types — albumin, immunoglobulins, low-molecular-weight proteins — which may not all be equally detected by a single assay. In conditions like myeloma kidney, reliance on total protein measurement without specific light chain detection may underestimate disease severity [72]. For both clinical and research purposes, aligning protein and creatinine measurement methods with the patient's underlying condition, and standardizing timing of sample collection, improves both the precision and interpretability of the P/C ratio.

Creatinine

Creatinine is a breakdown product of creatine phosphate from muscle metabolism, produced at a relatively constant rate in individuals with stable muscle mass. It is freely filtered by the glomerulus and not significantly reabsorbed, making it a useful endogenous marker for estimating glomerular filtration rate (GFR) and for normalizing urinary protein excretion in the protein-to-creatinine (P/C) ratio [73]. Because creatinine excretion is relatively stable over 24 hours, it serves as a surrogate for daily urine volume in spot measurements, compensating for variations in urine concentration due to hydration status or diuresis.

Despite its stability, creatinine excretion varies according to age, sex, muscle mass, and certain health conditions. Individuals with low muscle mass — such as the elderly, malnourished, or those with chronic illnesses — tend to have lower creatinine excretion, potentially inflating the P/C ratio for a given protein concentration [74]. Conversely, individuals with greater muscle mass, such as athletes, may have higher creatinine excretion, which can result in an underestimation of proteinuria severity when using the P/C ratio. This variability underscores the importance of interpreting ratio results in the context of the patient's clinical profile.

Dietary factors and physical activity can also influence creatinine excretion. A high-protein or meat-rich meal can transiently increase serum and urinary creatinine levels, while intense physical activity can increase creatinine generation due to muscle metabolism [75]. These factors, along with circadian rhythms in renal handling of solutes, can contribute to minor fluctuations in urinary creatinine concentration between morning and midday samples. As a result, sample timing may affect the calculated P/C ratio even if the actual amount of protein excreted remains constant.

In research and clinical practice, accurate measurement of urinary creatinine is critical for the reliability of P/C ratios. Enzymatic assays offer improved specificity over the traditional Jaffe method, particularly in dilute urine or in the presence of interfering substances [76]. For consistent interpretation, collection protocols should specify whether first-morning voids or midday samples are used, especially in comparative studies evaluating the correlation between spot P/C ratios and 24-hour proteinuria. Standardizing creatinine measurement methods and accounting for patient-specific factors ensures that the P/C ratio remains a dependable surrogate for daily protein excretion.

Diagnostic Utility

The **protein-to-creatinine ratio (P/C ratio)** has become an established tool in nephrology for estimating proteinuria without requiring the patient to perform a cumbersome 24-hour urine collection. Multiple studies have demonstrated that the P/C ratio from a spot urine sample strongly correlates with 24-hour urinary protein excretion across diverse populations, including adults, children, and pregnant women [77]. Its convenience,

reproducibility, and cost-effectiveness have contributed to its widespread adoption in both outpatient and inpatient settings.

A major diagnostic advantage of the P/C ratio is its ability to provide rapid quantification of proteinuria, facilitating timely clinical decision-making. For example, in suspected glomerulonephritis or nephrotic syndrome, the P/C ratio can guide the urgency of further investigations or initiation of treatment [78]. Similarly, in pregnancy, where rapid assessment is critical, spot P/C ratio testing has been incorporated into several guidelines for diagnosing preeclampsia, often replacing 24-hour urine collection as the first-line quantification method [79].

The diagnostic accuracy of the P/C ratio is generally high, but it varies with clinical context. In nephrotic-range proteinuria, correlation with 24-hour protein is excellent. However, in patients with borderline or low-grade proteinuria, variability in creatinine excretion and diurnal changes in protein output can lead to discrepancies [80]. For instance, first-morning samples may underestimate daily protein excretion in cases of orthostatic proteinuria, while midday samples may overestimate it in sedentary individuals with stable proteinuria patterns. These variations can affect whether a patient meets a diagnostic threshold for disease or treatment initiation.

In chronic kidney disease (CKD), serial P/C ratio measurements are valuable for monitoring disease progression and therapeutic response. Reductions in P/C ratio following interventions such as renin–angiotensin–aldosterone system (RAAS) blockade correlate with reduced risk of CKD progression and cardiovascular events [81]. In glomerular diseases, rising P/C ratios can signal disease relapse or inadequate immunosuppression, prompting timely therapeutic adjustments.

Nevertheless, the P/C ratio is not without limitations. It may be misleading in patients with abnormal creatinine excretion, such as those with very low muscle mass, high muscle turnover, or altered renal tubular handling of creatinine [82]. For these individuals, complementary methods — such as timed urine collections or alternative biomarkers like the albumin-to-creatinine ratio (ACR) — may provide more reliable information. When using the P/C ratio, standardizing the timing of sample collection (morning vs midday) and understanding its disease-specific performance are essential for maximizing its diagnostic utility.

Spot Urine P/C Ratio and Its Role in Kidney Diseases

The spot urine protein-to-creatinine ratio (P/C ratio) has emerged as a practical and reliable alternative to the 24-hour urine protein measurement for the evaluation of proteinuria in a wide spectrum of kidney diseases. Its main advantage lies in its convenience: a single voided sample can provide a reasonably accurate estimate of daily protein excretion, facilitating rapid diagnosis and monitoring without the logistical challenges of timed collections [83]. This feature is particularly useful in outpatient nephrology clinics, where patient compliance with 24-hour collections is often suboptimal, and in inpatient settings where quick decision-making is required.

In **glomerular diseases** such as minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and membranous nephropathy, the P/C ratio correlates well with 24-hour protein excretion, especially in cases of moderate-to-high proteinuria [84]. In these conditions, changes in the P/C ratio over time can help monitor response to immunosuppressive therapy or detect relapse early. For example, a rapid rise in P/C ratio in a patient with lupus nephritis often precedes clinical deterioration, allowing for timely treatment adjustment.

In **chronic kidney disease (CKD)**, the P/C ratio serves as both a diagnostic and prognostic marker. Persistent proteinuria quantified by the P/C ratio is a strong predictor of CKD progression, and its reduction is a therapeutic goal in many renoprotective strategies [85]. Serial P/C measurements can track the effectiveness of interventions such as RAAS blockade, sodium-glucose cotransporter-2 (SGLT2) inhibitors, or dietary protein restriction. A sustained decline in the P/C ratio is associated with slower decline in estimated glomerular filtration rate (eGFR) and reduced cardiovascular risk.

The P/C ratio also plays a critical role in **screening and monitoring for secondary causes of kidney injury**, such as diabetic nephropathy and hypertensive nephrosclerosis. In diabetic nephropathy, progression from microalbuminuria to overt proteinuria can be detected using either the albumin-to-creatinine ratio (ACR) or P/C ratio, with the latter offering the advantage of capturing non-albumin proteins that may also be elevated in advanced disease [86]. In hypertensive nephrosclerosis, persistent low-to-moderate proteinuria detected via P/C ratio supports the diagnosis and reinforces the need for strict blood pressure control.

A key consideration in all these contexts is **timing of sample collection**. Morning samples may provide a more stable baseline for longitudinal monitoring due to reduced influence from posture, dietary intake, and physical activity. Conversely, midday samples may be more sensitive for detecting conditions like orthostatic proteinuria or fluctuating protein excretion related to hemodynamic changes [87]. The choice between morning and midday sampling should be guided by the clinical question at hand, with consistency in timing critical for serial monitoring in individual patients.

Limitations of the P/C ratio include potential inaccuracy in patients with abnormal creatinine excretion due to muscle mass extremes, acute kidney injury, or certain metabolic states. Moreover, while the P/C ratio captures total urinary protein, it does not differentiate between protein types, which may be clinically important in diseases like multiple myeloma or tubular proteinuria. For these scenarios, complementary tests such as urine electrophoresis, immunofixation, or specific light chain assays should be employed alongside the P/C ratio [88].

Overall, the spot P/C ratio is a versatile and clinically valuable tool in nephrology, offering an efficient method for quantifying proteinuria across a broad range of kidney diseases. Its optimal use requires awareness of its methodological limitations, careful interpretation in context, and attention to timing of sample collection, especially when results are being compared to the gold-standard 24-hour protein measurement.

P/C Ratio and Diabetic Nephropathy

Diabetic nephropathy is one of the leading causes of chronic kidney disease (CKD) and end-stage kidney disease worldwide. Proteinuria, particularly albuminuria, is a hallmark of diabetic kidney injury and a key predictor of disease progression and cardiovascular risk [89]. Traditionally, the albumin-to-creatinine ratio (ACR) has been the preferred screening tool for early detection of microalbuminuria in diabetes. However, the protein-to-creatinine ratio (P/C ratio) has gained recognition for its broader detection capabilities, capturing not only albumin but also non-albumin proteins that may appear as tubular injury develops in advanced disease [90].

The P/C ratio correlates well with 24-hour proteinuria in patients with both type 1 and type 2 diabetes, making it a valuable tool for estimating total protein excretion without the inconvenience of timed urine collections [91]. In the early stages of diabetic nephropathy, proteinuria levels may be low and dominated by albumin, but as glomerular damage progresses, a broader spectrum of proteins is detected, increasing the relevance of total

protein measurement. Moreover, diabetic nephropathy often progresses silently, and routine P/C ratio monitoring can help detect subtle changes that might otherwise be missed between annual screening intervals.

From a prognostic standpoint, higher P/C ratios are associated with faster decline in estimated glomerular filtration rate (eGFR) and greater risk of end-stage kidney disease in diabetes [92]. This makes the P/C ratio not only a diagnostic tool but also a key marker for risk stratification and treatment monitoring. A significant reduction in P/C ratio following initiation of renin–angiotensin–aldosterone system (RAAS) inhibitors or sodium-glucose cotransporter-2 (SGLT2) inhibitors correlates with improved renal outcomes and reduced cardiovascular risk, highlighting its role in guiding therapeutic decisions.

Sample timing is an important consideration when using the P/C ratio in diabetic nephropathy. First-morning voids tend to produce more stable measurements, as they are less affected by recent physical activity, postural changes, and dietary intake [93]. Midday samples may yield slightly higher P/C ratios due to increased protein excretion during the active part of the day, but this variation is typically modest in stable disease. However, for accurate longitudinal monitoring, maintaining consistency in sample timing is crucial, particularly in research or when assessing small changes over time.

Despite its strengths, the P/C ratio should be interpreted alongside the ACR in diabetic nephropathy, especially for early disease detection. While P/C ratio captures broader proteinuria, ACR remains more sensitive for detecting microalbuminuria, which is the earliest clinically detectable stage of diabetic kidney injury [94]. Using both ratios together can provide a more comprehensive picture of renal injury, from the earliest stages through to advanced proteinuric disease.

Conclusion

Proteinuria remains a cornerstone biomarker in nephrology, providing diagnostic, prognostic, and therapeutic guidance across a spectrum of kidney diseases. While the 24-hour urine protein measurement has long been considered the reference standard, its practical limitations have driven widespread adoption of the spot urine protein-to-creatinine ratio (P/C ratio) as a convenient, reliable surrogate. The evidence consistently demonstrates strong correlations between P/C ratio values and 24-hour protein excretion, particularly in patients with moderate-to-high proteinuria, across diverse conditions including diabetic nephropathy, lupus nephritis, hypertensive nephrosclerosis, and glomerulonephritis.

The timing of urine sample collection is a critical but often underappreciated factor influencing P/C ratio results. Morning samples tend to be more stable and less affected by posture, diet, or activity, making them suitable for longitudinal monitoring and research. Midday samples, however, may better capture activity-related protein excretion, as in orthostatic proteinuria, but can overestimate proteinuria in some contexts. Recognizing these diurnal variations and standardizing collection timing improves diagnostic accuracy, reduces variability, and facilitates meaningful comparisons over time.

In clinical practice, the choice between morning and midday P/C ratios should be informed by the patient's underlying condition, the clinical question being addressed, and the need for reproducibility. Disease- and context-specific threshold values can enhance agreement with 24-hour proteinuria, but

interpretation must also account for patient-specific factors affecting creatinine excretion, such as muscle mass and dietary habits. For certain conditions, complementary tests like albumin-to-creatinine ratio (ACR), electrophoresis, or light chain assays may be necessary to fully characterize urinary protein composition.

Ultimately, the spot urine P/C ratio offers a practical, cost-effective, and clinically valuable method for proteinuria assessment, provided its limitations are acknowledged and protocols are standardized. Future research should aim to refine optimal sampling strategies, validate disease-specific thresholds, and explore the integration of P/C ratio with novel urinary biomarkers. Such efforts will strengthen its role not only as a surrogate for 24-hour proteinuria but also as a tool for precision medicine in nephrology.

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