The Kinetics of Cystatin C: A Marker for Dialysis Adequacy

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Medical Biophysics

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THE KINETICS OF CYSTATIN C: A MARKER FOR DIALYSIS ADEQUACY

(Thesis format: Integrated-Article)

by

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Graduate Program in Medical Biophysics

A thesis submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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THE UNIVERSITY OF WESTERN ONTARIO
Abstract

When 90% or more of native kidney function is lost, renal replacement therapy must be initiated to sustain life. Renal transplantation is the preferred method, but availability is limited. The ideal dialysis prescription remains elusive. Small molecular weight molecules (such as urea and creatinine) have been used as markers of both kidney (native and transplant) and dialysis toxin clearance (function), but there are pitfalls in using these markers to assess total 'renal' dose (kidney plus dialysis). Body weight, gender and other factors also affect the concentrations of these small molecules, but not cystatin C. Furthermore, cystatin C has been shown to be a better marker for estimating kidney function than creatinine, and is associated with cardiovascular morbidity and mortality. Studies have shown that it is removed by dialysis. Therefore, we investigated the use of cystatin C, a naturally occurring endogenous protein, as a marker for estimating dialysis adequacy and renal clearance. This investigation was comprised of four studies to understand the kinetics of cystatin C in patients with advanced kidney disease with or without dialysis. We found that the amount of cystatin C reduction was influenced positively by hemodialysis blood flow rate and treatment time, and negatively by ultrafiltration rate. We further demonstrated that renal hyperfiltration significantly influenced the error of creatinine-based glomerular filtrate rate equation, but not for the cystatin C equation. Therefore, cystatin C appears to be a useful marker for the assessment of kidney function in patients with advanced kidney disease but not yet on dialysis. This was taken further in our third study where we developed an equation, which gave a better estimate of residual renal function than previously published equations in patients on dialysis but who have some remaining kidney function. Finally, we confirmed our hypothesis that cystatin C is cleared during dialysis by both diffusion and convection. It is distributed mainly in the extracellular space but equilibrates slowly between the extravascular and intravascular spaces. Furthermore, we have shown that cystatin C while cleared by dialysis is stable between dialysis treatments rather than being influenced by
a single dialysis treatment. It is a marker for both dialysis and renal clearances and, thus, gives a stable index of total renal clearance.

The long term goal will be to define the cystatin C threshold level that influences patient morbidity and mortality and to allow better dialysis prescriptions for patients with varying (and changing) residual renal function.

Keywords
Cystatin C, Dialysis, Dialysis Adequacy, Hemodialysis, Nuclear Glomerular Filtration Rate, Residual Renal Function
Co-Authorship Statement

Chapter 2 has been adapted from a version of a manuscript submitted to the Clinical Journal of the American Society of Nephrology, 2011. The title of this manuscript is “Cystatin C Reduction Ratio Depends on Normalized Blood Liters Processed and Fluid Removal during Hemodialysis”, by: Huang, Filler, Yasin, and Lindsay. Huang, Filler, and Lindsay formulated the research idea and designed the study. Huang collected the data. Huang and Yasin performed the statistical analysis. The data were interpreted by Huang, Filler, Yasin and Lindsay. Each author contributed important intellectual content during the manuscript drafting and revising and accepted accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Chapter 3 has been adapted from the paper entitled “Hyperfiltration Affects Accuracy of Creatinine eGFR Measurements”, published in the Clinical Journal of the American Society of Nephrology, 2011 by: Huang, Sharma, Yasin, Lindsay, Clark and Filler. Huang, Filler, and Lindsay formulated the research idea and designed the study. This is a post-hoc analysis of a published study. Huang and Yasin performed the statistical analysis. The data were interpreted by Huang, Sharma, Lindsay, Clark and Filler. Each author contributed important intellectual content during manuscript drafting or revision and accepted accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Chapter 4 has been adapted from a version of a manuscript submitted to the Peritoneal Dialysis International, 2011. The title of this manuscript is “Residual Renal Function Calculated from Serum Cystatin C Measurements and a Knowledge of the Standard Weekly Kt/V (Urea)”, by Huang, Filler, and Lindsay. Huang, Filler, and Lindsay formulated the research idea and designed the study. Huang collected the data. Huang performed the statistical analysis. The data
were interpreted by Huang, Filler, and Lindsay. Each author contributed important intellectual content during manuscript drafting or revision and accepted accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Chapter 5 has been adapted from a version of a manuscript submitted to the *American Journal of Kidney Disease*, which has been published as a Research Letter in the journal. The title is “The Kinetics of Cystatin C Removal by Hemodialysis”, Huang; Tirona; Reid-Wilkinson; Thomson; Filler; Stodilka; Lindsay. Huang, Filler, Stodilka and Lindsay formulated the research idea and designed the study. Huang, and Reid-Wilkinson collected the data. Huang performed the statistical analysis. The data were interpreted by Huang, Tirona, Thomson, Stodilka and Lindsay. Each author contributed important intellectual content during manuscript drafting or revision and accepted accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Huang takes responsibility for that all four studies: that they have been reported honestly, accurately, and transparently; that no important aspects of the studies have been omitted, and that any discrepancies from the studies as planned have been adequately explained.
William Arthur Ward stated that, “The mediocre teacher tells. The good teacher explains. The superior teacher demonstrates. The great teacher inspires.” The last describes my supervisor, Dr. Robert Lindsay. He took me under his wing from the start and has helped me in every step throughout my training. He is always there to support me as a trainee, researcher and friend. I think he has truly gone above and beyond what a typical supervisor does. His passion for science and medicine has truly inspired me to become a better researcher and clinician. Thank you, Bob – any of my past and future accomplishments would not be possible without your inspiration.

I am also very grateful to my co-supervisor, Dr. Robert Stodilka, and my PhD mentorship advisor, Dr. Guido Filler. I met them as a trainee and a student, and both of them spent time to guide me in the right direction for my research development and help me to form research collaborations. Their advice and support were very helpful.

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Throughout my life, I have been blessed with loving family. I would like to dedicate this thesis to my family:

To my grandparents, I wish they could physically be here to see my accomplishment.

To my parents, Sherman and Lily, who taught me by example and are always there for me.

To my brother, George, who has supported me through every single step of my life.

To my sister, Flora, who is my best friend and my best supporter. Through my life journey, you have always been there during both the highs and the lows.
Table of Contents

Abstract........................................................................................................................................... ii
Co-Authorship Statement .................................................................................................................... iv
Acknowledgments ............................................................................................................................... vi
Table of Contents ............................................................................................................................... viii
List of Tables .................................................................................................................................... x
List of Figures .................................................................................................................................... xi
List of Appendices ............................................................................................................................... xiii
List of Abbreviations .......................................................................................................................... xiv

1. CHAPTER 1. GENERAL INTRODUCTION ................................................................................. 1
   1.1. INTRODUCTION TO RENAL CLEARANCE ......................................................................... 1
   1.1.1. Renal and Urinary Collecting System ............................................................................... 1
   1.1.2. Glomerular Filtration, Filtration Fraction and Chronic Kidney Disease ......................... 2
   1.1.3. Methods of Measuring Glomerular Filtration Rate ......................................................... 5
   1.1.4. Methods of Estimating Glomerular Filtration Rate: Biomarkers .................................... 8
   1.2. INTRODUCTION TO RENAL REPLACEMENT THERAPY ........................................... 13
   1.2.1. History and Development of Renal Replacement Therapy ............................................. 13
   1.2.2. History and Development of Hemodialysis .................................................................... 13
   1.2.3. History and Development of Peritoneal Dialysis ............................................................. 15
   1.3. COMPONENTS OF HEMODIALYSIS .................................................................................. 16
   1.3.1. Vascular Access ............................................................................................................... 17
   1.3.2. Dialysate Circuit .............................................................................................................. 17
   1.3.3. Blood Circuit ................................................................................................................... 18
   1.3.4. Dialyzer ........................................................................................................................... 18
   1.3.5. Hemodialysis Machines .................................................................................................. 22
   1.4. HEMODIALYSIS MODALITIES ....................................................................................... 23
   1.5. THE PHYSIOLOGY OF HEMODIALYSIS CLEARANCE .................................................. 24
   1.5.1. Basic Concepts of Diffusion and Convection .................................................................... 24
   1.5.2. Chronic Hemodialysis Access and Blood Flow Rate ...................................................... 26
   1.5.3. Dialyzer .......................................................................................................................... 31
   1.5.4. Dialysate and Its Flow Rate ............................................................................................ 33
   1.5.5. Hemodialysis Frequency and Duration .......................................................................... 38
   1.5.6. Convective Clearance ...................................................................................................... 38
   1.6. THE KINETICS OF SOLUTE CLEARANCE BY HEMODIALYSIS .................................. 40
   1.6.1. Body Compartments ........................................................................................................ 40
   1.6.2. Compartmental Modeling ................................................................................................ 41
   1.6.3. Uremic Toxins ................................................................................................................ 47
   1.6.4. Urea Clearance and Kinetic Modeling ............................................................................. 49
   1.6.5. Urea as a Marker for Dialysis Adequacy ......................................................................... 58
   1.6.6. Cystatin C as a Marker for Renal Clearance ................................................................. 60
   1.7. Cystatin C as a New Marker for Dialysis Adequacy .......................................................... 62
### Table of Contents

2. **Chapter 2. Cystatin C Reduction Ratio Depends on Normalized Blood Liters Processed and Fluid Removal** .................................................... 88  
   2.1. INTRODUCTION ........................................................................... 88  
   2.2. MATERIAL AND METHODS ............................................................. 90  
   2.3. RESULTS .................................................................................... 93  
   2.4. DISCUSSION ............................................................................... 99  

3. **Chapter 3. Hyperfiltration Affects Accuracy of Creatinine eGFR Measurement** ................................................. 107  
   3.1. INTRODUCTION ............................................................................. 107  
   3.2. MATERIALS AND METHODS .......................................................... 108  
   3.3. RESULTS ................................................................................... 111  
   3.4. DISCUSSION ............................................................................... 115  

4. **Chapter 4. Short Communication: Residual Renal Function Calculated from Serum Cystatin C Measurements and A Knowledge of The Standard Weekly Kt/V (Urea)** .............................................. 125  
   4.1. INTRODUCTION ............................................................................. 125  
   4.2. MATERIAL AND METHODS .......................................................... 126  
   4.3. RESULTS ................................................................................... 127  
   4.4. DISCUSSION ............................................................................... 129  

5. **CHAPTER 5. THE KINETICS OF CYSTATIN C REMOVAL BY HEMODIALYSIS** .................................................. 133  
   5.1. INTRODUCTION ............................................................................. 133  
   5.2. METHODS .................................................................................. 134  
   5.3. RESULTS ................................................................................... 139  
   5.4. DISCUSSION ............................................................................... 142  
   5.5. CONCLUSIONS .......................................................................... 145  

6. **CHAPTER 6. LIMITATIONS, FUTURE WORK AND SIGNIFICANCE** ................................................................. 150  
   6.1. OVERVIEW AND SUMMARY ......................................................... 150  
   6.2. LIMITATIONS OF CURRENT WORK .............................................. 152  
   6.3. FUTURE WORK ........................................................................... 154  
   6.4. SIGNIFICANCE ............................................................................ 157  

Appendices ......................................................................................... 161  
Curriculum Vitae ............................................................................... 168
List of Tables

Table 1-1. Commonly Used Creatinine-Based eGFR Equations ................. 10
Table 1-2. Commonly Used Cystatin C-Based eGFR Equations .................. 12
Table 1-3. Advantages and Disadvantages of Unmodified Cellulosic, Modified Cellulosic and Synthetic Hemodialysis Membranes .................. 21
Table 1-4. Different Types of Hemodialysis Membranes ......................... 22
Table 1-5. Factors Influencing Solute Concentrations in Dialysis Patients 25
Table 1-6. Constituents of Plasma and Dialysate. 3 ............................... 34
Table 1-7. The Time-Averaged Concentrations of Urea (mmol/l) and Dialysis Durations (minutes) for the Four Groups in the National Co-operative Dialysis Study. 161 .......................................................... 51
Table 2-1. Baseline Characteristics ....................................................... 94
Table 2-2. The Correlation Analysis and the Multivariable Analysis between CCRR, URR and CRR, and Other Variables ........................................ 97
Table 3-1. Statistics of the Most Important Measured and Calculated .......... 112
Table 3-2. Bland & Altman Results Summarized for Agreement of Various eGFR Formulae with the Measure Isotope GFR. ............................... 114
Table 3-3. Error by Level of eGFR (eGFR-GFR/GFR) for Various eGFR Formulae ............................................................. 114
Table 3-4. Spearman Rank Correlations between the Error of the GFR Estimate Models (BTP, Cystatin C, Schwartz) with FF (filtration fraction) 114
Table 5-1. Patients’ Baseline Characteristics (N=9) ................................ 140
Table 5-2. The Mean and Median Dialyzer Clearance (K, mL/min) for Cystatin C (CysC), Urea (Ur) and Creatinine (Cr). ................................. 141
Table 5-3. Assigned and Estimated Parameters for the Two-Pool Model of Cystatin C kinetics ................................................................. 142
Table 6-1. Recommended Stages for Biomarker Development and Testing .......................................................................................... 155
List of Figures

Figure 1-1. A Simple Schematic Diagram of Hemodialysis Circuit ...........23
Figure 1-2. Diffusion of Molecules in Two Solutions Through a Semi-permeable Membrane .................................................................26
Figure 1-3. The Relationship Between Small Solute Clearance and Total Surface Area of a Dialyzer ..........................................................33
Figure 1-4. An Schematic Diagram of a Water Purification System: the Reverse Osmosis System ..........................................................34
Figure 1-5. The Schematic Interpretation of Counter-Current and Parallel Flow Concentration Gradient ...................................................37
Figure 1-6. Relationship Between Nominal Blood Flow Rate and Blood Water Urea at Dialysate Flow Rate of 500 mL/min ....................................37
Figure 1-7. The Relationship Between Diffusional Clearance and the Blood Flow for Various Molecular Weight Molecules Using High-Flux Dialyzers ..39
Figure 1-8. Total Body Compartment .......................................................41
Figure 1-9. The Well Stirred and the Parallel Tube Models .......................45
Figure 1-10. The Single Compartmental Model of Hemodialysis Clearance 46
Figure 1-11. Multi Compartmental Model of a Solute Disposition in the Body ............................................................................................47
Figure 1-12. The Relationship Between Diffusional and/or Convective Clearance Rate and Molecular Weight of the Solute .........................49
Figure 2-1. Mean Cystatin C Levels During Hemodialysis Sessions ...........95
Figure 2-2. Cystatin C, Urea, and Creatinine Reduction Ratios (CCRR, URR and CRR, Respectively) ..............................................................96
Figure 2-3. Correlation Between the Calculated and the Measured Cystatin C Reduction Rate (CCRR) Based on a Model Using the Ultrafiltration Volume (UF [L]) and the Normalized Liters Processed (LP/kg) ..................98
Figure 2-4. Kinetic Model of Creatinine, Urea and Cystatin C during Hemodialysis .......................................................................................101
Figure 3-1. The Relationship Between the Percentage Error of the Schwartz, Filler and Benlamri eGFR and the Measured GFR, Plotted Against the Filtration Fraction (FF).................................................................................................................................115

Figure 3-2. Schematic Interpretation of Relationship Between the Filtration Fraction and Efferent Blood Flow.................................................................................................................................119

Figure 4-1. Correlation Analysis of the Hoek Residual Renal Function (RRF) and the Measured RRF, and ΔCysC RRF and Measured RRF ($r^2 = 0.69$, and 0.81, respectively; $p \leq 0.0001$)..................................................................................................................................128

Figure 6-1. The Pathophysiology of Cardiovascular Morbidity and Mortality in Dialysis Patients..................................................................................................................................................154
List of Appendices

Appendix A: Human Sciences Research Ethics Board Approval ............161

Appendix B. Copyright Material and Permission for the Four Publication Papers .................................................................162

Appendix C. Copyright Material and Permission for the Figures .............166
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2-M</td>
<td>β2-Microglobulin</td>
</tr>
<tr>
<td>A1C</td>
<td>Glycated Hemoglobin A1C</td>
</tr>
<tr>
<td>AGE</td>
<td>Advanced Glycation End-Products</td>
</tr>
<tr>
<td>BTP</td>
<td>Beta Trace Protein</td>
</tr>
<tr>
<td>BSA</td>
<td>Body Surface Area</td>
</tr>
<tr>
<td>BUN</td>
<td>Blood Urea Nitrogen</td>
</tr>
<tr>
<td>CAPD</td>
<td>Continuous Ambulatory Peritoneal Dialysis</td>
</tr>
<tr>
<td>CysC</td>
<td>Serum Cystatin C</td>
</tr>
<tr>
<td>CCRR</td>
<td>Cystatin C Reduction Ratio</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic Kidney Disease</td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>Chronic Kidney Disease Epidemiology Collaboration Equation</td>
</tr>
<tr>
<td>CrCl</td>
<td>Creatinine Clearance</td>
</tr>
<tr>
<td>CRR</td>
<td>Creatinine Reduction Ratio</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation (Standard Deviation/Mean)</td>
</tr>
<tr>
<td>EID</td>
<td>Effective Ionic Dialysance</td>
</tr>
<tr>
<td>FF</td>
<td>Filtration Fraction</td>
</tr>
<tr>
<td>FHN</td>
<td>Frequent Hemodialysis Network</td>
</tr>
<tr>
<td>ECV</td>
<td>Extracellular Volume</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular Filtration Fate</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated Glomerular Filtration Rate</td>
</tr>
<tr>
<td>IDMS</td>
<td>Isotope Dilution Mass Spectrometry</td>
</tr>
<tr>
<td>K</td>
<td>Clearance in mL/min</td>
</tr>
<tr>
<td><strong>KtA</strong></td>
<td>Mass Transfer Coefficient of the Dialyzer</td>
</tr>
<tr>
<td>-------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td><strong>KDOQI</strong></td>
<td>Kidney Disease Outcome Quality Improvement</td>
</tr>
<tr>
<td><strong>Kt/V</strong></td>
<td>Dimensionless Parameter for Assessing Hemodialysis Efficiency</td>
</tr>
<tr>
<td><strong>Kt/V_{urea}</strong></td>
<td>Dimensionless Parameter for Assessing Hemodialysis Urea Efficiency</td>
</tr>
<tr>
<td><strong>eKt/V_{urea}</strong></td>
<td>Equilibrated Kt/V of Urea</td>
</tr>
<tr>
<td><strong>ln (x)</strong></td>
<td>Natural Logarithm of x (log_e (x)), where e = 2.718281828459</td>
</tr>
<tr>
<td><strong>Log (x)</strong></td>
<td>Logarithm of x to base of 10 (log_{10} (x))</td>
</tr>
<tr>
<td><strong>LP</strong></td>
<td>Liters Processed</td>
</tr>
<tr>
<td><strong>LP/kg</strong></td>
<td>Liters Processed Normalized by Body Weight</td>
</tr>
<tr>
<td><strong>MDRD</strong></td>
<td>The Abbreviated 4-variable Modification of Diet in Renal Disease estimated Glomerular Filtration Rate</td>
</tr>
<tr>
<td><strong>NCDS</strong></td>
<td>National Co-operative Dialysis Study</td>
</tr>
<tr>
<td><strong>Q_b</strong></td>
<td>Blood Flow Rate</td>
</tr>
<tr>
<td><strong>Q_p</strong></td>
<td>Plasma Flow Rate</td>
</tr>
<tr>
<td><strong>Q_d</strong></td>
<td>Dialysate Flow Rate</td>
</tr>
<tr>
<td><strong>SCr</strong></td>
<td>Serum Creatinine</td>
</tr>
<tr>
<td><strong>Sp Kt/V_{urea}</strong></td>
<td>Single Pool Kt/V of Urea</td>
</tr>
<tr>
<td><strong>Std Kt/V_{urea}</strong></td>
<td>Weekly “Standardized” Kt/V of urea</td>
</tr>
<tr>
<td><strong>SUr</strong></td>
<td>Serum Urea</td>
</tr>
<tr>
<td><strong>TNF</strong></td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td><strong>UKM</strong></td>
<td>Urea Kinetic Modeling</td>
</tr>
<tr>
<td><strong>URR</strong></td>
<td>Urea Reduction Ratio</td>
</tr>
</tbody>
</table>
$^{51}\text{Cr-EDTA}$  Chromium 51-labeled Ethylenediaminetetraacetate

$^{99m}\text{Tc-DTPA}$  Technetium 99m-labeled Diethylenetriaminepentaacetic Acid
1. CHAPTER 1. GENERAL INTRODUCTION

1.1. INTRODUCTION TO RENAL CLEARANCE

1.1.1. Renal and Urinary Collecting System

Most humans have two kidneys, which are located in the retroperitoneal space on each side of the abdominal aorta. An adult kidney weighs about 115-170 g (11 cm x 6 cm x 3 cm).¹ It is bean-shaped and contains approximately 400,000 to 800,000 nephrons in the renal cortex.² Each functional unit of the kidney is called a nephron, which consists of a glomerulus and a tubule. Blood is filtered through the glomeruli (each one approximately 6.0 M microns in size) into the Bowman's capsule. This filtered fluid (approximately 180 L in an adult) is then processed during its transit through the tubule. The tubule can be separated into four parts (proximal tubule, loop of Hénle, distal tubule and collecting duct) and each part has different transporters and channels to maintain water, electrolytes and the acid-base balance. The final waste products and fluid from the collecting duct are drained into the renal pelvis. This is further emptied into the ureter through peristalsis initiated by special pacemaker cells and squirted into the bladder as urine. Once a certain bladder pressure is reached, the urine is voided through the urethra.

The major blood supply to each kidney comes through a renal artery. Because of the important function for maintaining electrolytes, and excreting toxins and fluid, kidneys need to have exceptionally high blood flow to tissue ratio. In fact, it has the highest ratio of any organ. The total blood flow to both kidneys is approximately 25% of the total cardiac output (1.25 L/min, or 350 mL/min/100 g of tissue).¹ This renal circulation is separated into two capillary networks where the glomerular vascular bed serves the purpose of filtration and the peri-tubular capillary bed serves as metabolic support.² In addition to excreting metabolic waste products, the kidneys have multiple additional functions: They regulate water and electrolyte balance, contribute to the control blood pressure, maintain
acid-base balance, produce erythropoietin, and convert vitamin D to its active form 1,25-dihydroxy-vitamin D.³

### 1.1.2. Glomerular Filtration, Filtration Fraction and Chronic Kidney Disease

Although a kidney has multiple tasks, kidney function is typically assessed by the glomerular filtration rate and, to a lesser extent, renal blood flow.⁴ It is important to measure accurately or estimate the glomerular filtration rate for early detection and monitoring of kidney function impairment, determining drug dosages based on renal clearance and assessing eligibility for kidney donation.

A glomerulus contains a network of many capillaries and it has unique structural support to hold the capillaries, the endothelium with numerous fenestrations, the glomerular basement membrane and cells such as podocytes and mesangial cells. The diameter of an endothelial fenestration is approximately 70–100 nm.⁵ The glomerular basement membrane forms both a size and charge barrier to filtration. The podocytes probably form the most important filtration barrier for plasma proteins.⁶ In normal conditions, urine formation starts when the blood from the afferent arterioles filtrates through the glomerular capillaries that consist of a filtrate with small plasma proteins and electrolytes. The amount of the filtered blood per time interval normalized to an idealized body surface area (1.73m²) is called the glomerular filtration rate (GFR, mL/min/1.73m²), which is determined by the hydrostatic pressure and colloid osmotic (oncotic) pressure across the capillary membrane, and the capillary filtration coefficient in the glomerulus.³ Filtration occurs by means of the transcapillary pressure difference:

\[
\text{Single Nephron GFR} = K_f (\Delta P_h - \Delta \pi)
\]

**Equation 1-1**

where \(K_f\) is a coefficient determined by hydraulic conductivity and the surface area, \(\Delta P_h\) and \(\Delta \pi\) are the hydrostatic pressure and the oncotic pressure differences between the capillary and the Bowman’s space (the beginning of the tubular component of a nephron), respectively. If the hydraulic pressure...
difference is smaller than the oncotic pressure, there is no filtration. The hydraulic pressure differences ($\Delta P_h$) between the capillary and the Bowman's space do not alter much at the beginning and the end of the glomerular capillary. However, the oncotic pressure difference ($\Delta \pi$) rises from 18 to 34 mmHg along the glomerular capillary.\(^5\) This is due to plasma filtration into the Bowman's space, a fluid which has no protein passes. The oncotic pressure increases within the capillary and thus is reduced in the Bowman's space. Therefore, the amount of filtration reduces along the capillary pathway.

\[
Single \text{ Nephron GFR} = Q_{\text{afferent}} \left(1 - \frac{\pi_A}{\Delta P_h}\right) \quad \text{Equation 1-2}
\]

where $\pi_A$ is the initial capillary oncotic pressure, and $Q_{\text{afferent}}$ is the afferent blood flow rate. Assuming $\pi_A$ and $\Delta P_h$ are unchanged constant, then the single nephron GFR varies, according to the $Q_{\text{afferent}}$.\(^5\) In general, the single nephron GFR, afferent and efferent blood flow rate, oncotic and hydraulic pressure are stable: this renal autoregulation is controlled by changes in afferent and efferent arteriolar resistance.\(^7\)

It is impractical, or even impossible, to measure GFR at the glomerular level. The GFR measurement is the overall glomerular filtration rate in one or both kidneys in a subject. A normal GFR in an adult is approximately 125 mL/min.\(^1\) Therefore, an average adult has a glomerular filtration rate of 180 L per day. If this filtered fluid is not reabsorbed, the patient will lose a significant number of electrolytes. As a result, the majority of the filtered electrolytes (such as sodium and potassium) and plasma fluid are reabsorbed. Currently, kidney function is reported based on GFR, either measured or estimated. The ideal markers for assessing GFR should be freely filtered by glomeruli without tubular secretion and reabsorption.\(^3\) This will be discussed in the next section.

Another variable that is closely linked to GFR is the renal filtration fraction (FF). FF is the amount of renal plasma flow that is filtered. It is calculated by the following equation:\(^1\)
A normal FF is approximately $18.7 \pm 3.2\%$ in healthy young adults between the ages of 20 and 30 years.\(^8\) When the FF is above the reference interval of 18-22%, it is considered hyperfiltration.\(^9\) Hyperfiltration can occur at the individual glomerular level in a situation where GFR is decreased. Early development of diabetic nephropathy is commonly associated with hyperfiltration and is a maladaptation process.\(^10\) This can lead to poor renal outcome.\(^11\) Reducing hyperfiltration through medications, such as angiotensin receptor blockers or angiotensin converting enzyme inhibitors, may prevent or reduce the rate of renal function decline.

As kidney function is commonly measured by GFR, the National Kidney Foundation has proposed five stages of Chronic Kidney Diseases (CKD).\(^12\) Measurements of urinary sediments, markers of renal damage, renal imaging and renal pathologic abnormalities can help identify kidney diseases where the GFR is not significantly altered. Therefore, the CKD Stages 1 and 2 are defined as kidney damage, which are measured by using urinary, imaging or pathologic methods, with normal or increased GFR ($\geq 90$ mL/min/1.73m\(^2\)) and with mildly decreased GFR ($60-89$ mL/min/1.73m\(^2\)). The CKD Stages 3-5 are defined mainly by GFR measurements: $30-59$ mL/min/1.73m\(^2\), $15-29$ mL/min/1.73m\(^2\), and $<15$ mL/min/1.73m\(^2\), respectively.

The CKD staging system has some issues and it has received some major criticisms.\(^13\) It does not consider the underlying pathophysiology of renal failure. Furthermore, proteinuria (protein in the urine) is an important renal prognostic indicator: the current CKD staging system does not take it into account. Therefore, Tonelli et al. have proposed a new classification system based on both the GFR and the proteinuria level.\(^14\) This classification has not yet been widely adapted. For physicians and health care providers, it is important to know that the management of CKD patients should be individualized and should not be based solely on the CKD stages.
1.1.3. Methods of Measuring Glomerular Filtration Rate

Over the last 80 years, several methods have been developed to assess GFR. Each of these methods has its pros and cons - some are more invasive and time-consuming, while others may not be sufficiently sensitive or specific. Here, we will discuss the methods for assessing GFR.

(1) The Inulin Study

Inulin is a fructose polymer made from the Jerusalem artichoke that does not have non-renal elimination, no plasma protein binding, and is neither absorbed or excreted by the tubule. It has the characteristics of an ideal renal marker for GFR measurements. Therefore, inulin clearance is considered the gold standard for measuring GFR. This method was initially developed in 1935 by Homer Smith. In the traditional method, an intravenous infusion of inulin is given after a bolus injection until a steady state is reached. Urinary inulin clearance is then measured. Because it is time consuming, modified versions of the traditional inulin clearance were developed. Several hours of inulin infusion is the ideal method for measuring clearance, and to minimize error, catheterization is best for accurate urine collection. Despite the use of these methods, there is a 10% inter-assay variability with inulin measurements due to analytical challenges and the inhomogeneity of the biomarker, especially when older biochemical methods are employed, rather than mass spectrometry. Because of its invasiveness (catheterization) and difficulties with the availability of inulin, an inulin study is rarely performed and is limited to a research setting.

(2) The Nuclear GFR Study

In the 1970's, nuclear medicine techniques replaced the inulin clearance method. The techniques, which use radio-labeled markers that have similar properties to inulin, have produced findings comparable to inulin GFR clearance studies of patients with GFRs above 20 mL/min/1.73m². A bolus of clearly measured and suitable compound, which was injected through a venipuncture, is commonly
utilized. The rate of decreasing plasma concentration of the compound, after adjusting for its inherited decay rate, is measured and is used to calculate renal GFR. In Europe, chromium 51-labeled ethylenediaminetetraacetate ($^{51}$Cr-EDTA) is the most widely used radio-labeled isotope. Technetium 99m-labeled diethylenetriaminepentaacetic acid ($^{99m}$Tc-DTPA) is the most commonly used GFR marker in North America.\textsuperscript{21-23} Although some studies have observed systematic differences between $^{51}$Cr-EDTA and $^{99m}$Tc-DTPA, these differences are small and $^{99m}$Tc-DTPA is recommended as an acceptable alternative to $^{51}$Cr-EDTA.\textsuperscript{20,21,24-27} Other exogenous markers that have been utilized are $^{125}$Iodine iothalamate, iothalamate and iohexol. The latter two have been used without being radiolabeled. All markers except iothalamate have a small amount of plasma protein binding.

For calculating GFR clearance using radioactive isotopes, some centers utilized a single-compartmental model.\textsuperscript{16} An example of the one-compartmental linear approach used log-transformation of the counts in each of the three samples:\textsuperscript{28}

$$
GFR_{\text{plasma}} = \left[ \frac{10000 \times S_l}{T_i - T_{i-1}} \times \ln \left( \frac{P_{i-1}}{P_i} \right) \times \exp \left( \frac{T_{i-1} \ln P_i - T_i \ln P_{i-1}}{T_i - T_{i-1}} \right) \right]^{0.979} \quad \text{where } i = 1, 2, 3 \text{ (sample)}
$$

$$
GFR_{\text{plasma}} = \text{median}(GFR_1, GFR_2, GFR_3) \times \left( \frac{1.73}{\text{BSA}} \right) \quad \text{where } \text{BSA} = 0.007184 \times \left( \text{height}^{0.725} \times \text{weight}^{0.425} \right)
$$

\textbf{Equation 1-4}

where $T_i$ is the time of the sampling, BSA is the body surface area, and $P_i$ is the plasma concentration of the isotope adjusted for the isotopic decay. This method can lead to overestimation of the GFR. Although this issue may be overcome by delay sampling, it is time-consuming.\textsuperscript{29-32} The lack of assessment of isotope extravasation, and the lack of information to demonstrate equilibration between intravascular and extracellular compartments, could explain the inaccuracy of the plasma isotopic GFR method. In patients with expanded extracellular volume (ECV), it will over-estimate GFR. Finally, not taking into account the extra-renal metabolism and/or excretion of the isotopic markers, i.e. hepatobiliary, can also over-estimate GFR. Since plasma isotopic GFR techniques commonly utilize a
single bolus-injection technique, it is important that the entire quantity of radioisotope enters the intravascular space, since extravasation can lead to the overestimation of GFR.

There are certain compartmental methods where the timing of the blood sampling can result in different values. It has been considered that the slope-intercept method, restricting the blood samples to the second of the two exponential components, provides the best compromise between accuracy and reliability, in addition to its simplicity.\textsuperscript{33} The Brochner-Mortensen technique has been developed to correct the systematic error of the slope-intercept technique.\textsuperscript{34,35} Appropriate pharmacokinetic approaches using the actual time point of sampling and Bayesian estimation for the calculation of the GFR from the isotope counts are important.\textsuperscript{36} The frequency and timing of collected samples matter, and are significant for accurate plasma isotopic GFR determination.\textsuperscript{21,22} It is generally recognized that at least 3 sampling points are required.

The accuracy of plasma isotopic GFR measurement when the GFR is below 20-30 mL/min/1.73m\textsuperscript{2} is limited.\textsuperscript{37,38} Therefore, the plasma isotopic GFR method can over-estimate GFR unless sampling time is extended; this may not be practical, especially for the dialysis population. To overcome this limitation, trials assessing GFR-estimation equations have used the urinary clearance isotopic GFR method.\textsuperscript{39} The urinary clearance can be calculated using the following method:

\[
GFR_{\text{Urinc}} = \frac{U_i \times V_i}{(T_i - T_{i-1}) \times \exp \left[0.5 \times \left(\ln P_i + \ln P_{i-1}\right)\right]} \quad \text{where } i = 1, 2, 3 \text{ (sample)}
\]

\[
GFR_{\text{Urinc}} = \text{median} \left( GFR_1, GFR_2, GFR_3 \right) \times \left( \frac{1.73}{B\text{SA}} \right) \quad \text{where } B\text{SA} = 0.007184 \times \left( \text{height}^{0.725} \times \text{weight}^{0.425} \right)
\]

Equation 1-5

where \(T_i\) is the time of the sampling, \(U_i\) is the urinary concentration of the isotope adjusted for isotopic decay, \(B\text{SA}\) is the body surface area, \(V_i\) is the urinary volume, and \(P_i\) is the plasma concentration of the isotope adjusted for the isotopic decay. However, few studies have been performed to resolve the
question as to whether urinary isotopic GFR could serve as a suitable replacement method.

(3) Endogenous Biomarkers to Estimate GFR

Although using exogenous markers, such as inulin and nuclear isotopes, to measure GFR is considered more accurate; the method is invasive and time-consuming, and not practical for day-to-day use. Therefore, endogenous biomarkers are commonly used to estimate. The ideal biomarkers in patients with chronic kidney diseases should confirm the level of renal function, measure the total “renal clearance” and predict the outcomes of “renal health”. Once the potential biomarkers are identified, they need to go through vigorous development and testing. In stage 1, pre-clinical research identifies promising markers that require further exploration. In stage 2, the potential biomarker is tested in human beings to determine if it can distinguish individuals severely affected with the disease from those who are healthy. In stage 3, retrospective studies establish whether the biomarker detects disease before the clinical diagnosis becomes evident. In stage 4, the biomarker undergoes prospective evaluation to determine the performance characteristics of the test in a setting in which it will be clinically applied. Finally, in stage 5, the focus is on the use of biomarkers to assess in the natural course of illness. When biomarkers are used for screening, it should be shown in randomized controlled trials that the application of interventions earlier in the process is indeed beneficial. Several biomarkers have been used to estimate glomerular filtration rate and have gone through at least stage 3 or 4; they include small plasma solutes such as creatinine, and endogenous small molecular weight proteins such as cystatin C, beta-trace protein and B₂ microglobulin.

1.1.4. Methods of Estimating Glomerular Filtration Rate: Biomarkers

(1) Creatinine
Creatinine is a small molecular weight solute (113 Dalton). It is the product of creatine and phosphocreatine, and is filtered by glomeruli. Proper and Mandel in 1937 proposed that it could be used for assessing kidney function. There are various methods and reference ranges for serum creatinine measurements. Recently, the isotope dilution-mass spectrometry (IDMS) reference method has improved and standardized the accuracy of creatinine measurements by eliminating some of the analytical problems. Measuring the creatinine clearance (mL/min/1.73m²) using 24-hour urinary creatinine measurements approximate GFR. However, it is not a true measure of GFR because there is some tubular secretion of creatinine. The equation for calculating 24-hour urinary creatinine clearance (CrCl) is as follows:

\[
CrCl = \frac{U_{Cr} \times V_{\text{urine}}}{P_{Cr}} \times \frac{1000}{1440} \times \frac{1.73}{BSA}
\]

where \(U_{Cr}\) (mmol/L) is the urinary creatinine concentration, \(V_{\text{urine}}\) is the urinary volume (L/24 hours), \(P_{Cr}\) (mmol/L) is the plasma creatinine concentration and BSA is the body surface area. This method is not routinely used because collecting 24-hour urinary creatinine clearance is cumbersome for patients. Timed urine collections are also notoriously inaccurate. Furthermore, creatinine is secreted by tubule. Therefore, creatinine clearance overestimates GFR by approximately 10% of the total excretion. A method called cimetidine creatinine clearance, where cimetidine treatment is used to block tubular secretion of creatinine, is impractical. To adjust for the problems, an equation to estimate CrCl was developed by Cockcroft and Gault (CG CrCl) and was published in 1976. 

\[
CG\ CrCl = \frac{(140 - \text{Age}) \times (\text{weight in Kg}) \times (0.85 \text{ if Female})}{72 \times \text{Serum creatinine}}
\]

The Cockcroft-Gault equation requires the weight of the patients, whereas some of the estimated GFR equations do not. Therefore, it is much easier to generate laboratory GFR results using the Modification of Diet in Renal Disease (MDRD) study equation along with the creatinine values. There have been more published
studies on using other creatinine-based estimating GFR (eGFR) equations than the Cockcroft-Gault equation. As a result, the Cockcroft-Gault equation is not as commonly used. There are several different eGFR equations. In the pediatric population, the most commonly used equation is the Schwartz equation. In the adult population, there are a few commonly used eGFR equations. See Table 1-1 for a summary.

Table 1-1. Commonly Used Creatinine-Based eGFR Equations

<table>
<thead>
<tr>
<th>Equations (GFR mL/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original MDRD&lt;sup&gt;39&lt;/sup&gt; eGFR = 170(Scr)&lt;sup&gt;-0.999&lt;/sup&gt;Age&lt;sup&gt;-0.176&lt;/sup&gt;BUN&lt;sup&gt;-0.170&lt;/sup&gt;Alt&lt;sup&gt;0.318&lt;/sup&gt;(0.762 if F)(1.180 if A.A.)</td>
</tr>
<tr>
<td>Abbreviated MDRD&lt;sup&gt;39&lt;/sup&gt; eGFR = 175(Scr)&lt;sup&gt;-1.154&lt;/sup&gt;Age&lt;sup&gt;-0.203&lt;/sup&gt;(0.742 if F)(1.212 if A.A.)&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>CKD-EPI&lt;sup&gt;48&lt;/sup&gt; eGFR = 141 (min(Scr&lt;sub&gt;K1&lt;/sub&gt;)&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt; max(Scr&lt;sub&gt;K1&lt;/sub&gt;)&lt;/sup&gt;&lt;sup&gt;-1.209&lt;/sup&gt;)0.993&lt;sup&gt;Age&lt;/sup&gt;(1.018 if F)(1.159 if A.A.)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Schwartz&lt;sup&gt;47&lt;/sup&gt; eGFR = (k x Height)/Scr&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Scr: serum creatinine (mg/dL); BUN: blood urea nitrogen concentrations (mg/dL); albumin (g/dL); F: female; A.A.: African-American. *This equation should be used when creatinine measurements have been calibrated to be traceable to IDMS. **K is 0.7 for females and 0.9 for males; <sup>a</sup> is -0.329 for females and -0.411 for males; min: minimum of Scr/K or 1; and max: maximum of Scr/K or 1. ***k is 0.33 for pre-term infant, 0.45 for full term infant and 0.55 for children of age 1-12.

However, creatinine is not a perfect GFR marker. Its production is affected by age, gender, ethnicity, and nutritional status. Because creatinine reflects individual body muscle mass, there is a large variability of its blood level among patients, independent of renal function. For example, disease such as spina bifida and neuromuscular disease can lead to unusually low creatinine levels. The estimated creatinine-based GFR, especially when the true GFR is greater than 60 mL/min/1.73m², can lead to the over diagnosis of chronic kidney disease. Over the last few years, almost all of the laboratories in Canada widely implemented the reporting of eGFR, rather than just creatinine values. Studies have shown that there was an increase in referral rate to nephrologists. Despite these issues, it is still the most commonly used biomarker to assess GFR. However, there is a need for better GFR biomarkers.
(2) Cystatin C

Serum cystatin C is a mid sized molecule with molecular weight of 13 kDalton. It is positively charged with an isoelectric point of 9.3 and is an inhibitor of cysteine proteases. This protein is produced at a very constant rate by all nucleated cells. The secretion of cystatin C is affected only by a few states, such as inflammation, uncontrolled hyperthyroidism and large doses of glucocorticoids. Furthermore, age-dependency reference intervals have been suggested. Currently, cystatin C levels are measured by automated and rapid particle-enhanced immunoturbidimetric (PETIA) and immunonephelometric (PENIA) methods rather than the original radioimuno- or enzyme-linked immunosorbant assays. This allows rapid and more precise measurements.

Approximately 94% of cystatin C is freely filtered in the glomeruli, and 99% of this filtered cystatin C is degraded in the tubular cells. In a thorough meta-analysis, Dharnidharka et al. demonstrated that cystatin C is an excellent marker for kidney function assessment in patients with chronic kidney disease. Similar observations about the superiority of cystatin C-based eGFR measurements have been noted by Stevens et al. in chronic kidney disease. White et al. further showed that cystatin C is also superior to creatinine in assessing kidney function in adult patients after renal transplantation. Several cystatin C estimating equations are summarized in Table 1-2. We recently published the results of a study, which compared all cystatin C-based estimating equations to the creatinine-based estimating equations (the abbreviated 4-variable Modification of Diet in Renal Disease and the Chronic Kidney Disease Epidemiology Collaboration Equations). We found that in this heterogeneous sample, the cystatin C-based Hoek equation performed the best overall. Stevens et al. also showed the superiority of cystatin-C-based GFR measurement. Furthermore, White et al. demonstrated that cystatin C-based equations, in particular the Filler equation, performed better than the creatinine-based GFR equation in renal transplant patients.
Table 1-2. Commonly Used Cystatin C-Based eGFR Equations

<table>
<thead>
<tr>
<th>Cystatin C-based GFR estimating equation (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filler\textsuperscript{70} Log eGFR = 1.962 + [1.123 log (1 / cystatin C)]</td>
</tr>
<tr>
<td>Grubb\textsuperscript{71} eGFR = 84.69 cystatin C\textsuperscript{-1.680} (1.384 if &lt; 14 years)</td>
</tr>
<tr>
<td>Hoek\textsuperscript{77} eGFR = -4.32 + (80.35/cystatin C)</td>
</tr>
<tr>
<td>Larsson\textsuperscript{72} eGFR\textsuperscript{*} = 77.239 cystatin C\textsuperscript{-1.2623}</td>
</tr>
<tr>
<td>Le Bricon\textsuperscript{73} eGFR = 78/cystatin C + 4</td>
</tr>
<tr>
<td>Rule\textsuperscript{74} eGFR = 66.8 cystatin C\textsuperscript{-1.30}</td>
</tr>
<tr>
<td>Boekenkamp\textsuperscript{75} eGFR = 137/cystatin C -20.4</td>
</tr>
<tr>
<td>Zappitelli\textsuperscript{76} eGFR = 75.94/cystatin C\textsuperscript{1.17} x (1.2 if renal transplant)</td>
</tr>
</tbody>
</table>

\textsuperscript{eGFR: estimating Glomerular filtration Rate}

Recently, the CKD-EPI investigators and the Chronic Kidney Disease in Children investigators developed equations that used both creatinine and cystatin C. They showed that the combined creatinine–cystatin C equation performed even better than either cystatin C- or creatinine-based equations.\textsuperscript{79,80} However, it has not been commonly used in clinical practice.

(3) Other Potential Markers

There are other potential GFR biomarkers; however, they have not been studied as extensively as creatinine and cystatin C. One example is beta-2 microglobulin, which has been used to estimate GFR. However, its use as a GFR marker has been limited because of its strong association with inflammation.\textsuperscript{16} Another example is Beta Trace Protein (BTP), which was mentioned briefly above. It has a molecular weight of 23-29 kDalton and has been studied to estimate GFR.\textsuperscript{21} It is expressed in all tissues except the ovaries.\textsuperscript{81} Some studies have demonstrated a good correlation between BTP levels and the inulin clearance.\textsuperscript{82} However, its use in estimating GFR is still preliminary and more studies are needed.
1.2. INTRODUCTION TO RENAL REPLACEMENT THERAPY

1.2.1. History and Development of Renal Replacement Therapy

When 90% or more of usual kidney function is lost, either kidney transplantation or dialysis is required to sustain life. As of December 31, 2012, there were 41,252 Canadians living with end-stage kidney disease and 23,814 persons in Canada (58%) were receiving dialysis; the remaining 42% had a functioning transplanted kidney.\(^{83}\) Hemodialysis is the most common form of dialysis, which is typically delivered three times a week with a machine connected to a patient’s vascular system. Mortality remains high (approximately 18 to 20% per year) despite improvements in technology for dialysis, development of new pharmaceutical agents, and nearly 50 years of experience. Although dialysis can sustain life, it rarely restores health. Patients undergoing dialysis have considerable complications often requiring hospitalization, and relatively poor functional status and health-related quality of life. The “renal health” of the dialysis patient relates to that component of health directly or indirectly impaired by loss of kidney function and, hence, possibly restored by effective (“adequate”) dialysis therapy.

1.2.2. History and Development of Hemodialysis

Dialysis uses very basic concepts, such as osmosis and diffusion, to clear extra fluid and substances from the body. The first person to describe this process was Thomas Graham\(^{84}\), known as the ‘Father of Dialysis’. He first studied diffusion in gases and later performed a series of experiments in liquids. He predicted that ‘dialysis’ would be an important treatment for renal failure. Subsequently, Fick\(^{85}\) published a quantitative description of diffusion.

Hemodialysis is a procedure that circulates blood from the body over an external circuit, exchanges substances between blood and dialysate through a semi-permeable membrane, and returns the ‘purified’ blood to the patient. The process is done outside of the body. Abel\(^{86}\) and colleagues created the first artificial
kidney in 1913. They dialyzed anesthetized animals using vividiffusion machines, which consisted of tubes with semipermeable membranes. He was also the first person to use an anticoagulant, Hirudin, to prevent clotting in the dialyzer. Subsequently, Haas\(^\text{87}\) documented the first human hemodialysis treatment in 1924. Although none of their patients survived, they started the use of the Collodion dialyzer membrane and was the first person to use heparin to prevent dialyzer clotting. Heparin is still the most commonly used method of anticoagulation in hemodialysis therapies.

Kolff\(^\text{88}\) in 1943 developed a more practical rotating drum hemodialysis machine and working dialyzer, and performed the first hemodialysis treatment where the patient survived. Although the basic concept of dialysis has not changed, the technical improvement in this rotating drum became the first major breakthrough in clinical hemodialysis. Kolff used a new material called cellophane, where the blood travelled through the inside the cellophane membrane tube and rotated through the dialysate fluid. The substances were removed and/or exchanged through the cellophane membrane. At this point, dialysis was to provide life support while waiting for recovery from acute renal failure. Kolff\(^\text{89}\) developed the next-generation dialysis machine, known as the Kolff-Brigham Kidney. These machines were used in the Korean War and significantly improved the survival rate of the soldiers. Kolff’s dialysis machine did not allow for excess fluid removal. It was Alwall\(^\text{90,91}\) who modified the machine and allowed a negative pressure to be applied for fluid removal. He also invented the arteriovenous shunt for hemodialysis in a rabbit in 1948. He later collaborated with a businessman and founded one of the major dialysis companies, Grambro, Inc. Improvements in equipment design and dialyzer occurred over the next few decades, but the basic concepts of dialysis, based on diffusion, have not changed.

Before the 1960’s, chronic hemodialysis was believed to be impossible. This is because when hemodialysis started, each hemodialysis session required physicians to cut down to the vessels to create vascular access. The damaged
veins and arteries made it difficult to find access to a patient’s blood after several sessions of treatment. The challenge of creating a reusable vascular access in humans was solved by Scribner. He worked with Quinton\textsuperscript{92} in the 1960’s who designed a U-Shaped Teflon tube that connected between artery and vein. Quinton later started a business called Quinton Instruments, which sold his inventions, including treadmills for cardiac stress tests. However, the Scribner’s shunt has problems, especially with clotting. Appell, Cimino, and Brescia\textsuperscript{93} pioneered the arterial-venous fistula for chronic vascular access that is used for hemodialysis patients today. An arterial-venous fistula is created by arterializing a vein by connecting it to an artery. These major improvements and advances in technology have helped the development of our current hemodialysis machines and equipment.

1.2.3. History and Development of Peritoneal Dialysis

Peritoneal dialysis was the first renal replacement therapy to be applied clinically. In 1877,\textsuperscript{94} Wegner documented osmotic ultrafiltration in Germany and infused a concentrated glucose solution into the peritoneal cavity of animals to increase fluid removal. During a peritoneal dialysis treatment, the peritoneal solution (dialysate) is infused into the peritoneal cavity, which is the space between the parietal peritoneum (the part that lines the abdominal wall) and the visceral peritoneum (the part that lines the visceral gastrointestinal tracts). The peritoneal membrane serves as the ‘membrane’ that separates the dialysate from the mesenteric blood flow. The surface area of the peritoneum membrane, which is covered by mesothelial cells, is approximately 1.00-2.07 m\textsuperscript{2} in adults.\textsuperscript{95} The interstitial layers are embedded with microvessels, or peritoneal blood supplies. As a result, both diffusion and convection clearance can be achieved between the peritoneal blood and dialysate fluid.

In 1923, Ganter\textsuperscript{96} applied this therapy clinically and treated a woman with obstructive uremia by infusing 1.5 L of saline into her abdominal cavity. She showed some temporary improvement, but died later. Like hemodialysis, intermittent peritoneal dialysis treatments prior to the 1960’s were used as short-
term renal replacement therapies due to a lack of permanent peritoneal dialysis catheters.\textsuperscript{97-99} It was Tenckhoff\textsuperscript{100} who designed a permanent and safe catheter, which allowed the development of chronic peritoneal dialysis. Later, Popovich and Moncrief\textsuperscript{101} using glass containers had the first clinical application of continuous ambulatory peritoneal dialysis (CAPD). They also developed a comprehensive calculation for dialysis clearance using the volume and the dwell time of dialysate solutions.\textsuperscript{102} The major comorbidity of these patients was peritonitis (infection of the peritoneum). It was known that the risks were related to the patients’ peritoneal dialysis technique and the connecting system. The peritonitis rate has significantly improved since Oreopoulos introduced peritoneal dialysate into plastic bags and used the Y connecting system, introduced by Buoncristiani,\textsuperscript{103,104} with the flush-before-fill technique. These simple concepts have dramatically reduced the peritoneal dialysis peritonitis complication rate. Although the automated cycler system was developed in 1962 by Boen, it has only become a common peritoneal dialysis technique over the last 10-20 years.\textsuperscript{105} Today, the common peritoneal dialysis prescription uses an automated cycler machine at night, which allows approximately 4 exchanges in 8-10 hours overnight, and up to two exchanges over 4-14 hours during daytime.

\textbf{1.3. COMPONENTS OF HEMODIALYSIS}

Both peritoneal dialysis and hemodialysis use the concepts of diffusion and convection clearance. Peritoneal dialysis uses the peritoneal blood flow, which allows molecules and fluid exchange to occur between the blood and dialysate in the peritoneal cavity. The dialysate fluid is drained and then the new dialysate fluid is infused. It is considered a continuous dialysis therapy. Similarly, hemodialysis uses the diffusion and convection clearance mechanisms. However, the blood is first removed from the body. As a result, fluid and molecules are exchanged outside of the body through a hemodialysis machine and a dialyzer. Dialysate runs in the counter-current direction in the dialyzer to maximize the concentration gradient. The ‘clean’ blood is then returned back to the patients. The therapy is usually performed three times per week, with four
hours each session. However, prolonged and/or frequent hemodialysis is performed only in a small population of patients, usually in the home setting. The focus of this chapter will be on hemodialysis, including its components and mechanisms, dialysis and patient physiology, and the concepts of dialysis adequacy and kinetics. The five major components of hemodialysis are: a vascular access, a dialysate circuit, a blood circuit, a dialyzer and a hemodialysis machine.

1.3.1. Vascular Access

A vascular access for a patient allows a gate for the blood to pass into the hemodialysis circuit. It is considered the Achilles’ heel of hemodialysis. Without a permanent vascular access, chronic hemodialysis cannot occur. However, the access can fail due to infection, stenosis, thrombosis or fibrin sheath formation. The commonly used vascular accesses currently are: arterial-venous fistulae (created by connecting a patient’s artery to a vein), arterial-venous graft (created by connecting patient’s artery to a vein, through a synthetic hollow tube), and central venous catheter (inserted into an internal jugular vein, a femoral vein, or a sub-clavian vein). To lower the infection rate, the central venous catheter usually tunnels under the skin for chronic use.

1.3.2. Dialysate Circuit

Another component of hemodialysis is a dialysate circuit. It is the circuit where the dialysate leaves its source/supply and passes through the dialyzer. Dialysate is the clean fluid that exchanges the electrolytes, toxins and fluid with the blood. Dialysate is generated from a water supply (i.e. from the city) after meeting certain universal standards. Because of the large water exposure, it is important that the water goes through a vigorous detoxing process, using a combination of water softener, activated carbon filter, reverse osmosis, and/or distillation. The clean water can be delivered to the dialysis machine, mixing with the concentrated electrolytes (such as sodium, potassium and bicarbonate) to form dialysate, which allows hemodialysis to occur. Its composition of sodium,
potassium and bicarbonate can be adjusted. To ensure the patient is not exposed to a hyper- or hypo-osmolar dialysis solution, which can be fatal, the dialysate conductivity (usually 12-16 mS/cm) is monitored in modern hemodialysis machines. During a 4-hour hemodialysis treatment, the patient is exposed to ~100 L of water.

1.3.3. Blood Circuit

The third component is a blood circuit. This is a circuit where the blood leaves the patient from the vascular access, passes through the dialyzer, and returns back to the patient. To allow for an efficient hemodialysis treatment with the current hemodialysis regime (four-hours thrice weekly), the blood flow rate needs to be at least 250 mL/min in adults, and is usually between 300-400 mL/min. In children, 5-8 mL/kg/min are targeted. The inflow bloodline (arterial) connects from the patient’s vascular access to the dialyzer. The outflow bloodline (venous) returns the blood from the dialyzer back to the patient. Before the roller pump was used in modern hemodialysis machines, the inflow bloodline would need to connect to an artery and the outflow bloodline was connected to a vein. The blood flow rate depended on the blood pressure difference between the artery and the vein. This pressure difference also allowed ultrafiltration to occur. With modern technology, the roller pump is placed in the arterial line to generate ‘negative’ pressure. Therefore, both the arterial and venous lines can be placed in the venous system of a hemodialysis patient. Blood flow rate is a function of the roller pump rotation rate and the bloodline pump segment volume. However, because of the negative pressure generated by the roller pump, air can get into the bloodline if there is a breakage in the bloodline system. An air detector is placed in the venous line as a safety measure before the blood is returned back to the patients, in order to avoid an air embolism.

1.3.4. Dialyzer

A dialyzer is where dialysis occurs. It contains many thin semi-permeable membrane hollow fibers with many small pores. It allows the molecules and fluid
to be exchanged between the blood and the dialysate. The blood flows within the hollow fibers in one direction and the dialysate flows outside the hollow fibers in the opposite direction. This counter-current flow maintains some concentration gradient between the blood and the dialysate. As the dialyzer membrane acts as a separator between the blood and the dialysate, there are two liquid phases: the transport (solute transport due to diffusion and convection) and the contact phases (blood material interaction with the dialyzer material). There is also adsorption of proteins on the membrane, which more commonly occurs for the hydrophobic molecules. One of the issues with dialyzer membranes is activation of the coagulation system leading to dialyzer thrombosis. As the dialyzer membrane is a foreign body, the blood coagulation system may be activated when blood comes into contact with the membrane. Dialyzer membrane thrombosis can lead to significant reduction in blood flow and diffusion capacity. The use of heparin has significantly reduced clot formation on the dialyzer membrane.

Dialyzer membranes have undergone significant development in the last century. The first human dialysis was performed using cellulose membranes. Cellulose is a polysaccharide polymer that consists of repeating units of cellobiose monomer. Each cellobiose monomer constitutes two glucose molecules, which contains three hydroxyl groups (-OH) that can lead to chemical reactions forming esters or ethers. Kolff began to use cellophane materials for dialysis. In the 1960s, cuprophane had become popular.\textsuperscript{106,107} Both cellophane and cuprophane are types of cellulosic membranes.\textsuperscript{48} Cuprophan is a low flux membrane with a wall thickness of 5-20 µm. Although it is good for small solute clearance, it has very low sieving capacity for large solute. It is also considered bio-incompatible. Bioflux, on the other hand, has a similar structure to cuprophan, but has larger pore diameters. Therefore, it has higher flux and has better clearance for medium solutes. Cuprammonium rayon was introduced to increase the removal of higher molecular weight molecules. It replaced the hydroxyl groups with hydrogen bonds. The bio-incompatibility of these membranes is because of the hydroxyl groups (-OH), which can activate the complement system and lead to
inflammatory reactions (interleukin-1, 6, and 8, and TNF-α) with activation of complement factors. Some patients develop anaphylactoid reaction (C3a and C5a) and leukopenia during dialysis. Therefore, there was a big movement to develop more biocompatible membranes.

The modified cellulosic dialyzers were developed and acetate or hemophane was used to replace the hydroxyl groups on the cellulosic backbone. Hemophane has 5% of the hydroxyl groups substituted to diethyl-amino-ethyl groups. It has reduced complement activation to some degree, but it is also considered a low flux membrane. Synthetically modified cellulose has 20-25% of the hydroxyl groups substituted by benzyl groups. It is approximately 8-9 µm in thickness. It is considered more biocompatible as compared to hemophane and cuprophan. Celluose acetate (and di- and tri-acetate) substitutes the hydroxyl group with CH₃CO radicals. The cuprammonium-rayon polyethylene glycol substitutes the hydroxyl groups with polyethylene glycol chains.

There was a significant increased in the use of synthetic membrane dialyzers over the last 10-20 years.¹⁰⁸ Now, the more commonly used dialyzers in North America contain synthetic membranes (based on synthetic polymers).¹⁰⁹ These materials are more biocompatible and heat resistant with endotoxin retention. They are more permeable. The natural stiffness of the material provides membrane strength and overall the structural support.¹¹⁰ Cellulose membranes have the thinnest membrane and the synthetic membranes are much thicker.¹¹¹ By contrast, the membrane inner diameters are relative similar between cellulosic and synthetic membrane (170-230 µm). The common synthetic membranes are polyacrylonitrile, polysulfone and polyamide. One of the major issue that needs to be addressed for cellulose dialyzer membranes is complement activation. Synthetic dialyzer membranes are less likely to bind to complement-regulating protein and are considered more biocompatible. The development of synthetic membranes was started in 1970 using polyacrylnitrile materials, with the desire to increase higher molecular weight solutes clearance.¹¹¹ Subsequently, polysulfone membrane has become the most widely used synthetic dialyzer.
Some of the polysulfone membrane was developed using a steam sterilization technique with another safety feature, endotoxin retention. The various proportion of hydrophilic and hydrophobic composition different between different polysulfone dialyzers. The advantages and disadvantages of the different types of membrane are summarized in Table 1-3 and the different types of hemodialysis membranes are summarized in Table 1-4.

**Table 1-3. Advantages and Disadvantages of Unmodified Cellulosic, Modified Cellulosic and Synthetic Hemodialysis Membranes**

<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unmodified Cellulosic</strong></td>
<td>High diffusive membrane transport for small molecular solutes</td>
<td>Higher complement activation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower clearance of middle and large molecular solutes</td>
</tr>
<tr>
<td><strong>Modified Cellulosic</strong></td>
<td>Increased removal of middle molecular solutes</td>
<td>Higher complement activation</td>
</tr>
<tr>
<td><strong>Synthetic</strong></td>
<td>High water permeability for ultrafiltration; Better middle molecular solute removal</td>
<td>Thick membrane wall</td>
</tr>
</tbody>
</table>
Table 1-4. Different Types of Hemodialysis Membranes

<table>
<thead>
<tr>
<th></th>
<th>Unmodified Cellulosic</th>
<th>Modified Cellulosic</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-Flux</td>
<td>Cuprophan</td>
<td>Cellulose (di) acetate</td>
<td>Polysulfone</td>
</tr>
<tr>
<td></td>
<td>Saponified cellulose ester</td>
<td>Hemophan</td>
<td>Polyamide</td>
</tr>
<tr>
<td></td>
<td>Cuprammonium-rayon</td>
<td>Cuprammonium-rayon polyethylene glycol</td>
<td>Polyethersulfone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Synthetic modified cellulose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cellulose tri acetate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin E</td>
<td></td>
</tr>
<tr>
<td>High-Flux</td>
<td>Bioflux</td>
<td></td>
<td>Polyester polymer alloy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Poly-acrylonitrile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polyarylethersulfone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethylene vinyl-alcohol copolymer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polycarbonate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>polyether copolymer</td>
</tr>
</tbody>
</table>

1.3.5. Hemodialysis Machines

Finally, the hemodialysis machine is the controlling center for all these components. It has a computer system and a monitor, pressure sensors, flow sensors, and an air detector. More modern hemodialysis machines also include other devices, such as online ionic effective dialysance methodology, blood pressure monitoring, and relative blood volume measurements. Figure 1-1, summarizes all the necessary components of the hemodialysis therapy.
Figure 1-1. A Simple Schematic Diagram of Hemodialysis Circuit

1.4. HEMODIALYSIS MODALITIES

Hemodialysis can be performed at home, in hospitals (in-center), or in satellite units (hemodialysis units associate with major dialysis centers but not inside hospitals). The duration and the timing of hemodialysis treatments can vary between 2-24 hours per day and it can be performed during daytime and nighttime. Below are some of the common hemodialysis settings and techniques/modalities:

- **Intermittent hemodialysis therapies (3-4 days per week)**
  - Conventional hemodialysis (3-4 hours per session): in-center, satellite units, or home
  - Extended-hours hemodialysis (6-8 hours per session): in-center or home
- **Quotidian hemodialysis therapies (5-7 days per week)**
  - Short-hours daily (2-2.5 hours per session) – in-center or home
• Nocturnal (8-10 hours per night) – home

• **Slow continuous dialysis therapies (24 hours per day)**
  • Continuous renal replacement therapy, usually with venous to venous access and less commonly with arterial to venous access
    o Hemodialysis only: Continuous venovenous hemodialysis – intensive care units
    o Hemofiltration only: Continuous venovenous hemofiltration – intensive care units
    o Both hemodialysis and hemofiltration: Continuous venovenous hemodiafiltration – intensive care units
  • Slow continuous ultrafiltration (SCUF) with no dialysis or replacement fluid - in-center

1.5. THE PHYSIOLOGY OF HEMODIALYSIS CLEARANCE

1.5.1. Basic Concepts of Diffusion and Convection

Diffusional clearance occurs when solutes from solution A (blood) move to solution B (dialysate) and are driven by concentration differences through a semipermeable membrane (Figure 1-2). Molecules and ions dissolved in the solutions are in constant motion. More of the molecules on average moves from high concentration solution to low concentration solution. Only molecules that are smaller than the pore size can pass through the membrane. Water molecules can also ‘diffuse’ through a semi-permeable membrane. The term ultrafiltration refers to the situation where water molecules move from solution A (blood) to solution B (dialysate). This process is driven by either a hydrostatic or an osmotic gradient through a semipermeable membrane. The hydrostatic pressure difference between the two sides of the semi-permeable membrane is opposed by osmotic pressure. As a result, convectional clearance can occur when water moves from one solution to another solution through the semipermeable membrane (i.e. blood to dialysate), and the water is accompanied by other solutes. This process is called convection or solvent drag.
Dialysis clearance is defined as the volume of blood from which all solutes in question are reduced during a specified time period. The total amount of solute removal depends on the duration of the therapy. This is similar to renal clearance in the healthy kidney (discussed earlier). Conventional hemodialysis (3 times per week and 4 hours per session) is highly efficient in removing small solutes, and it has a high clearance rate per session. However, because it is an intermittent therapy that occurs only 3 times per week and 4 hours per session, its weekly clearance is similar to continuous therapy by peritoneal dialysis. The factors that influence hemodialysis solute clearance are listed in Table 1-5, and are grouped as dialysis-related, patient-related, and solute-related factors. Some of the key variables to consider when discussing hemodialysis clearance are blood flow rate, dialysate and its flow rate, dialyzer characteristics, ultrafiltration rate, and dialysis duration and frequency. Each of these key factors will be discussed in this section. Solute characteristics are key to its clearance rate, which is also influenced by its compartmental distribution. This will be discussed in the next section.

### Table 1-5. Factors Influencing Solute Concentrations in Dialysis Patients

<table>
<thead>
<tr>
<th>Dialysis-related</th>
<th>Patient-related</th>
<th>Solute-related</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Body weight and total body water</td>
<td>Compartmental Distribution</td>
</tr>
<tr>
<td>Frequency</td>
<td>Solute intake/absorption/generation</td>
<td>Protein Binding</td>
</tr>
<tr>
<td>Blood flow</td>
<td>Residual renal function</td>
<td>Charge</td>
</tr>
<tr>
<td>Dialysate flow</td>
<td>Blood viscosity</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>Dialyzer characteristics*</td>
<td></td>
<td>Steric configuration</td>
</tr>
<tr>
<td>Membrane absorption</td>
<td></td>
<td>Intracellular Concentration</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*surface area, pore size, hydrophilic/hydrophobic
1.5.2. Chronic Hemodialysis Access and Blood Flow Rate

To reach adequate weekly hemodialysis with conventional hemodialysis treatment, the blood flow rate needs to be 300-400 mL/min for patients with minimal renal function. They will need at least 3-4 hours of treatment per session. It is of paramount importance to have reliable vascular access. Because of the need for high blood-flow rate, chronic hemodialysis was not possible before the development of chronic vascular access techniques. Prior to the 1960’s, a surgical procedure to cut down the arterial and venous vessels was required prior to each hemodialysis treatment. Therefore, patients would eventually run out of vessels to allow vascular access. In the 1960’s, this issue was resolved by the development of Scribner’s shunt. Subsequently, the development of other vascular accesses, such as an arteriovenous fistula, an arteriovenous graft, and a central venous catheter, allowed chronic hemodialysis to be possible.
Connecting a vein to an artery creates an arteriovenous fistula. With the use of an artificial graft, an arteriovenous graft can be created. The advantage of an arteriovenous graft over a fistula is that it is available within a few weeks after its surgical creation. However, it has shorter access survival time as compared to fistula. There is also a higher risk of graft infection, thrombosis and stenosis. Therefore, the arteriovenous fistula is still the preferred chronic vascular access for hemodialysis patients over the arteriovenous graft. Another common vascular access in hemodialysis patients is the central venous catheter. It is most commonly placed through the internal jugular vein with cuffed tunneled catheters just under the skin. Other insertion sites such as subclavian and femoral veins have been used. Rarely, translumbar catheters to inferior vena cava have been used in patients with challenging vascular access. Each catheter has two lumens, one is the venous port and one is the arterial port. These two ports/lumens are within the venous system of the patient. Formation of a thrombus and/or a fibrin sheath at the tip of the catheter can cause delayed catheter dysfunction or a reduction in blood flow rate, and/or lead to recirculation. Sometimes, the blood flow rates are significantly affected and the arterial and venous lumens need to be reversed. This further increases the amount of access recirculation and reduces the solute clearance. Infection is another common complication when using the central venous catheter. Because the arteriovenous fistula method is consistently associated with the lowest risk of mortality versus arterio-venous grafts or catheters, therefore, the arteriovenous fistula is considered the preferred vascular access for hemodialysis patients. The discussion below will focus on the arteriovenous fistula.

After the arteriovenous fistula creation surgery, there is an increase in shear stress in the vasculature because of the local substantial increase in blood flow. As a fistula matures, the diameter of the proximal part of the vein will grow. Experimental models showed that this is followed by compensatory changes, which are related to the release of nitro-oxide, prostacyclin and other mediators, as well as changes in endothelial cells and the remodeling of cellular and extracellular components of the wall. These adaptations may take 2-3 months to
Surgical technique is quite critical to the success of fistula creation. A well-developed fistula can usually reach a flow rate of 645 ± 332 mL/min in the forearm and 1336 ± 689 mL/min at the brachial or upper arm. By contrast, some patients may develop a high blood flow rate in their fistula (>2,000 mL/min or access flow rate/cardiac output >30-35%). If the flow rate of the arteriovenous fistula is very high, patients can develop left ventricular hypertrophy and/or high output cardiac failure. A patient with total cardiac output of 7,400 mL/min and vascular access flow of 4,100 mL/min can reduce the systematically functional cardiac output. A group of high vascular access flow patients (3,135 ± 692 mL/min) demonstrated a significant reduction in functional cardiac output after access creation. Left ventricular mass regression, however, can be seen after ligation of the arteriovenous fistula. This can significantly compromise cardiac function. By contrast, a high output state does not seem to increase mortality. In fact, a study has shown that high access flow rate was associated with increased survival.

Blood flow rate at the needling sites of the fistula will depend on the arterial flow prior to entering the fistula (stenosis at the anastomosis site), collateral vascular system (taking away the blood flowing to the fistula), and the flow resistance within the fistula (stenosis of the fistula), and at the distal part of the venous system (central stenosis). One of the common issues with an arteriovenous fistula is reduction in access flow rate due to stenosis. As the stenosis gets more severe, the access flow rate can be reduced; in addition, the hemodialysis clearance will be reduced. Severe arteriovenous fistula stenosis is also a sign of a failing fistula. This can also increase clotting risk and/or lead to acute thrombosis of the fistula. If the stenosis is distal to the arterial needle during hemodialysis, recirculation can occur. When the arteriovenous fistula flow rate is less than the extracorporeal blood flow rate (<300-400 mL/min), recirculation will occur.

Therefore, routine monitoring of the access function is recommended; measurement of the access blood flow rate is the preferred method. The gold
standard for measurement of the access blood flow \((Q_a; \text{mL/min})\) is an indicator dilution technique.\(^{120}\) A dilutant, such as sodium, is injected into the venous line, and it can be detected in the dialyzer inlet immediately after. The indicator is the ultrasound velocity of the blood and the dilution is by saline solution. Another technique, such as thermodilution, has also been used: it is well validated and established and employs Krivitski’s theory:

\[
Q_a = Q_b \times \left(\frac{1-R}{R}\right)
\]

Equation 1-8

where \(Q_b\) is the blood flow (mL/min) through the dialyzer and \(R\) is the proportion of circulated blood through the access induced by reversing the arterial and the venous dialysis line. Other indicator dilution methods exist.\(^{121}\) Another phenomenon can be observed in an arteriovenous fistula: cardiopulmonary recirculation. This results when the ‘clean blood’ bypasses the systemic microcirculation from cardio and pulmonary vessels and returns to the extracorporeal circuit. Therefore, retrograded ‘clean blood’ will flow and mix with the blood that enters the hemodialysis machine. This can affect the clearance. The amount of cardiopulmonary recirculation is proportional to the arteriovenous fistula flow rate and is inversely proportional to the cardiac output.

Repeated needle entries into the fistula prior to each hemodialysis treatment allow access to high blood flow during hemodialysis. The viscosity of blood is 3 times higher than that of water. This is because the hematocrit of the blood is \(\sim 40\%\) (the percentage of the blood that is cells). In addition, there are proteins in the plasma, although these effects on viscosity are much smaller. Unlike in simple fluids, blood viscosity varies by shear rates, and shear stress affects the particles and other contents of blood, such as hemoglobin. Therefore, the half-life of red blood cells is shorter in hemodialysis patients, because a small amount of hemolysis can occur with each hemodialysis treatment. Blood flow \((Q_b)\) through a vessel is dependent on the pressure difference \((\Delta P)\) between the two ends of the vessels and the vascular resistance \((R)\). This follows Ohm’s Law:
Newtonian fluid (i.e. water) is a fluid in which the viscous stresses and the strain rate are related by a constant viscosity tensor. It does not relate to the flow rate. The Poiseuille’s law provides a helpful measure of the dependence of flow on viscosity and tube geometry. The Poiseuille’s law is described as the following:

\[ Q_b = \frac{\Delta P}{R} \]  
Equation 1-9

\[ R = \frac{9\mu L}{\pi r^4} \]  
Equation 1-10

where \( R \) is the resistance, \( \mu \) is the blood viscosity, \( L \) is the access length, and \( r \) is the access radius.\[^{107}\] For example, with a reduction in \( r \) of 50%, \( R \) will increase by 16 times. Although Poiseuille’s law is a widely used equation for fluid flow resistance measurements, it has certain restriction to Newtonian fluid with laminar flow in a straight tubes. Blood is a non-Newtonian fluid. This relationship will only serve as an estimate for the blood flow, especially in the dialysis tubing where the flow is laminar. In addition, the Reynolds number for straight, smooth, laminar flow of Newtonian fluid is usually \( \sim 2300 \). For blood, the Reynolds number is related to the blood flow, and tube diameter. In the extracorporeal circuit in dialysis patients where the tube diameter is 4.5 mm and the viscosity is 3.5 mPa/S (with hematocrit of 0.38), the Reynolds number is below 500 for the blood flow \(< 600 \text{ mL/min} \) (i.e. blood flow of 400 mL/min has Reynolds number \( \sim 600 \)).\[^{107}\] These are important factors to consider when assessing the blood flow rate of the fistula, as well as the extracorporeal blood flow rate within the connecting tubing and the dialyzer.

In the extracorporeal circuit, there is generally laminar flow. However, the flows at the cannulation and connector sites are turbulent. The flow rate is proportional to the pressure differences between the inlet and the outlet of the circuit, and inversely proportional to the blood viscosity and the resistance of the circuit. Historically, the pressure differences between the artery and vein were used as the driving force in the extracorporeal circuit of a hemodialysis system. However, today’s hemodialysis machines use a pump-driven extracorporeal system. Most of these machines use rotary peristaltic pumps. A pump that allows flow reversal...
has also been developed. This has been used in single-needle hemodialysis, especially in nocturnal hemodialysis patients and/or in patients with a new fistula.

The stroke volume can be calculated based on the following equation\textsuperscript{122}:

\[
\text{Stroke Volume} = \frac{D d^2 \pi}{4} \quad \text{Equation 1-11}
\]

A typical pump has the diameter of its pump bed (D) of 8.8 cm and the tubing cross-section diameter (d) as 0.8 cm. The blood flow rate will be the product of the stroke volume per revolution and the rotation speed of the roller. Some hemodialysis machines, such as the Fresenius A2008C\textsuperscript{®}, have a built in system to calculate the flow.

Although the blood flow rate is typically set at 400 mL/min during a hemodialysis treatment, the actual blood flow rate is lower than 400 mL/min. Recently, in one of our published studies (see Chapter 5), we measured the true blood flow rate and compared this to the machine blood flow rate.\textsuperscript{123} This was measured by the Transonic HD01 monitor (Transonic Systems, Ithaca, NY). When the hemodialysis machine blood flow rates were set at 200 mL/min and 400 mL/min, the actual blood flow rates were 179 ± 2 and 331 ± 6 mL/min, respectively.

**1.5.3. Dialyzer**

Each dialyzer contains 7000-14000 hollow fibers. Resistance in the dialyzer can affect the blood flow rate. The smaller inner fiber diameter provides a shorter diffusive distance for the solute, but it has a higher shear rate as compared to the larger size fiber.\textsuperscript{120} The membrane of the hollow fibers consist of millions of pores that allow diffusion and ultrafiltration to occur. These pores allow the fluid and solutes to diffuse across the membrane through concentration gradients. The pore-related characteristics such as pore size and pore density influence the permeability of the solute. Water permeability is strongly related to the pore radius only. The larger the pores, the less transmembrane pressure is required for fluid transport. However, the larger the pores, the more the larger molecules,
such as protein (albumin), can diffuse through. This will have a significant clinical consequence.

By contrast, one way to increase the diffusional clearance is to increase the surface area of the membrane. The synthetic hollow fibers have an inner diameter of approximately 180-220 µm, and a length of 20-24 cm. The surface area of a small fiber can be expressed as the following:

\[ S = 2\pi rL \]  

**Equation 1-12**

The total surface area of the dialyzer (assuming 10000 fibers) is approximately 1.51 m². The surface area of an adult size dialyzer is generally in the range of 1.6-2 m². As the dialyzer surface area increases, the diffusional clearance will also increase. This can be demonstrated in Figure 1-3.

The absorption of the dialyzer is commonly ignored. However, this should be considered when assessing solute clearance. Generally, this will depend on the charge, size and other characteristics of the molecule. However, the characteristics and types of dialyzers can also influence the solute absorption.
1.5.4. Dialysate and Its Flow Rate

Dialysate is an important part of the hemodialysis treatment. In current use, dialysate water comes from purified water. A healthy person drinks ~1.5 – 2 L of water per day which is 10 -15 L per week, but a hemodialysis patient can be exposed to ~ 400 L of water per week. Dialysate water is exposed to blood directly; thus barriers such as intestinal protection are eliminated. Water contaminants, including particles (ion, sand, and clay), chemicals, and microorganisms/endotoxins (virus and fungi) need to be removed.

For dialysate water, water purification is commonly done by using city water purified through distillation, reverse osmosis, ultrafiltration and/or deionizer systems. Because a distillation system is expensive and given the large amount of purified water required for hemodialysis units, ultrafiltration (using various sizes of filters), reverse osmosis and/or deionizer are more commonly used. In addition, softeners to remove calcium and magnesium, and activated carbon to removed organic contaminants, chlorine and chloramine, are common devices employed in the water purification systems.

Reverse osmosis is an effective way of removing organic and inorganic solutes. As water flows through the system, it is pushed through a very tight membrane. Although the osmotic gradient at the ‘rejected’ side is much higher than the ‘permeated’ side, the hydraulic pressure is high enough to push the fluid against the osmotic force. These membranes are quite similar to dialyzers. By monitoring the conductivity of the ‘permeated’ water, as the conductivity increases, it provides an indication of the poor performance of the membrane.

A deionizer system removes inorganic ions by an ionic exchange process. The water passes through a cationic resin, where the cation will exchange with H⁺. Then, the water passes through the anionic resin and the anion will exchange...
with OH\(^{-}\). Again the resistivity of the water will be monitored. The resin will need to be changed if the resistivity of the water decreases. Instead of resin, an electric deionizer can also be used. A deionizer system will need to be followed by ultrafiltration system because it is not effective in removing organic or bacterial toxins.

The quality of dialysate water is crucial. Aluminum toxicity has led to many deaths, and chloramine toxicity can lead to acute hemolysis, and microcystis aeroginosa overgrowth can also lead to inflammation and increase risk of infection. Currently, the recommended bacteria levels of dialysate water are < 100 colony-forming units per milliliter of water. Endotoxin levels should be < 0.1 EU/mL. The hardness of water should be < 1 ppm. The pressure between the pre- and post-water softener, carbon tank and deionizer should not drop more than 10 PSI. The resistivity of the reverse osmosis system and the deionizer system need to be monitored. An example of a reverse osmosis system is shown in Figure 1-4 and the constituents of dialysate are summarized in Table 1-6.

![Figure 1-4. An Schematic Diagram of a Water Purification System: the Reverse Osmosis System.](image)

**Table 1-6. Constituents of Plasma and Dialysate.**

\(^3\)
<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Dialysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na⁺; mEq/L)</td>
<td>142</td>
<td>140</td>
</tr>
<tr>
<td>Potassium (K⁺; mEq/L)</td>
<td>5.0</td>
<td>1.0-4.0</td>
</tr>
<tr>
<td>Calcium (Ca²⁺; mEq/L)</td>
<td>3.0</td>
<td>2.5-3.5</td>
</tr>
<tr>
<td>Magnesium (Mg²⁺; mEq/L)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Chloride (Cl⁻; mEq/L)</td>
<td>107</td>
<td>105</td>
</tr>
<tr>
<td>Bicarbonate (HCO₃⁻; mEq/L)</td>
<td>24.0</td>
<td>35.7</td>
</tr>
<tr>
<td>Lactate (mEq/L)</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Phosphate (HPO₄²⁻; mEq/L)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>88</td>
<td>0</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.5</td>
<td>7.0</td>
</tr>
</tbody>
</table>
In order to ensure the required concentration of the electrolytes in the dialysate solution, a monitoring system is required. Conductivity is the measured of electrical current conduction. Conductance is measured in S (Siemens) and resistance is measured in Ω (Ohm). Specific resistivity or conductivity is the resistance or the conductance of a cube of the substance in S/cm or Ω x cm units, respectively. Molar conductivity is the specific conductivity divided by the solute concentration. However, conductivity can also be used to measure concentration. At 25°C, the conductivity of sodium chloride of 103 mmol/L is equal to 10.68 mS/cm.\textsuperscript{125} Therefore, conductance was built into the hemodialysis machines. This allows measurements of electrolyte concentration (mainly sodium chloride), but has also been applied to measure single hemodialysis clearance (Kt/V) and vascular access flow. One way to optimize solute clearance is to maintain the concentration between blood and dialysate by using the counter-current dialysis method, see Figure 1-5. It has been shown that hemodialysis clearance can be increased either by increasing the dialysate or the blood flow rate.

Generally, the blood and dialysate flow rates are set at 400 mL/min and 500-800 mL/min, respectively. However, in Figure 1-6, we can see that the clearance rate of small solutes does not increase linearly with the increased in blood flow rate. As the flow rate gets higher, the efficiency of clearance increases at a slower rate. Therefore, once the blood or dialysate flow rate reaches 400 mL/min or 800 mL/min, respectively, the benefit of further increasing blood or dialysate flow rates is limited.
Figure 1-5. The Schematic Interpretation of Counter-Current and Parallel Flow Concentration Gradient

Figure 1-6. Relationship Between Nominal Blood Flow Rate and Blood Water Urea at Dialysate Flow Rate of 500 mL/min
(Reprint with Permission from Daugirdas)
1.5.5. Hemodialysis Frequency and Duration

The duration and frequency of hemodialysis treatments can also affect a patient’s total solute clearance. Although conventional thrice-weekly, four-hours hemodialysis is the current standard of care, frequent and/or prolonged hemodialysis (quotidian) is being used more often clinically, especially in the home setting. Gotch\textsuperscript{127} has developed a method to estimate weekly urea clearance (weekly standard Kt/V of urea, weekly Std Kt/V\textsubscript{urea}) in hemodialysis patients. This will be discussed in detail in a later section (Urea as a Marker for Dialysis Adequacy). Briefly, the initial intent of developing this method was to have a technique to compare small-solute clearance between conventional hemodialysis (intermittent) and peritoneal dialysis (continuous). However, the weekly Std Kt/V\textsubscript{urea} has also been used to compare various hemodialysis modalities, especially in a research settings.\textsuperscript{128} The weekly Std Kt/V\textsubscript{urea} of a 70 kg patient, who is on conventional hemodialysis (4 hours thrice weekly) with dry-weight of 68 kg, and pre- and post-dialysis urea levels of 20 mmol/L and 6 mmol/L, is 2.18. By increasing the frequency to 6 sessions per week, the Std Kt/V\textsubscript{urea} will increase to 4.54.

1.5.6. Convective Clearance

Convective clearance is another major mechanism for solute or toxin clearance. Ultrafiltration is important for maintaining volume homeostasis, which affects blood pressure control. However, it is also important for middle molecular weight solute removal when using convectional clearance. In hemodialysis, the pressure gradient is generated by hydraulic pressure and in peritoneal dialysis, which is generated by osmotic pressure gradient. The membrane permeability to water is called the ultrafiltration coefficient (UF\textsubscript{coeff}; mL/hr/mmHg),

\[
UF_{coeff} = \frac{Q_f}{TMP}
\]

\textbf{Equation 1-13}

where Q\textsubscript{f} (mL/min) is the ultrafiltration rate, and TMP (mmHg) is the transmembrane pressure gradient. A dialyzer is considered to be low flux if its
UFcoeff is < 10 mL/hr/mmHg, it is considered high flux if \( \geq 10 \) mL/hr/mmHg. This is different from a ‘high-performance’ dialyzer, where the UFcoeff is > 8-15 mL/hr/mmHg in the US (and >10-20 mL/hr/mmHg in Europe), with a large surface area (\( \geq 2\text{m}^2 \)). During ultrafiltration, convectional clearance takes place. Generally, the amount of small solute is equal to the concentration of the solute in plasma and the amount of fluid that is removed. However, for larger molecules, if the sieving coefficient is < 1, the amount of solute removal will be less than the solute concentration times the amount of fluid removal. See Figure 1-7. Some observational studies have shown that the removal of middle molecules may be related to better survival rates.\textsuperscript{129} The benefit of a high-flux dialyzer is not as clear. The largest hemodialysis trial that compared high- and low-flux dialyzers did not show any survival benefit.\textsuperscript{130-135} We will now discuss some of the “uremic” solutes.

\textbf{Figure 1-7. The Relationship Between Diffusional Clearance and the Blood Flow for Various Molecular Weight Molecules Using High-Flux Dialyzers (Adapted from Advanced Renal Education Program\textsuperscript{136})}
1.6. THE KINETICS OF SOLUTE CLEARANCE BY HEMODIALYSIS

1.6.1. Body Compartments

In an average adult patient, water is 60% of total body weight. For example, a 70-kg man has about 42 L of total body water, where 28 L is in the intracellular compartment, 11 L in the interstitial space of extracellular compartment and 3 L in the intravascular space. Blood contains approximately 60% plasma, which is mostly water, 40% red blood cells, and <1% white blood cells and platelets; the average blood volume in a 70 kg man is about 5 L. However, this percentage may change associated with age and renal disease (Figure 1-8) The plasma and the interstitial compartments have similar ionic compositions (sodium, potassium, calcium, magnesium, chloride, phosphate, bicarbonate and others such as sulfates which are not routinely measured), but there are more proteins in the plasma. This leads to a higher concentration of cation than anion in the plasma, because proteins are negatively charged. Furthermore, the most abundant cation in interstitial fluid is potassium (K+) whereas sodium (Na+) is the most abundant cation in the intravascular space. Other nonelectrolyte solutes are phospholipids, cholesterol, glucose, urea, lactate, uric acid, creatinine, and bilirubin.¹

The tissue-blood interface, which is commonly ignored, is an important factor, which can affect the dialysis clearance of solutes. There are inter-compartment solute mass transfers within the patient and the dialyzer clearance within the dialyzer when considering solute transport. Low inter-compartment solute mass transfer is responsible for solute disequilibrium during dialysis or solute rebound post-dialysis.¹²⁶ However, when slow, prolonged hemodialysis or continuous dialysis (such as peritoneal dialysis) is used, these risks are minimized. Solute generation in patients also can also influence the dialysis clearance measurement.
1.6.2. Compartmental Modeling

The concept of organ clearance, including the hepatics and pharmacokinetics of drug clearance, are based on an original concept describing urea clearance by the kidney.\textsuperscript{137-140} There are different techniques to model this clearance from organs. These include the compartmental and physiological models. These models include transport barriers, binding, and enzymatic activities.\textsuperscript{141} The physiological and compartmental models can both lead to similar inter-compartmental elimination rate constants. Compartmental models have been used to develop renal and dialysis clearance models. Before discussing more details on urea kinetic modeling, the basic concepts of compartmental models will be discussed.

\textbf{Figure 1-8. Total Body Compartment}
Two simple conventional models, the ‘well-stirred’ and the ‘parallel tube’ will be reviewed here (Figure 1-9). However, we will not be discussing other compartmental models, such as the series-compartment model and the distributed-model. The well-stirred model considers the organ (or device such as dialysis) as a well-stirred compartment. The concentration of the solute is in equilibrium with the blood. By contrast, the parallel tube model considers the compartment as a series of parallel tubes, where the concentration of solute declines along the tube. The well-stirred model considers 100% mixing in the compartment whereas the parallel tube model considers no mixing. A model that integrates intermediate mixing is the dispersion model.

We can consider dialysis as a simple single-compartmental model, such as the well-stirred model (a single compartment with the assumption that there is rapid equilibration, homogeneous throughout the whole tissue space and constant volume). The well-stirred model is simple and commonly used. Its clearance is based on the flow-limited approach; this equation is given by:

$$\frac{dc}{dt} = \frac{Q_b}{V} (c_{in} - \frac{c}{\lambda P_{1, p}})$$  \hspace{1cm} \text{Equation 1-14}

where $c$ is the concentration of the solute in the compartment, $c_{in}$ is the input concentration of the solute, $t$ is the time (min), $Q_b$ is the blood flow (mL/min), $V$ is the compartmental volume (mL), and $\lambda$ partition coefficient of the solute.

In hemodialysis patients, the clearance occurs at the semi-permeable membrane of the dialyzer. It is only the intravascular compartment of the extracellular space that is directly accessible for dialysis. The blood concentration of a substance should be corrected for blood water concentration. Commonly, the hematocrit is usually lower than those of the general population (36% vs. 42%). This means that a blood flow rate of 200 mL/min will have a plasma flow rate of 140 mL/min and an erythrocyte flow rate of 60 mL/min. However, urea is dissolved in both plasma water and red blood cells. Approximately 93% of plasma is water and 72% of an erythrocyte is water. However, urea also associates with the non-water portion of erythrocytes. Therefore, it is considered dissolved in a volume ~
80% of the erythrocytes.\textsuperscript{91} Without making this correction, urea removal will be overestimated by 10%. The common correction factor for clearance is 0.894.\textsuperscript{91} Therefore, $Q_b$ should correct for blood water as $Q_p$ (plasma water flow rate). For simplicity, we will leave it as $Q_b$ to illustrate the concept of solute clearance and compartmental modeling. Finally, solute generation will need to be considered.

The mass balance equation can be expressed as:

$$M_i = Q_b (C_b^i - C_b^f) = Q_p (C_d^f - C_d^i) \quad \text{Equation 1-15}$$

where $Q_d$ and $Q_b$ are the volume flow rates of the dialysate and blood (mL/min), $C_d^i$ and $C_b^i$ are the initial solute concentrations of dialysate and blood (mmol/L), and $C_d^f$ and $C_b^f$ are the final solute concentrations of the dialysate and blood (mmol/L). This equation can also be expressed as the following:

$$M_i = K_o A \frac{(C_d^f - C_d^i) - (C_b^f - C_b^i)}{ln(C_b^f - C_b^i)/(C_b^i - C_d^i)} \quad \text{Equation 1-16}$$

where $K_o A$ is the mass area transfer coefficient of the dialyzer (mL/min). It is also the property of the membrane. The solute can be considered to have the maximum clearance possible at infinite blood and dialysate flow rate.

However, the single compartmental model can over-estimate the solute removal in hemodialysis clearance (Figure 1-10). It assumes the body acts as a single compartment with well stirred distributing among all body compartments and rapidly distributed among all compartments. When the inter-compartment solute exchange rate is slower, the one compartmental model cannot fully represent the solute clearance. To consider the whole body, the model will need to take into account the volume distribution of the solute in the other compartments of the body, and not just the blood-water component. For example, urea is also dissolved in the extra-cellular and intra-cellular spaces. The inter-compartmental solute movement can also affect the solute concentration in the blood-water. Unless the solute equilibration rate between the compartments is rapid and 100%, there will be an overestimation of total solute clearance if the inter-
compartmental clearance is not considered. This can be observed with a post-dialysis solute concentration rebound. There are other components of the solute that need to be considered. These include its distribution at other compartments and inter-compartmental clearance, solute generation, and renal clearance.

The multi-compartmental model describes the body as composed of multiple compartments in which solute distributes with a mass transfer governed by the inter-compartmental clearance constants (Figure 1-11). Solute generation provides input into the compartments while elimination occurs through the vascular compartment by renal, non-renal pathways as well as through dialysis. Volume of distribution at steady-state ($V_{SS}$) was calculated from the following relationship:

$$V_{SS} = V_I \left(1 + \frac{K_{IE}}{K_{EI}}\right) \quad \text{Equation 1-17}$$

$V_I$ is the solute concentration in intravascular space. $K_{IE}$ is the solute inter-compartmental transfer clearance from intravascular space to extravascular space. $K_{EI}$ is the solute inter-compartmental transfer clearance from extravascular space to intravascular space. $K$ is a constant. The rate of change of solute mass in the multi compartmental model can be written as a differential equation: 149

$$\frac{dM_i}{dt} = G - K_D (C_{is} - C_D) - K_r (C_{is}) \quad \text{Equation 1-18}$$

where $K_D$ is dialysis clearance, and $K_r$ is the renal clearance (mL/min). $C_{IS}$ and $C_D$ are solute concentrations (mmol/L) in the intravascular space and dialysate.
Figure 1-9. The Well Stirred and the Parallel Tube Models
Figure 1-10. The Single Compartmental Model of Hemodialysis Clearance

$C_1$ and $V_1$ are the solute concentration and volume in plasma space. $C_2$ and $V_2$ are the solute concentration and the volume of dialysate space. $C_{in}$ and $C_{out}$ are the solute concentrations of plasma prior entering and after leaving dialyzer. By contrast, $C_{din}$ and $C_{dout}$ are the solute concentrations prior entering and after leaving the dialyzer. $Q_{pin}$ and $Q_{din}$ are the plasma and dialysate flow rates, respectively. $Q_{uf}$ is the ultrafiltration rate. $AQ_u$ is the amount of solute removal by ultrafiltration. ($C$: mmol/L, $V$: L)
Figure 1-11. Multi Compartmental Model of a Solute Disposition in the Body

The word ‘uremia’ is created from the Greek words, ouron (urine) and haima (blood). Uremia is the toxicity related to the retention of organic waste solutes; it was fatal before dialysis became available.\textsuperscript{150,151} It results in physiological and biological deterioration. Uremic compounds can lead to common symptoms such as fatigue, anorexia, nausea, neuropathy, sleep disturbance, amenorrhea, sexual dysfunction, increased protein catabolism, pruritus, and platelet dysfunction. There have been \textasciitilde{}130 to 300 uremic compounds identified. There is great interest in finding these ‘uremic toxins’, and the list continues to expand.\textsuperscript{152,153} However, with current dialysis therapies, not only the ‘bad’ solutes are removed, but ‘good’ solutes can also be removed. To compensate for the latter, as an
example, it is recommended that hemodialysis patients receive a multi-vitamin daily, as especially the water-soluble vitamins are removed by hemodialysis. The dosage of multi-vitamin is usually doubled for nocturnal hemodialysis patients.

Because urea is a small molecule, it is easily removed by dialysis and it can be used to measure renal function; it has been used as a dialysis clearance marker, especially since the National Corporative Dialysis Study, despite urea per se being not very toxic. In a normal kidney, the glomerulus filters molecules up to a molecular weight of 58 kDalton. While the dialysis membrane can clear some larger molecules, it is not up to the ability of inherited renal function. Generally, low molecular weight molecules (aka small molecular solutes) are characterized by molecular weights up to 300 Dalton, whereas middle molecular solutes range from 300 to 15 kDalton. For small molecular solutes (e.g. urea and creatinine), the hemodialysis clearance occurs mainly by diffusion. The surface area of the dialyzer is an important factor for diffusional clearance. For middle molecular solutes (e.g. cystatin C, B2-microglobulin and beta trace protein), a larger proportion of the clearance occurs by convective clearance (Figure 1-12).

In addition to urea and creatinine, β2-Microglobulin (β2-M) has gained some interest as a dialysis clearance marker. It is part of the major histocompatibility antigen, and one of the most studied middle size uremic molecules. A high-flux dialyzer removes it. Because of the β2-M distribution among several body compartments, there is a concentration rebound after a hemodialysis treatment. β2-M accumulation in dialysis patients can lead to dialysis-related amyloidosis, but other biological impacts are minimal. Although prior research has studied using β2-M as a clearance molecule, urea remains the primary clearance marker for the dialysis population.

Other examples of middle molecules have been studied extensively, including advanced glycation end-products (AGE). AGE (2-6 kDalton) is the product of reduced sugars that reacted with free amino acids. It is absorbed through the gastrointestinal tract; its level is affected by diabetes and age, in addition to renal
function. Increased oxidative stress and impaired renal function can lead to an accumulation of AGEs. AGE is mainly dialyzed by a high-flux dialyzer.\textsuperscript{157} Elevated AGE levels can lead to inflammatory responses and the release of cytokines.\textsuperscript{158} Some solutes such as albumins are usually not removed by hemodialysis. Therefore, the clearance of these protein-bound compounds (mainly albumin-bound) depends on the fraction of “unbounded” compounds and the rate at which the compounds dissociate from the proteins.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{The Relationship Between Diffusional and/or Convective Clearance Rate and Molecular Weight of the Solute}
\end{figure}

1.6.4. Urea Clearance and Kinetic Modeling

Dialysis clearance is defined as the hypothetical amount of blood that is totally cleared of a particular substance in one minute. The total clearance includes solute removal by both diffusional and convectional clearance. Again, diffusion and convection clearance are the main methods for hemodialysis solute removal. Although diffusion occurs at two sites, one at the dialyzer interface between dialysate and blood and the other at the capillary space between blood and

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Molecular Size in log scale (Dalton) & Diffusion & Convection & Combined \\
\hline
10 & \multicolumn{3}{c|}{250} \\
100 & \multicolumn{3}{c|}{200} \\
1,000 & \multicolumn{3}{c|}{150} \\
10,000 & \multicolumn{3}{c|}{100} \\
100,000 & \multicolumn{3}{c|}{50} \\
\hline
\end{tabular}
\caption{Clearance (mL/min) vs. Molecular Size in log scale (Dalton)}
\end{table}
extravascular compartments, diffusion at the dialyzer level follows the first order phenomenon. Fick’s law of diffusion states that:\textsuperscript{159}

\[ J_s = \frac{D_f}{\Delta X} A \cdot \Delta C \]  \hspace{1cm} \text{Equation 1-19}

where \( J_s \) is the rate of diffusion (mol/s), \( A \) is the surface area where diffusion occurs (m\(^2\)), \( \Delta C \) is the change in concentration (mol/m\(^3\)), \( \Delta X \) change in positions (m) and \( D_f \) is the diffusion coefficient (m\(^2\)/s). For a dialyzer with fixed surface area, the term \( K_0 A \) is used to describe the mass transfer area coefficient, with units in mL/min; it can also be interpreted as the solute clearance at maximum blood flow rate.

In the early days of hemodialysis, various molecules were proposed as uremic toxins.\textsuperscript{160} The middle molecule hypothesis had been competing with the method of using solely small molecules for dialysis dose determination. The theory came from the observation that peritoneal dialysis patients showed higher urea but less neuropathy. A prolongation of hemodialysis time arrests the peripheral neuropathy. However, the debate between using small molecules or middle molecules for assessing dialysis clearance was settled after the results of the National Co-operative Dialysis Study (NCDS).\textsuperscript{161} The NCDS included 165 patients in a 2 x 2 factorial randomized controlled study design. The patients were randomized to high or low time-average blood urea nitrogen concentration level groups (50 vs. 100 mg/dL) and longer or shorter treatment time groups (2.5-3.5 vs. 4.5-5 hours) thrice weekly. Treatment parameters, such as blood flow rate, were adjusted to match the target-time average blood urea nitrogen levels based on the following concept:\textsuperscript{162}

\[ C_{\text{post}} = C_{\text{pre}} \times e^{-\frac{Kt}{V}} \]  \hspace{1cm} \text{Equation 1-20}

where \( C_{\text{pre}} \) and \( C_{\text{post}} \) were the solute concentrations pre- and post-hemodialysis. In this study, the solute of interest is urea. Once again, \( K \) is the urea clearance, \( t \) is the time on dialysis and \( V \) is the volume of distribution of urea. The patients were randomized into one of the four groups. The study was designed to assess
the rate of hospitalization by comparing urea clearance and by comparing high and low-flux dialyzers, respectively.

The results of the NCDS showed that the hospitalization and mortality rates were higher in patients who had higher time-averaged concentrations of urea. This was not evident when comparing hospitalization rates between the groups who received different durations of dialysis treatment. One of the criticisms of the study is that the time-averaged concentrations were too high in the high-urea group and that was not the standard of care. Furthermore, the sample size was too small to detect any significant outcome difference between the groups undergoing different treatment durations (p=0.06). Despite these criticisms, urea has become the most commonly used solute for the measurement of dialysis efficiency.\(^{161}\) The use of middle molecules in dialysis clearance assessment has been reduced significantly.

Table 1-7. The Time-Averaged Concentrations of Urea (mmol/l) and Dialysis Durations (minutes) for the Four Groups in the National Co-operative Dialysis Study.\(^ {161}\)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time-Average Urea (mmol/l)</th>
<th>Dialysis Duration (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target</td>
<td>Achieved</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>33</td>
</tr>
</tbody>
</table>

**Urea Reduction Ratio**

After the results of NCDS were published, urea has become the main state of ‘dialysis adequacy’ measurement. However, the term ‘dialysis adequacy’ refers
to the achievement of treatment objectives that fulfill the definitions of adequacy and the overall health of dialysis patients is improved by dialysis. Frequent assessments and consultations by physicians, nurses, dieticians and physiotherapists, and regular blood works are suggested. Because some of these assessments do not have objective standards, physicians and health care providers have relied on ‘dialysis adequacy’, based solely on dialysis solute clearance, specifically urea. Here, the common methods of measuring dialysis clearance, mainly urea, will be discussed.

**Urea Reduction Ratio**

The simplest way to estimate dialysis urea clearance is to measure its concentration change before and after a hemodialysis treatment. The term ‘urea reduction ratio’ (URR) is used to describe this change in urea concentrations. A URR greater than 65% per treatment is considered the standard of care for conventional hemodialysis. The URR, however, does not take into account convectional clearance, urea generation, and urea equilibration from other compartments. Although the URR has its weaknesses, the URR method is easy to use and has been shown to be as meaningful as Kt/V urea, which will be discussed next.

\[
URR = \frac{Predialysis \, urea \, concentration - Postdialysis \, urea \, concentration}{Predialysis \, urea \, concentration}
\]

Equation 1-21

**Single-Pool Kt/V of urea by various equations**

The initial NCDS used time-average concentration of urea as a measure of dialysis adequacy. However, the levels were strongly influence by dietary protein intake. Gotch et al. reanalyzed the NCDS data and derived another dialysis clearance measurement tool called Kt/V of urea (Kt/V urea). Single pool Kt/V urea is now the most popular method of assessing a single hemodialysis clearance. Kt/V is a dimensionless parameter (where K = clearance in mL/min, t = time in minutes and V = volume of distribution in mL). The clearance K of a solute is influenced by the blood flow rate, the dialysate flow rate, the dialyzer membrane
resistance and the surface area of the dialyzer. By contrast, total removal of a solute is influenced by the product of K and total time on dialysis (Kt). This product is further adjusted by the volume of the distribution of the solute, which in the NCDS is urea. The volume of distribution of urea is equal to total body water. The single pool Kt/V, without considering solute generation and ultrafiltration, can be written as:

\[ C_{post} = C_{pre}e^{-Sp \frac{Kt}{V}} \]  

Equation 1-22

Therefore, this can also be related to the urea reduction ratio (URR):

\[ Sp \frac{Kt}{V_{urea}} = -\ln(1 - URR) \]  

Equation 1-23

However, solute generation, convective clearance, and volume of distribution for urea need to be considered. Daugirdas\(^{126}\) developed several methods to estimate Sp Kt/V\(_{urea}\), using the pre-dialysis and post-dialysis urea ratios. Again, the relationship between the URR and Sp Kt/V\(_{urea}\) is non-linear. The first and second-generation equations Daugirdas developed are described below:

\[ Sp \frac{Kt}{V_{urea}} = -\ln \left( (1 - URR) - 0.008t - f \frac{UF}{W} \right) \]  

Equation 1-24

\[ Sp \frac{Kt}{V_{urea}} = -\ln \left( (1 - URR) - 0.008t \right) + (4 - 3.5(1 - URR)) \times \left( 0.55 \frac{UF}{V} \right) \]  

Equation 1-25

where UF is ultrafiltration in liters, V is volume of distribution of urea in liters, t is duration on dialysis in hours, f is 0.7, 1, and 1.25 when Kt/V is >1.3, between 0.7 and 1.3 and <0.7 respectively, W is the post-dialysis weight (kg). Equation 1-25 has been also validated in children.\(^{166}\) In both equations, convective clearance is included.

Furthermore, Daugirdas\(^{126}\) has also developed other formulae that incorporate the total protein equivalent of total nitrogen appearance and residual renal function. The post-dialysis urea measurement is usually taken at 15-30 seconds after hemodialysis, although some recommend taking it 2 minutes post termination. As the immediate postdialysis urea samples do not reflect the
“equilibrated urea” levels, the urea concentration measured immediately post-dialysis is lower than the equilibrated sample. The urea has not equilibrated from other compartments, such as the muscle to blood (double pool), thus Sp Kt/V_urea overestimates the urea clearance.

In the equilibrated method blood urea samples are usually taken 30 minutes post-dialysis. This considers the potential urea disequilibrium problem and rebound of urea into vascular compartments, whereas the Sp Kt/V_urea method does not. This “double-pool” Kt/V_urea is also called equilibrated Kt/V_urea (e Kt/V_urea), which is usually 0.21 lower than the Sp Kt/V_urea. This difference diminishes as the dialysis duration increases. For example, continuous hemodialysis or peritoneal dialysis treatments are in an equilibrated condition. Daugirdas has also developed other formulae, depending on the sampling time and the types of accesses (arteriovenous or venovenous) that were shown to correlate well with the measured eKt/V_urea. Although it is important to understand the equilibrated and double-pool effects of urea, many hemodialysis studies, especially studies that looked at survival outcomes, such as the HEMO study (see below), were done using Sp Kt/V_urea. Therefore, the Sp Kt/V_urea method is still more commonly used than the eKt/V_urea.

\[
eKt/V_\text{urea} = Sp\ Kt/V_\text{urea} - 0.47 \times (K/V) + 0.017 \quad \text{Equation 1-26}
\]

Since the NCDS study showed that there was a significant benefit of reducing hospitalization rates in patients who had Sp Kt/V_urea values above 0.9-0.95, the next question was raised, ‘is there a benefit of a further increase in Kt/V_urea?’ This question was answered by the HEMO study. It was a multi-center, 2x2 factorial designed study that involved 1846 patients. The patients were randomized to target standard and high-dose Sp Kt/V_urea, and to use high- and low-flux dialyzers. The results showed that the high-flux dialyzer does not improve survival rates and that targeting Sp Kt/V_urea of 1.25 is as good as 1.65. Despite other observational studies that have shown a survival benefit in targeting higher Sp Kt/V_urea, the HEMO study did not shown any reduced mortality from increasing Sp Kt/ V_urea >1.25. Therefore, the KDOQI recommends targeting
intermittent hemodialysis clearance with a minimum Sp Kt/V$_{urea}$ of 1.2 or URR of 65%.  

Clinical information is required to measure and calculate Sp Kt/V$_{urea}$, including pre-dialysis and post-dialysis weight and urea levels, ultrafiltration volume, intra-dialysis and inter-dialytic time, and hematocrit. Only in the case of patients who are not in a steady-state (urea generation equal to urea clearance), a pre-dialysis urea sample is required prior to the next dialysis session. Otherwise, the computer model can estimate the third pre-dialysis urea level. The residual renal function and the protein equivalent of total nitrogen appearance is measured and calculated from the inter-dialytic urine collection. Urea kinetic modeling involves advanced mathematical calculation and, therefore, a computer-modeling program is usually required.

Urea kinetic modeling will calculate dialysis and renal clearance, and urea generation. The following are the steps involved in urea kinetic modeling: first, the program calculates the Kt/V$_{urea}$ of the hemodialysis treatment. This takes into consideration renal clearance and urea generation, in addition to dialytic urea removal by diffusion and convection. Second, an expected value can be calculated based on a validated nomogram. Finally, the expected Kt of urea is compared with the calculated Kt/V$_{urea}$. V is then back-calculated. The predicted V value based on anthropometrical calculation and the calculated V value are compared. If there is a discrepancy between these two V values, it can be due to access recirculation when the calculated V over-estimated the anthropometric V, or malnutrition, where the calculated V under-estimated the anthropometric V.

**Ionic dialysance**

Another measurement technique for hemodialysis clearance has been developed using the Effective Ionic Dialysance (EID) method. EID was first proposed by Petitclerc et al. Studies have demonstrated that EID provides a reliable surrogate for measuring either blood side or dialysate side urea clearance during the dialysis procedure. It corrects for access and cardiopulmonary
recirculation. Furthermore, it can be done on-line during the dialysis procedure; this requires the incorporation of two conductivity measurement cells within the dialysis circuit. Each cell will measure the conductivity of the dialysate solution in mS/cm. On-line dialysance requires the measurement of the conductivity of mobile electrolytes through the dialysis membrane, at the inlet and the outlet, at two different set points. The dialysate conductivity at the inlet at the prescribed value of dialysate sodium (the main determinate of conductivity) is \( X_1 \), the corresponding value at dialysate outlet is measured: this is \( Y_1 \). The dialysate conductivity is, then, increased to \( X_2 \) at the inlet by a step up in dialysate sodium concentration by 10 mmol/l (1 mS/cm approximately in conductivity units). The dialysate conductivity at the outlet is measured again after a 6-minute stable period; this corresponds to the value \( Y_2 \). Finally, the inlet conductivity is returned to \( X_1 \), and outlet conductivity is measured again to confirm \( (Y_1) \) after another 6-minute stable period. The EID measurement is completed and computed based on

\[
EID = (Q_d + Q_{uf}) \left(1 - \frac{Y_1 - Y_2}{X_1 - X_2}\right)
\]

Equation 1-27

where \( Q_d \) and \( Q_{uf} \) are the dialysate and ultrafiltration flow rates, respectively.

**Weekly Standardized Kt/V\textsubscript{urea}**

Using Sp Kt/V\textsubscript{urea} > 1.2 to target hemodialysis clearance is only applied to thrice weekly hemodialysis, and cannot apply to frequent hemodialysis and/or peritoneal dialysis. Therefore, a method to compare various dialysis therapies was developed by Gotch\textsuperscript{127} and Leypoldt et al.\textsuperscript{175} This dimensionless index was named the weekly “standardized” Kt/V\textsubscript{urea} (Std Kt/V\textsubscript{urea}).

The concept of Std Kt/V\textsubscript{urea} was based on two principal hypotheses. The peak concentration hypothesis was postulated by Keshaviah:\textsuperscript{176} that uremic toxicity correlate with the amount of time the patient spends with a urea concentration above a threshold level, rather than the time-average urea concentration. Urea concentrations in patients on intermittent hemodialysis change constantly, with
peak levels prior to hemodialysis and trough levels post-hemodialysis. By contrast, urea concentrations in patients on peritoneal dialysis are relatively stable. Therefore, when the peak urea concentrations are the same on intermittent thrice-weekly hemodialysis as on peritoneal dialysis, the time-averaged urea concentrations are lower for the thrice-weekly hemodialysis patients. However, Depner\textsuperscript{177,178} questioned the peak concentration hypothesis and stated that time-average urea concentrations are also important. It is the inefficiency of intermittent hemodialysis that explains the difference between continuous therapies and the intermittent thrice-weekly hemodialysis therapies. Firstly, the later part of a hemodialysis treatment would have less urea removal than the first part of the treatment. Therefore, the amount of urea removal has lessened as the therapy continues. Secondly, the urea disequilibrium during intermittent hemodialysis, which results in post-hemodialysis rebound, also provides evidence of the inefficiency of intermittent hemodialysis. Likely, both the Depner and Keshaviah theories are at least partially true.

Based on the peak concentration hypothesis, and the assumption that intermittent thrice-weekly hemodialysis and peritoneal dialysis are equal, Gotch developed the Std $Kt/V_{\text{urea}}$ equation, and Leypold et al.\textsuperscript{175} further derived an estimated equation that correlated with Gotch’s\textsuperscript{127} original equation. Std $Kt/V_{\text{urea}}$ is expressed as the relationship between urea generation (normalized protein catabolic rate, nPCR) and the peak urea concentration ($C_{\text{peak urea}}$):\textsuperscript{127}

$$Std \ Kt/V_{\text{urea}} = \frac{0.184(nPCR - 0.17)V \times 0.001}{C_{\text{peak urea}}} \times \frac{7 \times 1440}{V}$$ \hspace{1cm} \text{Equation 1-28}$$

where the term $0.184(nPCR - 0.17)V \times 0.001$ is equal to the urea generation rate in mg/min and $V$ is the total body water. The $C_{\text{peak urea}}$ takes into account the frequency, and the duration, of the dialysis treatments. As a result, a series of curves were generated based on this formula and a Std $Kt/V_{\text{urea}}$ of 2 attained by peritoneal dialysis is equivalent to a Sp $Kt/V_{\text{urea}}$ of 1.2 achieved by thrice-weekly hemodialysis. It is more effective to increase Std $Kt/V_{\text{urea}}$ by increasing the frequency of hemodialysis than to increase the Sp $Kt/V_{\text{urea}}$ per session. Despite
Depner's argument, Std $Kt/V_{urea}$ is the only tool available at this point to compare dialysis clearance between hemodialysis and peritoneal dialysis, and between various forms of frequent hemodialysis. However, it has to be kept in mind that this model has only been validated against clinical outcomes until the recent Frequent Hemodialysis Network (FHN) trials.\textsuperscript{128}

1.6.5. Urea as a Marker for Dialysis Adequacy

Currently, “hemodialysis adequacy” is monitored based on blood urea levels and symptoms of uremia and volume overload. Although the concept of the “middle molecule hypothesis” (or square meter hour hypothesis) was emerging, the NCDS shifted the focus to small molecular weight solute removal, mainly urea, as a dialysis adequacy marker. Again, the NCDS confirmed that urea reduction, which translated into single-pool $Kt/V_{urea}$ ($Sp\ Kt/V_{urea} > 0.9$), significantly reduced hospitalization rates.\textsuperscript{161} Therefore, conventional thrice weekly treatments targeting a $Sp\ Kt/V_{urea} \geq 1.2$ became the standard of care for the management of patients who are on hemodialysis. Subsequently, the HEMO study did not show any additional mortality benefit with targeting a $Sp\ Kt/V_{urea} > 1.6$ as compared to 1.2.\textsuperscript{135} These studies have confirmed that three times per week of a four-hour hemodialysis regime is ‘adequate’, based on small urea clearance. Because of these results, and for health-economic reasons, shorter and less frequent (4 hours thrice weekly) hemodialysis focusing on urea clearance, which had started in the United States, was then adopted by the rest of the world.

Current hemodialysis clearance measurements are based on urea; however, urea is not an ideal marker. Urea is used as a surrogate marker for the clearance of other uremic toxins. The clearance of other uremic solutes may be related to convectional clearance, with different production and extracellular distribution. Some of the larger molecules are protein-bound and are therefore less likely to clear by dialysis, especially by peritoneal dialysis therapy.\textsuperscript{179,180} Urea measurements are influenced by urea generation, urea rebound and recirculation. Several mathematical techniques and models have been developed to correct these errors.\textsuperscript{92}
Because of shorter and only thrice weekly treatments, the fluid and electrolyte management of hemodialysis have become difficult, with significant hemodynamic effects during and post-hemodialysis.\textsuperscript{181} In the HEMO study, the 5-year mortality rates in patients who were on hemodialysis were 60%\textsuperscript{135,182} The leading cause of death in dialysis patients is cardiovascular disease, and cardiovascular morbidity in this population is very high; heart failure developed in up to 50\% of the dialysis patients.\textsuperscript{183} A contributing factor may be that the prevalence of existing cardiovascular disease on the initiation of dialysis treatment is high.\textsuperscript{184} However, the chance of developing cardiovascular disease is also very high after the initiation of dialysis. Certainly, hypertension and chronic volume overload are common in dialysis patients.\textsuperscript{185} Ninety percent of hypertension responds to volume control.\textsuperscript{186,187} Volume overload leads to left ventricular hypertrophy, heart failure/dilation, and it is associated with other cardiovascular risk factors.\textsuperscript{188} The management of volume overload in dialysis patients is one of the major challenges for nephrologists.

Hemodialysis itself is also an independent risk factor for the development of de novo and recurrent heart failure. A phenomenon called myocardial stunning occurs in hemodialysis patients and can leads to transient left-ventricular dysfunction.\textsuperscript{189} McIntyre et al.\textsuperscript{190} showed that dialysis-induced cardiac dysfunction is associated with a reduction in global and segmental myocardial blood flow. In patients with no cardiac stunning, the survival rate after 1 year was 100\%. By contrast, in patients with cardiac stunning, the 1-year mortality rate was 28\%.\textsuperscript{191}

Clearly, there are a lot of limitations with current hemodialysis treatment. Before we can develop new techniques and devices to improve hemodialysis outcomes and therapies, we need to have some methods to compare various hemodialysis treatments. Using urea as the sole marker to assess ‘dialysis adequacy’ is not adequate. The Std $Kt/V_{\text{urea}}$ measurement has not yet been validated in large prospective or randomized controlled trials, and its use in comparing various dialysis modalities remains more theoretical than with clinically proven.
Therefore, an ideal dialysis adequacy marker for current hemodialysis therapies should be simple, cleared by kidney and dialysis, and associated with:

1. Mortality outcome,
2. Cardiovascular morbidities and mortalities,
3. Volume status of the patient, and
4. The amount of dialysis (time, frequency and duration)

We propose that we should examine middle molecules, in particular, cystatin C.

**1.6.6. Cystatin C as a Marker for Renal Clearance**

Cystatin C is known as a γ trace protein and a base protein inhibitor with a molecular weight of 13359 Dalton. It is produced at a constant rate by all nucleated cells and appears in human plasma and serum. It is freely filtered by the glomerulus and not secreted by the tubule. It may be influenced by high cell turn-over rate such as in hyperthyroidism and inflammation. However, unlike creatinine, it is not influenced by muscle mass, food intake or body surface area. It consists of one polypeptide chain with 120 amino acids (SSPGK PPRLV GGPMD ASVEE EGVRR ALDFA VGEYN KASND MYHSR ALQVV RARKQ IVAGV NYFLD VELGR TTCTK TQPNL DNCPF HDQPH LKRKA FCSFQ IYAVP WQGTM TLSKS TCQDA). It does not have any glycosylation. It has an isoelectric point of 9.3. It has disulphide bonds between residues 73 and 83, and 97 and 117. Its gene is located on chromosome 20 at p.11.2. Its plasma half-life is 20 minutes under normal renal function (blood concentration of 0.96 mg/L with range between 0.57-1.79 mg/L). Cystatin C measurement is based on enhanced immnnonephelometric assay using antibodies from a rabbit. It is calibrated using purified cystatin from human urine against recombinant rabbit; the measurement ranges from 0.3 to 10 mg/L.

Cystatin C has been shown to be superior to creatinine as a marker for kidney function assessment in patients with chronic kidney disease. In addition, cystatin C has been studied in the context of cardiovascular outcomes. One
study has shown that serum cystatin C is associated with cardiovascular survival in patients with chronic kidney disease stages 3 and 4. Other studies have shown that cystatin C levels are associated with vasospastic angina and cardiovascular outcomes independent of renal function, and a level >1.3 mg/L is a risk factor for the occurrence of fatal and non-fatal cardiovascular events. Xie et al. found that oxidants induce cystatin C elevation, which affects cardiac extracellular remodeling by regulating Cathepsin B activity. Furthermore, studies have found an association between cystatin C and monocyte levels, which can lead to atherosclerosis. The above studies have shown that cystatin C can be a marker for both kidney function and cardiovascular outcomes.

Unlike with its use in measuring residual renal function, there are few studies on the use of cystatin C in dialysis patients. Both Delaney and Hoek suggested that cystatin C levels mainly relate to residual renal function, rather than dialysis clearance, whereas Thysell, Lindström and Park demonstrated the kinetics of cystatin C in a single hemodialysis treatment. Thysell et al. showed that with low-flux hemodialysis, cystatin C concentrations rose after a single treatment by 4 ± 6.3%. Lindström et al. demonstrated cystatin C levels were reduced with one dialysis session, with the highest clearance occurring with hemofiltration, compared to hemodiafiltration and low flux hemodialysis. Park et al. recently also demonstrated more effective cystatin C clearance by high-flux hemodialysis when compared to low-flux hemodialysis. Thus it is safe to say that cystatin C, a middle molecule, is cleared by dialysis, but there is little information on the factors that influence this clearance. None of these studies demonstrated any association between cystatin C and dialysis dose; we are the first to do this.

Al-Malki published a cross-sectional pilot study of cystatin C in 35 functionally anephric patients receiving various forms of dialytic therapies (peritoneal dialysis, conventional thrice weekly hemodialysis, and frequent short daily and nocturnal hemodialysis). All patients underwent urea kinetic studies and had values calculated for Sp Kt/Vurea and weekly Std Kt/Vurea. The study showed that there was no correlation between pre-dialysis cystatin C levels and Sp Kt/Vurea...
values, but there was a significant inverse linear correlation with weekly Std Kt/Vurea values \( r = -0.49; p = 0.003 \). In other words, cystatin C was influenced by the weekly total dose of dialysis received, and lower pre-dialysis cystatin C levels are found with patients undergoing more intensive dialysis (e.g. nocturnal).

In our current study we have demonstrated that cystatin C is cleared by the kidney. As discussed above, previous studies have shown that it correlated with cardiovascular morbidity and mortality. However, there are very few studies, which have looked at its removal by hemodialysis.

### 1.7. Cystatin C as a New Marker for Dialysis Adequacy

We have proposed that cystatin C is a useful marker to gauge dialysis adequacy. To evaluate this, we would first need to assess the pathophysiological changes in its clearance as kidney function declines, and its ability to predict residual renal function in end-stage renal disease. Furthermore, we need to understand its removal during a single hemodialysis treatment. Finally, once we have confirmed that it is removed by hemodialysis, we would assess its kinetics, volume of distribution, rebound, and conventional and diffusion clearance during a single hemodialysis treatment therapy. We have performed the following 4 studies.

To better understand the removal of cystatin C in a single hemodialysis treatment, we conducted the study (Chapter 2): *Cystatin C reduction ratio depends on normalized blood liters processed and fluid removal during hemodialysis.*\(^{204}\) The aim of the study was to assess cystatin C kinetics in a single hemodialysis treatment. We measured cystatin C levels at pre-, mid- and post-hemodialysis treatment on three consecutive treatments in 15 hemodialysis patients with no residual renal function. We hypothesized that there is significant reduction in cystatin C level by high-flux hemodialysis treatment, and this reduction is significantly associated with the number of liters processed and the ultrafiltration volume.
To better assess the effects of hyperfiltration on cystatin C renal clearance, we conducted the study (Chapter 3): *Hyperfiltration affects accuracy of creatinine eGFR measurement.*\(^{205}\) In this post-hoc analysis study, 127 pediatric patients with chronic kidney disease not yet on dialysis were observed. The goal of the study was to see whether hyperfiltration affects the renal biomarkers, creatinine, cystatin C and beta trace protein. We hypothesized that the accuracy of small-molecules-based eGFR (creatinine) is significantly affected by hyperfiltration, whereas the middle-molecules-based eGFR (cystatin C and beta-trace protein) are not.

Currently the gold standard method of measuring residual renal function is using 24-hour urine mean urea and creatinine clearances. However, it is inconvenient for the patients. To determine if cystatin C levels can be used to measure both dialysis efficacy and residual renal function in dialysis patients, we conducted the study (Chapter 4): *Residual renal function calculated from serum cystatin C measurements and a knowledge of the weekly standardized Kt/V\(_{\text{urea}}\).*\(^{206}\) We recruited 15 patients and developed a cystatin C-based estimated residual renal function equation. The study aimed to assess whether cystatin C levels can be used to determine both dialysis efficacy and residual renal function in dialysis patients. We hypothesized that the difference between the measured cystatin C level and that estimated from the Al-Malki study, the Δcystatin C, would significantly correlate with residual renal function as measured by the average of urinary creatinine and urea clearances.\(^{203}\) We further hypothesized that this correlation would be significantly stronger than the association between the measured residual renal function and the residual renal function using the Hoek equation.\(^{201}\)

Finally, to better examine the kinetics of cystatin C removal over the course of single hemodialysis treatments, we conducted the study (Chapter 5): *The kinetics of cystatin C removal by hemodialysis.*\(^{123}\) The aim of the study was to calculate the diffusional and convectional clearances of cystatin C by hemodialysis, and to
estimate its volume of distribution and intra-compartmental equilibration rate constants.
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197. Evangelopoulos AA, Vallianou, N.G., Bountziouka, V., Katsagoni, C., Bathrellou, E., Vogiatzakis, E.D., Bonou, M.S., Barbetseas, J., Avgerinos, P.C.,


2. Chapter 2. Cystatin C Reduction Ratio Depends on Normalized Blood Liters Processed and Fluid Removal

To better understand the removal of cystatin C in a single hemodialysis treatment, here, we measured cystatin C levels at pre-, mid- and post-hemodialysis treatment on three consecutive treatments in 15 hemodialysis patients with no residual renal function. We found that the amount of cystatin C reduction was influenced positively by dialysis blood flow rate and treatment time, and negatively by ultrafiltration rate. From this we hypothesized that cystatin C behaves as a middle molecule with distribution likely in the extracellular compartment with a slow equilibration rate between interstitial space and intravascular space. This Chapter is similar to work already published in the Clinical Journal of the American Society of Nephrology in 2011. ¹

2.1. INTRODUCTION

Serum creatinine and urea are small molecules that are commonly measured to monitor renal function in patients with chronic kidney disease (CKD). Serum creatinine (SCr) is the most commonly used surrogate marker for assessing kidney function in patients with CKD stage I-IV. It has molecular weight of 113 Daltons and is a metabolic product of creatine and phosphocreatine.¹ The use of serum urea (SUr) is recommended by the Kidney Disease Outcomes Quality Improvement (KDOQI) clinical practice guideline to assess dialysis clearance.² It has molecular weight of 60 Daltons.³

The preferred assessment of hemodialysis (“dose”) efficiency is by urea kinetic modeling (UKM) calculating the dimensionless parameter Kt/V (urea) (where K = clearance in mL/min, t = time in minutes and V = volume of distribution in mL). Kt/V values may be given for single pool (Sp Kt/V) or double pool (equilibrated or

eKt/V) volumes of distribution; they depend upon the urea reduction ratio (URR) over a single hemodialysis treatment.\textsuperscript{4} To assess dialysis efficiency over a period of one week, Gotch\textsuperscript{5,6} derived a new dialysis assessment index named the weekly “standardized” Kt/V (Std Kt/V). Std Kt/V allows comparison of different dialysis modalities (e.g. peritoneal versus hemodialysis) and weekly treatment frequencies.

Cystatin C (CysC) is a low molecular weight protein (13 kDalton, 121 amino-acid residues) that is produced by all nucleated cells.\textsuperscript{7} It is positively charged with an iso-electric point of 9.3. CysC has attractive characteristics as a marker for assessing native kidney or dialysis clearance. Its plasma level is not influenced by age, gender and body mass index.\textsuperscript{8} CysC is distributed mainly extracellularly.\textsuperscript{9} Its production is relatively constant and it is freely circulating.\textsuperscript{10} However, it may be affected by conditions that alter cell turn-over rate, such as inflammation or thyroid dysfunction.\textsuperscript{11,12} It remains controversial whether glucocorticoid medications may change the serum CysC level.\textsuperscript{9,13} Estimated glomerular filtration rate (eGFR) by CysC has shown to be superior to eGFR by SCr for patients with chronic kidney disease.\textsuperscript{14-16}

There have been few studies of CysC in dialysis. In peritoneal dialysis patients, the study by Delaney et al.\textsuperscript{17} showed that CysC levels are mainly related to the residual renal function, rather than the dialysis clearance. Furthermore, the study by Hoek et al.\textsuperscript{18} demonstrated a good correlation between 1/CysC and residual renal function. To obviate the effect of residual renal function we examined predialysis or steady state serum CysC levels and found these to be influenced by the dialytic treatment modality and the Std Kt/V and hence were lowest in patients receiving 5-7 nights per week of hemodialysis as compared with conventional hemodialysis and peritoneal dialysis.\textsuperscript{19} There are a few studies using CysC reduction ratio (CCRR) to assess CysC hemodialysis clearance. Thysell et al.\textsuperscript{20} demonstrated that with low-flux hemodialysis, CysC concentrations rose after dialysis by 4 ± 6.3%. Furthermore, Lindström et al.\textsuperscript{21} compared CCRR after hemodiafiltration, hemofiltration and low-flux
hemodialysis. The post-treatment CysC concentrations were lowest after hemofiltration, and highest after low-flux hemodialysis. A recent study by Park et al.\textsuperscript{10} showed a more effective CysC clearance by high-flux hemodialysis compared to low-flux hemodialysis. Although these studies have demonstrated the potential value of CysC as an indicator of middle molecule clearance, the variables affecting CCRR were not identified.

In this study, we aimed at assessing the CysC hemodialysis clearance and handling compared to the two small molecules urea and creatinine. Given a largely extracellular distribution of CysC and a presumed slow equilibration between the intravascular and the extravascular volume because of its size, we hypothesized that there is significant reduction in CysC level by high-flux hemodialysis treatment, and this reduction is significantly associated with the number of liters processed and the ultrafiltration volume.

2.2. MATERIAL AND METHODS

Participants

In this cross-sectional, single center, open study of patients with end stage renal disease receiving hemodialysis therapy, a total of 15 patients were recruited. All patients provided written informed consent. All patients were on thrice weekly high-flux high-efficiency hemodialysis therapies. Only functionally anephric patients, defined as urine output < 250 mL per day, were included in the study. Patients were excluded if they did not consent to the study, or if during the previous three months hospitalization or dialysis prescription changes occurred. The study was approved by the Ethic Review Board at the University of Western Ontario (HSREB#16599E).

Experimental Procedure

All patients were dialyzed using high-flux high-efficiency polysulphone membrane dialyzers (Optiflux F160NR or F200NR Fresenius Inc., Toronto, Canada). Either central venous catheters or fistulas served as dialysis access. The blood flow
was between 300-400 mL/min and the dialysate flow was at 500 mL/min. The blood samples were taken through the patients’ dialysis access at the beginning, at the middle and at the end of their dialysis sessions. The blood samples were taken at all three hemodialysis sessions over a one-week period. In addition to CysC, SCr, and SUr, the pre-dialysis blood samples prior the first hemodialysis session included thyroid function (TSH) and C reactive protein (CRP) measurements. Blood samples were taken at mid-dialysis for CysC measurements. Finally, additional blood samples for CysC, SCr, and SUr were taken post-dialysis. The post-dialysis blood samples utilized the 15-second slow flow methodology to obviate urea dilution by recirculation. For the analyses, the averages of all three pre-, mid- and post-dialysis measurements were used.

CysC was measured by immune nephelometry using an N-latex cystatin C kit (Dade Behring, Mississauga, Canada) on a Behring BN ProSpec analyzer (Dade Behring Marburg, Germany) at the reference laboratory at the Children’s Hospital of Eastern Ontario in Ottawa. The co-efficient of variation (CV) of the CysC measurements has been previously established at 3.1% at 1.06 mg/L; 3.5% at 2.04 mg/L and 6.7% at 5.26 mg/L. CysC was reported as an absolute level in mg/L, rather than as eGFR. SCr was measured by modified Jaffe’s reaction, using the Synchron System Kits on a Beckman Coulter LX20 Pro (Beckman Coulter Inc, Brea, CA) with a normal adult reference interval of 55-120 µmol/L. CRP was measured by immunonephelometry (Dade Behring BN Prospec, Mississauga, Canada) with CV of 4.02% at the level of 12.79 mg/L and 4.48% at 50.87 mg/L. TSH was measured by direct chemiluminescence assay (Bayer Centaur Instrument, Germany).

The single hemodialysis treatment efficacy was taken as the Sp Kt/V calculated by UKM. It was carried out during the second hemodialysis session of the week. The Std Kt/V also calculated from UKM based on Gotch’s initial paper. Reduction ratios for CysC (CCRR), urea (URR) and creatinine (CRR) were calculated by taking the difference between pre- and post- levels, and divided by pre-levels. We assumed that the volume of distribution of CysC is different from
that of urea and creatinine yet still related to body weight. We also assumed the dialyzer clearance of CysC is mainly related to the dialysis circuit blood flow and total amount removed by time (surface area being similar for all). We, thus, hypothesized that CCRR will be related to the liters of blood processed (LP; L) during dialysis normalized by the target post-dialysis weight (LP/kg). LP (L) = dialyzer blood flow (Qb) (mL/min) x time (min). LP values were obtained at the end of each dialysis directly from the dialysis machine. The amount of ultrafiltration (L) during dialysis was recorded as it was also felt to influence CCRR by a) convective removal versus b) hemoconcentration of CysC.

**Statistical Analysis**

Statistical analysis was performed using the GraphPad Prism software version 4.03 for Windows (GraphPad Software, San Diego, CA, U.S.A.). For the multiple stepwise regression analysis, Medcalc version 11.2.1.0 (Medcalc Software bvba, Mariakerke, Belgium) was used. Contiguous data were analyzed for normal distribution with the Shapiro-Wilk normality test. Mean and standard deviation were reported for normally distributed data; otherwise, median, 25th, and 75th percentiles (inter quartile range) were given. The paired t-test for normally distributed variables and the Mann-Whitney test for non-normally distributed variables were used to compare between the pre- and post-dialysis CysC, SCr and SUr levels. We also compared pre-dialysis levels from the three dialysis sessions to assess the intra-patient variability. Depending on whether or not data were normally distributed, Pearson’s correlation or the non-parametric (Spearman’s rank) correlation analysis was used to assess the strength of relationship between CCRR, and URR, CRR, Sp Kt/V, Std Kt/V, TSH and CRP as well as LP/kg and UF. Pearson correlation coefficients were expressed as r-values and the significance level of the p-value was also recorded. A p value of <0.05 was considered significant. For the multiple regression analysis, we calculated the correlation coefficients $r^2$: this is the proportion of the variation in the dependent variable explained by the regression model. It can range from 0 to 1 and is a measure of the goodness of fit of the model.
2.3. RESULTS

A total of 15 patients were enrolled into the study, all functionally anephric with urine output <250 mL per day. All patients met the inclusion criteria without violating exclusion criteria with unchanged conventional thrice weekly in center high-efficiency and high-flux hemodialysis prescription and without hospitalizations over the last 3 months. The mean age ± standard deviation (SD) was 67.3 ± 11.2 years. The most common cause of end-stage renal disease was diabetes mellitus (53.3%). The median dialysis time was 3.75 (3, 4) hours per session. The mean pre- and post-dialysis CysC concentrations were 5.96 ± 0.94 mg/L and 4.66 ± 1.09 mg/L, respectively (Figure 2-1). All of the patients had 9 CysC values over the one-week interval except one patient who had a single missing post-dialysis CysC value. The mean Sp Kt/V was 1.51 ± 0.24, while the median Std Kt/V was 2.63 (2.15, 2.71). The median TSH (normal range 0.27 to 4.20 mIU/L) was 1.62 (1.31, 3.16) mIU/L. The mean CRP concentration (normal range ≤5.0 mg/L) was 20.51 ± 15.13 mg/L. The mean LP/kg and UF were 0.89 ± 0.21 L/kg and 2.84 ± 1.06 L. Clinical results are summarized in Table 2-1. The URR, the CRR and the CCRR were 70.2 ± 9.0 %, 64.5 ± 8.2 % and 26.1 ± 11.8% (p≤0.002), respectively (Figure 2-2).
Table 2-1. Baseline Characteristics

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<tr>
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<td>1.31, 3.16</td>
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*Expressed as mean if the variable is normally distributed by Shapiro-Wilk normality test
^Expressed as median if the variable is not normally distributed by Shapiro-Wilk normality test
®Expressed in total number and percentage; Sp Kt/V = Single pool Kt/V, Std Kt/V = Standardized Kt/V or weekly Kt/V, TSH = Thyroid stimulating hormone, CRP = C reactive protein, UF = Ultrafiltration volume, LP/kg = Normalized liter processed
Figure 2-1. Mean Cystatin C Levels During Hemodialysis Sessions. 
This figure shows for each of the 15 patients the cystatin C levels at the start, the middle, and the end of dialysis. Each value represents the average of three dialysis treatments.
Figure 2-2. Cystatin C, Urea, and Creatinine Reduction Ratios (CCRR, URR and CRR, Respectively).
The reduction ratio for each of the biomarkers is shown. The values for URR, CRR, and CCRR were 70.2% ± 9.0%, 64.5% ± 8.2%, and 26.1% ± 11.8%, respectively. By Paired t test, each post-dialysis biomarker concentration was significantly lower than the pre-dialysis value (P < 0.002).

There were no statistically significant correlations between the CCRR and Sp Kt/V, URR and CRR (p>0.151). The correlation coefficient was significant between CCRR and LP/kg (r=0.678, p=0.006). There also was a significant but negative correlation between CCRR and UF (r=-0.724, p=0.002). Multiple regression analysis with these two parameters provided a model that explained 81% of the variance (r²=0.811, p<0.001), CCRR = 0.127 + 0.331 LP/kg - 0.072 x UF (Figure 2-3). There was no correlation between pre-dialysis CysC, and Std Kt/V, TSH, CRP (p>0.166). As expected, there were strong correlations between Sp Kt/V, and URR (r=0.770, p<0.001) and CRR (r=0.727, p=0.002). URR and
CRR correlated weakly with LP/kg but not with UF. The results of correlation analyses are summarized in Table 2-2.

There were no significant differences between the three pre-dialysis CysC levels (Paired t-test, p>0.115) of the three dialysis sessions in the one week. For the pre-dialysis SUr, however, there was a significant difference between Session 1 and 3 (Mann-Whitney test, p=0.029). For the pre-dialysis SCr, there were significant differences between Session 2 and 3 (Paired t-test, p<0.001), and Session 1 and 3 (Paired t-test, p=0.005).

Table 2-2. The Correlation Analysis and the Multivariable Analysis between CCRR, URR and CRR, and Other Variables.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>Variable 3</th>
<th>Pearson Correlation Coefficients (r)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>CCRR</td>
<td>Sp Kt/V</td>
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<td>0.447</td>
</tr>
<tr>
<td>CCRR</td>
<td>URR</td>
<td></td>
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<tr>
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<td>CRR</td>
<td></td>
<td>0.363</td>
<td>0.184</td>
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<tr>
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<td>LP/kg</td>
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<td>0.006</td>
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<tr>
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<td>UF</td>
<td></td>
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<tr>
<td>UUR</td>
<td>Sp Kt/V</td>
<td></td>
<td>0.770</td>
<td>&lt;0.001</td>
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<tr>
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<td>LP/kg</td>
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<tr>
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<td>UF</td>
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<td>Sp Kt/V</td>
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<td>0.002</td>
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<td>LP/kg</td>
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<td>TSH</td>
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<tr>
<td>Pre-CysC</td>
<td>CRP</td>
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<td>0.339</td>
<td>0.216</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>Variable 3</th>
<th>Correlation Coefficients (r^2)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCRR</td>
<td>UF</td>
<td>LP/kg</td>
<td>0.811</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>URR</td>
<td>UF</td>
<td>LP/kg</td>
<td>0.359</td>
<td>0.072</td>
</tr>
<tr>
<td>CRR</td>
<td>UF</td>
<td>LP/kg</td>
<td>0.327</td>
<td>0.067</td>
</tr>
</tbody>
</table>

CCRR = Cystatin C reduction ratio, URR = Urea reduction ratio, CRR = Creatinine reduction ratio, Sp Kt/V = Single pool Kt/V, Std Kt/V = Standardized Kt/V or weekly Kt/V, Pre-CysC = Pre-dialysis Cystatin C level, TSH = Thyroid stimulating hormone, CRP = C reactive protein, UF = Ultrafiltration, LP/kg = normalized liter processed
The model explained 81% of the variance. This figure shows a highly significant linear correlation between the predicted CCRR and the measured CCRR ($r^2=0.811, \ p<0.001$). Note. The three patients with low CCRR have 4-5 L of fluid removal during hemodialysis treatments.
2.4. DISCUSSION

The purpose of this study was to evaluate the dialysis clearance and handling of CysC and the variables that affect its clearance in a single high-flux high-efficiency hemodialysis session. It is a continuation of the study by Al-Malki et al.\textsuperscript{19} There was significant CysC reduction through a single high-flux high-efficiency hemodialysis session. The CCRR was 26.1 ± 11.8%. This is lower than the small solutes clearance, with URR and CRR being 70.2 ± 9.0 % and 64.5 ± 8.2 %, respectively. There was no significant correlation between CCRR, and the small solute clearance (Sp Kt/V, URR and CRR). Multiple regression analysis with the LP/kg and UF provided a model that explained 81% of the variance ($r^2=0.811$, p<0.001), CCRR = 0.127 + 0.331 x LP/kg - 0.072 x UF. To the best of our knowledge, this is the first description of the parameters that influence CCRR.

Thysell et al.\textsuperscript{20} showed a paradoxical increase in post-dialysis CysC level in low-efficiency hemodialysis. This was likely due to hemoconcentration and slow equilibration of CysC between intravascular and extravascular spaces. A previous study demonstrated that CysC elimination was more efficient by hemodiafiltration compared to low-flux hemodialysis.\textsuperscript{21} Park et al.\textsuperscript{10} showed a significant difference between low- and high-flux dialyzers in CysC clearance. CCRR results were 11.5 ± 16.2% with low-flux dialyzers and 42.4 ± 6.3% with high-flux dialyzers, respectively, with a significant difference in CCRR between dialyzers (p<0.0001). The lower CCRR of 26% in our study despite very high blood flows may be explained by differences in UF rates, although no details were provided in the Park manuscript. Park et al.\textsuperscript{10} also revealed a weak correlation between CCRR, and URR and eKt/V. By contrast, there was a strong correlation with CCRR and $\beta$2-microglobulin clearance. These studies have demonstrated a significant reduction of CysC through hemodialysis but the variables that affect the CysC clearance were not assessed.
The results of our study suggest a very different handling of CysC compared to the clearances of the small solutes urea and creatinine, in a single high-flux high-efficiency hemodialysis treatment. All patients were functionally anephric, which eliminates the effects of residual renal function on CysC clearance. Three factors should affect CysC clearance: diffusive clearance – albeit small, convective clearance and the ultrafiltration volume. So what do we know about Cystatin C clearance to explain our findings? CysC is a middle molecule that distributed mainly extracellularly. It is also minimally protein bound with presumed slow redistribution between intravascular space and extravascular space because of its size. Unfortunately, little is known about the equilibration of CysC between the intra- and extravascular space; however, in view of what is known about other middle molecule redistribution, we can assume a slow equilibration. Zingraff et al. compared the clearance of radioiodinated serum amyloid P component (125I-SAP), a constituent for systemic amyloidosis deposits, in healthy subjects and chronic hemodialysis patients. In the hemodialysis patients, the decline was in a biexponential mode, rather than a single-exponential slope. There was also evidence of “tissue retention” of 125I-SAP in the extravascular space. This was enhanced in patients with symptomatic dialysis–related amyloidosis. By contrast, SUr and SCr are distributed both in extracellular (both intra- and extravascular) and intracellular spaces, with presumed rapid equilibration between all three compartments during hemodialysis. It is presumed that small molecules are mostly affected by diffusive clearance and relatively unaltered by UF because of rapid equilibration. By contrast, CCRR is affected by a combination of diffusive and convective clearance. The data of Park et al. suggest that convective clearance is much more important for CysC. Removal of some cystatin C by membrane adsorption as does occur with B2- microglobulin must also be considered. There is, as yet, no published information on this. This possibility needs to be explored.

Given these facts and after establishing the inverse correlation between UF and CCRR, we hypothesized that CysC, SCr, and SUr have different volumes of distribution within different fluid body compartments and had different inter-
compartmental rates of equilibration. We hypothesize that SUr and even more so SCr equilibrate quickly between the intra- and extravascular space, thereby remaining unaffected by UF. By contrast, CysC is only altered in the intravascular space by the dialysis, equilibrates slowly, and is largely affected by the sometimes substantial UF observed in our patients (max 5L) in this study. Figure 2-4 presents a hypothetical model for the different handling of the two molecule classes. This model is well supported by the fact that we can explain 81% of the variance by UF and liters processed.

Figure 2-4. Kinetic Model of Creatinine, Urea and Cystatin C during Hemodialysis.
Cystatin C is a middle molecule that is distributed mainly extracellular and minimally protein bound. It was presumed to have slow redistribution between intravascular space and extravascular space because of its size. By contrast, urea and creatinine are distributed both in extracellular (both intra- and extravascular) and intracellular spaces, with presumed rapid equilibration between all three compartments during hemodialysis. As a result, it is presumed that small molecules are mostly affected by diffusive clearance and relatively unaltered by UF because of rapid equilibration. By contrast, CCRR is affected by a combination of diffusive and convective clearance and by ultrafiltration, which may concentrate the intravascular content of Cystatin C.
Our study had a few limitations. It was a small pilot study of only 15 patients. All of the patients used high-flux dialyzers. The study results are not applicable to low-flux hemodialysis treatments. The previous study by Al-Malki et al.\textsuperscript{19} demonstrated a significant negative correlation between Std Kt/V and pre-dialysis CysC in functionally anephric patients. We did not find such a correlation and did not expect to due to the small samples size and the narrow range of Std Kt/V values obtained from patients on identical dialysis modalities. We did not assess CysC rebound post-hemodialysis. This was previously demonstrated in the study by Lindström et al.;\textsuperscript{21} there was a rise in CysC level by 12% in the hemodiafiltration group.

Why is CysC an attractive dialysis adequacy marker? By increasing small molecule clearance, the HEMO and the ADEMEX studies have failed to show any mortality benefit. By contrast, there is evidence that CysC levels associate with clinical outcome.\textsuperscript{26,27} CysC levels have been shown to correlate with cardiac mortality in patients with coronary heart disease.\textsuperscript{28} In patients with Stage III or IV chronic kidney disease, the CysC level is associated with all cause and cardiovascular disease mortality.\textsuperscript{29} If CysC level correlates with clinical outcome in the dialysis population regardless of the residual renal function, it may become an important dialysis adequacy parameter. As a result, further studies remain to assess this association and the target of a satisfactory CysC level.

In conclusion, this study is the first to define the parameters that determine CCRR. The total dialysis dose measured as normalized liters processed plus the ultrafiltration rate are the most important determinants for CCRR. This is novel. Based on molecular characteristics, we hypothesize on the differences that explain the different handling of SCr and SUr on the one hand and CysC on the other. The current study provides a first model for the kinetics of Cystatin C removal by dialysis. Further studies are indicated.
REFERENCES


3. Chapter 3. Hyperfiltration Affects Accuracy of Creatinine eGFR Measurement

To better assess the effects of hyperfiltration on cystatin C renal clearance, here, 127 pediatric patients with chronic kidney disease not yet on dialysis were observed. We demonstrated that there is a significant negative correlation between the errors for the Schwartz estimated glomerular filtration rate and the filtration fraction (the ratio of GFR and renal plasma flow). Both cystatin C and beta-trace protein were not affected by differences in filtration fraction. Therefore, cystatin C might be a useful marker for the assessment of kidney function in advanced kidney disease. This Chapter is similar to work already published in the Clinical Journal of the American Society of Nephrology in 2011.²

3.1. INTRODUCTION

Renal function measurement is often focused on the glomerular filtration rate (GFR) and, to a lesser extent, on the renal blood flow.¹ The gold-standard for measuring the GFR is inulin clearance.² However, nuclear medicine studies have replaced inulin clearance owing to convenience and absence of urine collection. In Europe, ⁵¹Cr-ethylenediamine tetra-acetic acid (EDTA) is the most widely used method for the determination of GFR, whereas in North America, the ⁹⁹Tc Diethylenetriamine penta-acetic acid (DTPA) renal scan enjoys the most widespread utilization.³⁻⁵ While less demanding than inulin clearance studies, nuclear GFR studies are still cumbersome, invasive and involve the handling of radiopharmaceutical substances. Endogenous markers for estimated GFR, such as serum creatinine and more recently Cystatin C, are hampered by diagnostic imprecision.⁶ Recently Beta trace protein (BTP) has been introduced as a surrogate marker for GFR measurement.⁷

Creatinine (113 Dalton, neutrally charged) is the metabolic product of creatine and phosphocreatine found in muscle, and therefore reflects muscle mass.⁸,⁹

Given the large variability of muscle mass, there is substantial inter-patient variability of serum creatinine concentration due to its high water solubility.\textsuperscript{10,11} Serum cystatin C has been shown to be an excellent marker for GFR.\textsuperscript{12,13} It is a small molecular weight protein (13 kDalton, positive charge with isoelectric point of 9.3) that was initially known as γ–trace protein and its amino acid sequence was determined in 1981.\textsuperscript{14,15} This protein is produced at a very constant rate and is affected by only a few conditions, such as uncontrolled hyperthyroidism.\textsuperscript{16} BTP (23-29 kDalton, mildly negatively charged with isoelectric point of 5.8-6.7, also known as prostaglandin D synthase), has been traditionally used as a marker of cerebrospinal fluid leakage.\textsuperscript{17,18} It is expressed in all tissues except the ovaries.\textsuperscript{19} Preliminary studies have confirmed a good correlation between serum BTP levels and GFR measurement by inulin clearance and nuclear medicine techniques.\textsuperscript{5,20}

Hyperfiltration is considered an abnormal increase in the glomerular filtration rate.\textsuperscript{21,22} However, this definition ignores the fact that hyperfiltration can take place in a single nephron even with globally decreased GFR. Other sources have defined hyperfiltration as the result of an increase in the glomerular capillary pressure.\textsuperscript{23,24} The filtration fraction (FF) is the ratio of GFR and effective renal plasma flow (ERPF). A normal filtration fraction is 18.7±3.2 percent in healthy young adults, between the ages of 20-30 years.\textsuperscript{25} Hyperfiltration should be considered if the filtration fraction is above the reference interval.

We were interested in whether hyperfiltration affects the diagnostic accuracy of commonly used eGFR measurements using creatinine, cystatin C and BTP in a pediatric population. The precision between the surrogate markers and the eGFR is reduced at higher GFR. One possible explanation for this phenomenon may be that some patients have hyperfiltering and others do not.

3.2. MATERIALS AND METHODS

(1) Participants
The study received approval of the local ethics boards and was in accordance with the ethical standards of the Helsinki declaration of 1975 (revised in 1983). Written consent was obtained in each case from the parents and in case of a consenting minor, from the patients as well. One hundred and twenty-seven of the patients had a $^{51}\text{Cr}$ EDTA renal scan with concomitant determination of the $^{131}\text{I}$ Hippurate clearance (ERPF), thus allowing for the calculation of the FF. Hyperfiltration should be considered if the filtration fraction is above 18.7±3.2.

Venous blood samples were obtained from 127 children with various renal pathologies, referred for determination of nuclear medicine GFR study. Patients were recruited consecutively and their ages ranged from 1.0 to 18.0 years with a mean of 11.5 ± 4.2 years. Thirty-five percent of patients were females. Mean height was 136.7 ± 28.4 cm (range 62.3 - 189.1 cm), mean weight was 40.2 ± 20.0 kg (range 6.5 – 104.0 kg) and mean body surface area was 1.22 ± 0.42 m$^2$ (range 0.33 - 2.20 m$^2$). The main indications for GFR measurements were: various forms of glomerulonephritis (44.7%), obstructive uropathy (19.9%), reflux nephropathy (13.6%), post renal transplantation (5.4%), and others (16.4%, including post hemolytic uremic syndrome, steroid sensitive nephrotic syndrome, cystinosis, orthostatic proteinuria, etc.).

### (2) Experimental Procedure

The methods for the simultaneous measurement of both GFR and ERPF using $^{51}\text{Cr}$ EDTA renal scan with concomitant determination of the $^{131}\text{I}$ Hippurate clearance have been described elsewhere.$^{25}$ GFR and ERPF were corrected to a standard body surface area of mL/min/1.73 m$^2$. For consistency, we mean by GFR and ERPF the corrected values per 1.73 m$^2$ body surface area throughout the manuscript. Filtration fraction was calculated as the ratio between $^{51}\text{Cr}$ EDTA GFR and $^{131}\text{I}$ Hippurate ERPF and was expressed in percent. Serum creatinine was measured with an enzymatic assay (Ortho Clinical Diagnostics). As enzymatic assays measure approximately 20% lower than the Jaffé method that was used in the original formula by George Schwartz, we used 20% higher constants (38 for children above 1 year of age, 48 for adolescent males) to
calculate the eGFR estimate according to Schwartz.\textsuperscript{26} We validated these revised constants for the Schwartz formula in our patient cohort. For adolescent males, the estimated constant was $49.4 \pm 10.5$, not significantly different from 48 ($p=0.3271$, one-sample t-test). For the non-adolescent male patients, the constant was $40.3 \pm 7.7$, again not significantly different from 38. We therefore used the constants 38 and 48. The formula reads:

$$\text{GFR estimate} = \frac{\text{Height [cm] \times constant}}{\text{serum creatinine [µmol/L]}}$$

\textbf{Equation 3-1}

The methods for the determination of Cystatin C (Siemens Diagnostics GmbH) and BTP (Siemens Diagnostics) were described in the previous study.\textsuperscript{5} Cystatin C eGFR was calculated using the Filler formula.\textsuperscript{27} For the BTP eGFR, we used a recently published and validated formula by Benlamri et al.\textsuperscript{28}

We calculated the error between the measured GFR and the estimated GFR for creatinine using Schwartz, cystatin C and BTP using \((\text{estimated GFR}_{\text{Parameter}} - \text{measured GFR})/\text{measured GFR}\).

(3) \textbf{Statistical Analysis}

All statistical analyses were performed using GraphPad Prism Software for Science Version 4.0c, San Diego, CA, USA. Standard regression and correlation analysis were applied. Normal distribution was assessed using the Shapiro Wilks test.

Agreement between methods was tested using the Bland and Altman plot method.\textsuperscript{29} The Bland and Altman plot is a statistical method used to compare two measurement techniques. In this graphical method the differences (or alternatively the ratios) between the two techniques are plotted against the averages of the two techniques. Horizontal lines are drawn at the mean difference, and at the mean difference plus and minus 1.96 times the standard deviation of the differences. If the differences within mean ± 1.96 SD are not
clinically important, the two methods may be used interchangeably. Standard deviation (SD) of the differences between the two assay methods is used to calculate the limits of agreement, computed as the mean bias plus or minus 1.96 times its SD. The bias is computed as the value determined by one method minus the value determined by the other method. If one method is sometimes higher, and sometimes the other method is higher, the average of the differences will be close to zero. If it is not close to zero, this indicates that the two assay methods are producing different results. Correlation analysis was performed using appropriate parametric (in case of normal distribution) or non-parametric tests (Spearman rank). In case of non-normal distribution, data are given as a median (25th percentile, 75th percentile). The percentiles in brackets are also known as inter-quartile range (IQR). A p-values <0.05 was considered statistically significant.

3.3. RESULTS

The 127 children had a median age of 11.9 (IQR 8.5, 14.9) years, weighed 39.9 (28.8, 54.3) kg, and had a height of 146.0 (131.0, 163.8) cm. Mean body surface area was 1.30±0.39 m². The mean measured $^{51}$Cr EDTA GFR was 100.6 ± 32.1 mL/min/1.73 m². The median $^{131}$I hippurate clearance (ERPF) was 588 (398, 739) mL/min/1.73 m². The mean filtration fraction was 17.7± 4.5% and hyperfiltration is considered if the filtration fraction is above 18.7±3.2. Median serum creatinine 56 (52, 74) µmol/L, whereas median cystatin C was 0.98 (0.83, 1.21) mg/L and median BTP was 0.76 (0.62, 0.98) mg/L. The results of the most important parameters are summarized in Table 3-1.
Table 3-1. Statistics of the Most Important Measured and Calculated

<table>
<thead>
<tr>
<th></th>
<th>$^{51}$Cr EDTA*</th>
<th>$^{123}$I- Hipp. *</th>
<th>FF [%]</th>
<th>Creat. [µmol/L]</th>
<th>Schwartz eGFR*</th>
<th>Cys C [mg/L]</th>
<th>CysC eGFR*</th>
<th>BTP mg/L</th>
<th>BTP eGFR*</th>
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<tr>
<td>Num.</td>
<td>127</td>
<td>127</td>
<td>127</td>
<td>127</td>
<td>127</td>
<td>127</td>
<td>127</td>
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<td>25%</td>
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<td>14.6</td>
<td>41.55</td>
<td>84.5</td>
<td>0.83</td>
<td>73.97</td>
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<td>Median</td>
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<td>93.72</td>
<td>0.76</td>
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<td>75%</td>
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<td>Mean Std. Dev.</td>
<td>100.6</td>
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<td>17.7</td>
<td>58.76</td>
<td>112.4</td>
<td>1.105</td>
<td>91.27</td>
<td>0.8935</td>
<td>102.4</td>
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<td></td>
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<tr>
<td>W</td>
<td>0.98</td>
<td>0.91</td>
<td>0.99</td>
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<td>0.95</td>
<td>0.86</td>
<td>0.98</td>
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<td>0.99</td>
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<td>P&lt;0.01</td>
<td>0.06</td>
<td>P&lt;0.01</td>
<td>0.332</td>
</tr>
</tbody>
</table>

Hipp. = Hippurate, FF = Filtration fraction, Creat. = Creatinine, eGFR = estimated glomerular filtration rate, CysC = Cystatin C, BTP = beta trace protein, W = Wilk constant. Num = number of patients, 25% = 25th percentile, 75% = 75th percentile, and Std. Dev. = standard deviation. *unit for GFR = mL/min/1.73 m²
Bland and Altman analysis revealed a bias of 10.8±21.2%, with a 95% limit of agreement from -0.8 to 52.4% between the Schwartz formula eGFR and the measured GFR. For cystatin C, the bias was -9.6±21.6% with a 95% limit of agreement from -52.0 to 32.7%, and for BTP, the bias was 1.4±28.3 with a 95% limit of agreement from -54.0 to 56.8% (Table 3-2).

The median (IQR) relative error ((eGFR-GFR)/GFR) for the Schwartz formula was +12 (IQR -4, +24)%, whereas the median error for Cystatin C eGFR was -9 (IQR -21, +6)%, and for BTP eGFR was +5 (IQR -16, +25)% (Table 3-3).

There was no significant correlation between the FF and the error for Cystatin C eGFR and BTP eGFR, whereas there was a significant negative correlation between the error for the Schwartz eGFR and the FF (Figure 3-1). Further, a significant negative correlation existed between FF and GFR, Schwartz GFR, Cystatin C eGFR and BTP eGFR. Clinically, this suggests that most patients with a lower GFR have hyperfiltration, whereas only some hyperfilter with normal GFR. Table 3-2 summarizes the correlation analysis (Spearman rank). There was no significant correlation between the error for the Cystatin C eGFR and BTP eGFR and the FF. On the other hand, a significant negative correlation existed between FF and GFR, Schwartz GFR, Cystatin C eGFR and BTP eGFR.
Table 3-2. Bland & Altman Results Summarized for Agreement of Various eGFR Formulae with the Measure Isotope GFR.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Schwartz eGFR</th>
<th>Cystatin C eGFR</th>
<th>BTP eGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias</td>
<td>10.8</td>
<td>9.6</td>
<td>1.4</td>
</tr>
<tr>
<td>SD %</td>
<td>21.2</td>
<td>21.6</td>
<td>28.3</td>
</tr>
<tr>
<td>95% limit of agreement</td>
<td>-0.80 to 52.4</td>
<td>-52.0 to 32.7</td>
<td>-54.0 to 56.8</td>
</tr>
</tbody>
</table>

Table 3-3. Error by Level of eGFR (eGFR-GFR/GFR) for Various eGFR Formulae.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Schwartz eGFR</th>
<th>Cystatin C eGFR</th>
<th>BTP eGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median error</td>
<td>+12.0</td>
<td>-9.0</td>
<td>+5</td>
</tr>
<tr>
<td>25&lt;sup&gt;th&lt;/sup&gt; percentile error</td>
<td>-4.0</td>
<td>-21.0</td>
<td>-16.0</td>
</tr>
<tr>
<td>75&lt;sup&gt;th&lt;/sup&gt; percentile error</td>
<td>+24.0</td>
<td>+6.0</td>
<td>+25.0</td>
</tr>
</tbody>
</table>

Table 3-4. Spearman Rank Correlations between the Error of the GFR Estimate Models (BTP, Cystatin C, Schwartz) with FF (filtration fraction)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Schwartz %error</th>
<th>CysC %error</th>
<th>BTP %error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of XY Pairs</td>
<td>127</td>
<td>127</td>
<td>127</td>
</tr>
<tr>
<td>Spearman r</td>
<td>-0.2365</td>
<td>-0.08541</td>
<td>-0.1089</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>-0.3988 to -0.05968</td>
<td>-0.2607 to 0.09535</td>
<td>-0.2826 to 0.07185</td>
</tr>
<tr>
<td>P value (two-tailed)</td>
<td>0.0074</td>
<td>0.3397</td>
<td>0.2232</td>
</tr>
<tr>
<td>P value summary</td>
<td>**</td>
<td>Ns</td>
<td>Ns</td>
</tr>
</tbody>
</table>
Figure 3.1. The Relationship Between the Percentage Error of the Schwartz, Filler and Benlamri eGFR and the Measured GFR, Plotted Against the Filtration Fraction (FF).

There was a significant negative correlation (Spearman $r=-0.2365$, $p=0.0074$). For the non-linear regression model, we used a one-phase exponential decay model with the constants $\text{SPAN}=2295$, $K=0.0001440$, $\text{PLATEAU}=-2295$, $\text{HalfLife}=4815$.

3.4. DISCUSSION

The main finding of the study was that creatinine-based eGFR was influenced by the FF, whereas the accuracy of the eGFR from the Filler’s equation using serum cystatin C and the Benlamri’s equation using serum BTP was unaffected. In fact, there was a significant negative correlation between error of eGFR calculated from the Schwartz’s formula and the measured GFR and the FF. To the best of our knowledge, this is the first study that demonstrates that the error between Schwartz formula eGFR and measured GFR is altered by hyperfiltration. By contrast, eGFR based on low molecular weight proteins was not altered by hyperfiltration.

This finding is novel and has significant implications. Previous studies have focused on the errors in eGFR from various surrogate markers and their respective formulas, to the nuclear GFR studies.$^{30,31}$ Consistently, better agreement was found in the low GFR range, whereas the precision between
measured GFR and surrogate marker eGFR worsened with normal and high GFR values. It was therefore logical to assess the effect of hyperfiltration on the diagnostic performance of surrogate eGFR markers. Previous studies did not include the ERPF or the FF.

What does this mean? It appears that a small molecular weight soluble substance can be affected by hyperfiltration that weakens its diagnostic performance as a GFR marker, whereas low molecular weight proteins are unaffected. This would render serum creatinine a less accurate marker for eGFR in the presence of hyperfiltration. As GFR may remain constant in the early stages of CKD while the nephron endowment deteriorates secondary to a renal disease, patients with a normal GFR may or may not be hyperfiltrating. In advanced CKD, all remaining nephrons hyperfilter. It is therefore conceivable that the degree of hyperfiltration may serve as the main explanation for the reduced precision of any surrogate GFR marker in the normal and high GFR range. Admittedly, the correlation was only 0.24, which was significant, but not very impressive. The study was not designed to discover a strong correlation between the error of a creatinine-based eGFR formula and the filtration fraction. Rather, it was designed to test the hypothesis whether some of variance of the scatter can be explained by the degree of hyperfiltration. The clinical significance of our findings lies in the fact that indeed some of the imprecision of creatinine-based eGFR can be explained by hyperfiltration, and especially patients early in the course of diabetic nephropathy and IgA nephropathy may have significant hyperfiltration. In the low GFR range, the phenomenon becomes less important, but our data suggest that creatinine handling may be altered by the filtration fraction to a degree that it renders the marker less favorable when compared to the low molecular weight eGFR markers.

Of course, the question arises as to why the small molecule creatinine is handled differently than the small molecular weight proteins cystatin C and BTP. All surrogate eGFR markers have different charges and isoelectric points. As cystatin C and BTP are handled identically, electric charge is unlikely to explain
the negative correlation between the error in eGFR for creatinine in the measurement of the FF. One possible explanation for this increase in error in creatinine based eGFR using the Schwartz’s formula is that creatinine is also secreted by the renal tubule along with the excretion from glomerular filtration while there is minimal or no tubular reabsorption of creatinine.\textsuperscript{34} Cystatin C and BTP are exclusively eliminated through glomerular filtration. Therefore, with low filtration fraction, there is more blood flow in the efferent arteriole and subsequently more creatinine available in the peritubular capillaries for tubular secretion. This may lead to an increase of tubular secretion at lower FF, thereby creating an overestimation in the eGFR. It should be noted that the difference between the 25\textsuperscript{th} and 75\textsuperscript{th} percentile (i.e. IQR, a measure of precision) was inferior for BTP, suggesting that of the two low molecular weight proteins, Cystatin C should be preferred.

When the filtration fraction is increased, there is a decrease in efferent blood flow with a subsequent decrease of creatinine available for tubular secretion. Therefore, the eGFR from the creatinine based formula correlates better with measured GFR at higher filtration fraction. The proposed differential handling of creatinine with lower and higher filtration fraction is demonstrated in Figure 3-2.

As tubular secretion does not modify cystatin C and BTP concentrations, the FF is unaffected by the error between the eGFR errors for both cystatin C and the beta trace protein based formulae.

Our study has limitations. The first limitation is related to the nuclear medicine methods chosen to determine GFR and ERPF. No separate gold standard such as inulin clearance and para-aminohippuric acid (PAH) clearance were used to evaluate the accuracy of the nuclear medicine methods. Nuclear medicine methods are known to be imperfect measures of GFR and ERPF. Inulin and PAH clearance studies are no longer performed in most tertiary centers. However, the methods were validated and performed as described in as the standard of care.\textsuperscript{25} While earlier studies comparing inulin clearance and \textsuperscript{51}Cr EDTA clearance mostly reported correlations upon introduction of the nuclear medicine methods, a
recent study from Medeiros et al.\textsuperscript{36} used appropriate testing for agreement with Bland and Altman analysis for an identical method to ours and revealed narrow limits of agreement and a difference (bias) of 2.8 and 2.7 mL/min, respectively. They concluded that \textsuperscript{51}Cr-EDTA-Clearance was a reliable method to measure GFR compared with Inulin clearance. The authors are unaware of any modern studies employing Bland and Altman analysis to study agreement between \textsuperscript{131}I Hippurate clearance with PAH clearance, however, a study from 1980 demonstrated identical results with PAH clearance and slightly better performance of \textsuperscript{131}I Hippurate clearance that we used in our study when compared to \textsuperscript{123}I Hippurate clearance.\textsuperscript{36} Furthermore, this study was conducted in a pediatric population and it is unclear whether these findings can be generalized to all ages. In children, eGFR is calculated using the Schwartz’s formula that is based on creatinine and patient height. Adult eGFR calculations based on serum creatinine such as the \textit{Modification of Diet in Renal Disease study} (MDRD) equation and the Cockcroft-Gault equation. In children, these formulae cannot be used before the findings of this study can be generalized.\textsuperscript{37}

The eGFR errors using the Schwartz’s formula change with the state of FF. It is impractical to measure FF in every patient. FF can only be measured if GFR and ERPF are determined simultaneously. In Canada, \textsuperscript{131}I or \textsuperscript{121}I paraaminohippuric acid are not commercially available. Furthermore, for a simultaneous nuclear medicine method, two different isotopes with gamma and beta radiation are required, which is impractical in North America because \textsuperscript{51}Cr EDTA is not commercially available.\textsuperscript{38} While we have a general rule of thumb that the tubular
secretion for creatinine is approximately 10% of the total excretion, this may not be applicable for different states of hyperfiltration. The creatinine based eGFR formulas may be unpredictable in terms of the eGFR errors. Other limitations include a relatively low sample size of 127 patients and with a small proportion of patients with low GFR. Our study casts significant doubts on the accuracy of serum creatinine in patients with a variable degree of hyperfiltration. Early in the course of disease, hyperfiltration may or may not be operant. In case of a GFR > 150 mL/min/1.73 m², hyperfiltration can be assumed, but in case of normal GFR, hyperfiltration may or may not occur. Our study suggests that a surrogate marker for eGFR should be based on a low molecular weight protein rather than serum creatinine.

The question of the clinical applicability of these findings remains to be established. Short of performing a proper inulin and para-aminohippuric acid clearance study, filtration fraction is not easily measurable. Clinically, we
assume hyperfiltration whenever the GFR is high. Our study suggests that also all patients with a low GFR hyperfilter. The importance of this study lies less in the clinical applicability of the effect of hyperfiltration on creatinine excretion, but rather points to an important factor that explains some of the scatter when using surrogate markers for the estimation of eGFR. The study also suggests that CysC is less affected by hyperfiltration than creatinine.

In conclusion, this study showed that creatinine based Schwartz’s formula is influenced by filtration fraction. The errors of eGFR negatively correlate with filtration fraction. Only the eGFRs based on low molecular weight proteins (Filler’s equation using the cystatin C and the Belami’s equation using the beta trace protein) are unaffected at different levels of FF. Further studies are required to test the result in adult populations with other creatinine based formulae.
REFERENCES


4. Chapter 4. Short Communication: Residual Renal Function Calculated from Serum Cystatin C Measurements and A Knowledge of The Standard Weekly Kt/V (Urea)

Currently the gold standard method of measuring residual renal function is using 24-hour urine mean urea and creatinine clearances. However, it is inconvenient for the patients. To determine if cystatin C levels can be used to measure both dialysis efficacy and residual renal function in dialysis patients, we recruited 15 patients and developed a cystatin C-based estimated residual renal function equation. This equation was a better estimate of residual renal function than previously published equations with $r^2=0.81$ ($p<0.0001$). For our equation, we found that the cystatin C residual renal function estimating equation performed better when we incorporated the weekly dialysis clearance. This Chapter is similar to work already published in the *Peritoneal Dialysis International*, 2011.3

4.1. INTRODUCTION

The residual renal clearance was found to be a predictor of survival in dialysis patients.1,2 It is important to monitor and to preserve residual renal function (RRF; mL/min/1.73m$^2$) in dialysis patients.3,4 Cystatin C (CysC) is a low molecular weight protein. The studies by Delaney et al.5 and Hoek et al.6 showed strong correlations between serum CysC levels and residual renal functions (RRF) in dialysis patients. In the Hoek study, an estimating equation was developed: estimated RRF (mL/min) = 22/CysC – 0.70. A recent study by Al Malki et al. showed a significant inverse relationship between serum CysC levels and 'Weekly Standardized' Kt/V (Std Kt/V) values in functionally anephric patients: Std Kt/V = 7.254 - 0.703 CysC.7

3 Huang SH, Filler G, Lindsay RM. Residual Renal Function Calculated from Serum Cystatin C Measurements and a Knowledge of the Standard Weekly Kt/V (Urea). Perit Dial Int. 2012 Jan-Feb;32(1):102-4. PMID: 22302925
In this study, we aimed to assess the role of serum CysC levels and dialytic clearance in measuring RRF. We hypothesized this difference between the measured CysC and that estimated from the Al Malki equation would significantly correlate with RRF as measured by the average of urinary creatinine and urea clearance. We also postulated that this correlation might be stronger than Hoek’s RRF, which uses 1/CysC values alone.

4.2. MATERIAL AND METHODS

This is a cross-sectional, single center pilot study of patients with end stage renal disease receiving peritoneal dialysis and conventional thrice weekly high-flux hemodialysis therapy. Blood and urine samples were collected prospectively. All patients provided written informed consent. Patients with recent changes in dialysis prescription within the last 3 months were excluded. The study was approved by the Ethic Review Board at the University of Western Ontario (HSREB#16598E).

Seven of the 15 patients were on peritoneal dialysis. Eight of 15 patients were on conventional high-flux hemodialysis therapies (3-4 hours thrice weekly). The serum CysC, urea and creatinine levels were measured. For the hemodialysis patients, the pre-dialysis blood samples were used to measure serum CysC on the mid-week hemodialysis session, although our recent study demonstrated that pre-dialysis CysC values do not vary between hemodialysis sessions. Serum CysC levels were measured by immune nephelometry using an N-latex cystatin C kit (Siemens Healthcare Diagnostics Ltd.) on a Behring BN ProSpec analyzer (Dade Behring Marburg, Germany) at the reference laboratory at the Children’s Hospital of Eastern Ontario in Ottawa, with established coefficient of variation.

We obtained 24-45 hours of urinary output collection. The RRF were measured by average urinary creatinine and urea clearance, which were further adjusted for body surface area (BSA) using the DuBois’ formula (mRRF; mL/min/1.73m²). For the peritoneal dialysis patients, a 24 hours collection of peritoneal effluent was obtained and the total urea loss was measured. From these, the daily urea
clearance was calculated and the Std Kt/V values were derived (7 times daily K, L) employing the Watson equation for V.$^3,10$ For the hemodialysis patients, the single hemodialysis treatment efficacy was taken as the single pool Kt/V (Sp Kt/V) calculated by Urea Kinetic Modeling and the Std Kt/V was derived.$^4,11,12$

All 15 patients had their RRF estimated using the Hoek equation.$^6$ By rearranging the Al Malki equation, we can use the Std Kt/V to predict the pre-dialysis cystatin C levels (expected pre-dialysis cystatin C). The expected CysC levels did not incorporate RRF.$^7$ The differences between the expected CysC levels and the measured CysC levels were defined as ΔCysC values. The ΔCysC estimated RRF equation was derived using ΔCysC value.

Statistical analysis was performed using the GraphPad Prism software version 4.03 for Windows (GraphPad Software, San Diego, CA, U.S.A.). Mean and standard deviation (SD) were reported for normally distributed data; otherwise, median, 25$^{th}$, and 75$^{th}$ percentiles (inter quartile range) were given. A linear regression equation was derived from ΔCysC values. Pearson’s correlation analysis was used to assess the strength of the relationship between measured RRF and ΔCysC values, and the measured RRF and estimated RRF, using both the Hoek equation and the ΔCysC equation. The Bland-Altman test was used to calculate the bias and the standard deviation of the bias between the estimated RRF and measured RRF. A p value of <0.05 was considered significant.

**4.3. RESULTS**

A total of 15 patients had completed measurements. The mean age ± standard deviation was 63 ± 15 years. The three most common causes of end-stage renal disease were hypertensive nephropathy (33%), diabetic nephropathy (27%) and glomerulonephritis (14%). The mean measured pre-dialysis CysC concentration was 4.57 ± 1.02 mg/L. The mean Std Kt/V values, with and without the consideration of RRF, were 2.61 ± 0.67 and 1.65 ± 0.59, respectively. The mean measured RRF was 1.73 ± 0.67 mL/min/1.73m$^2$. 

127
There was a statistically significant correlation between measured RRF and ΔCysC values as $r^2=0.81$ (p< 0.0001). The association between measured RRF and ΔCysC values was: measured RRF (mL/min/1.73m$^2$) = 0.3601 ΔCysC + 0.5034. The ΔCysC estimated RRF values were plotted against measured RRF and data are shown in Figure 4-1. The bias was $0.001 \pm 0.290$ mL/min/1.73m$^2$. The correlation coefficient between Hoek RRF and measured RRF was $r^2 = 0.69$ (p < 0.0001), see Figure 1, with a bias of $2.70 \pm 0.847$ mL/min/1.73m$^2$.

![Graph showing correlation between Hoek RRF and measured RRF, and ΔCysC RRF and measured RRF.](image)

**Figure 4-1.** Correlation Analysis of the Hoek Residual Renal Function (RRF) and the Measured RRF, and ΔCysC RRF and Measured RRF ($r^2 = 0.69$, and 0.81, respectively; p ≤ 0.0001).
4.4. DISCUSSION

Hoek et al.\(^6\) derived an equation to obtain RRF using serum 1/CysC values in both hemodialysis and peritoneal dialysis patients. However, they ignored the dialytic clearance of CysC. In our study, we showed strong correlation between measured RRF (mL/min/1.73m\(^2\)) and ΔCysC levels (\(r^2=0.81\), \(p< 0.0001\)), which considered only the dialytic clearance. The linear relationship between the two parameters was expressed as measured RRF = 0.3601 ΔCysC + 0.5034. The bias was 0.001 ± 0.290 mL/min/1.73m\(^2\) (\(p=0.40\)). Figure 4-1 showed that the regression lines for ΔCysC estimated RRF, which was closer to the line of identity compared to the Hoek’s.

Why does the Hoek RRF overestimate measured RRF? This is likely due to the difference in Std Kt/V values in our study population and the Hoek study population. There was a significantly higher mean pre-dialysis CysC level in the Hoek study (5.8-6.1 mg/L) as compared to ours (4.6 ± 1.20 mg/L), even though the measured RRF in our study was lower than Hoek’s (1.73 ± 0.67 mL/min/1.73m\(^2\) vs. 2.7-3.3 ± 1.3-1.5 mL/min/1.73m\(^2\), respectively). This indicated that the mean Std Kt/V, without consideration of renal clearance, in the Hoek study, was lower than our study. Therefore, it is not surprising that the Hoek RRF systemically over-estimated the residual renal function in our study population. Incorporating Std Kt/V can eliminate this systematic error.

The accuracy is limited regarding the nuclear medicine isotopic glomerular filtration rate method when it is below 30 mL/min/1.73m\(^2\).\(^{13}\) Therefore, we used average urinary creatinine and urea clearance as our reference RRF. We did not find a correlation between Std Kt/V and CysC, and did not expect to because of the small sample size and the narrow range of Std Kt/V values obtained from patients on identical dialysis modalities. Although Sjostrom et al. suggested that renal clearance of CysC is in a form of hyperbolic function (1/X), we were unable to incorporate the Std Kt/V to derive RRF without using the Al Malki equation. The major limitation of the study is its small size. Furthermore, both the Al Malki
equation, which was used to derive the ΔCysC, and the estimated RRF equation in this study need further validation. Another limitation of the study is the small and narrow range of the RRF values. However, this study was designed to support our operational hypothesis. We found the importance of incorporating Std Kt/V into the estimated RRF equation, as this can result in systematic bias when the study population has different Std Kt/V values. We plan to conduct a larger study with wide ranges of Std Kt/V values to validate the ΔCysC equation.


5. CHAPTER 5. THE KINETICS OF CYSTATIN C REMOVAL BY HEMODIALYSIS

There have been only a limited number of published studies to examine the kinetics of cystatin C removal over the course of single hemodialysis treatments. We hypothesized that the primary method of cystatin C clearance would be convective clearance and that its volume of distribution would be similar to the extracellular volume. We analyzed 10 hemodialysis sessions with high-flux dialyzers from 9 patients, finding that indeed cystatin C is cleared during dialysis by both diffusion and convection. It is distributed mainly in the extracellular space but equilibrates slowly between the extravascular and intravascular spaces. This Chapter is similar to work already published in the American Journal of Kidney Disease in 2015.4

5.1. INTRODUCTION

The mainstay for calculating dialysis dose, and determining its adequacy as delivered, is by clearance measurement of solutes that accumulate in uremic patients. Urea (Ur) kinetic modeling is the most commonly described method. 1 Nevertheless, there are pitfalls in using Ur clearance to assess hemodialysis dose: (1) studies have not shown any benefits of further increasing Ur clearance once a threshold level has been reached (single pool Kt/Vurea of 1.2), and (2) estimations of dialysis dose using Ur clearance with hemodialysis of variable duration and frequency (standardized weekly Kt/Vurea) have not been validated.2,3 Therefore, there is an interest in assessing alternative quantitative methods and biomarker solutes in the optimization of dialysis dose.

Cystatin C (CysC), a middle molecular protein (13.4 kDalton), is dialyzable when a high-flux dialyzer is used. It is cleared from the blood though diffusion and convection processes.4-6 Al-Malki et al.5 showed that pre-dialysis CysC levels in anuric dialysis patients depended on the intensity of treatment; patients treated

using nocturnal hemodialysis had lower levels than those who underwent conventional thrice-weekly treated patients. Although there was a significant inverse correlation between the pre-dialysis blood CysC levels and the standard weekly $\text{Kt/V}_\text{urea}$ yet there is no relationship between this parameter and any per-treatment urea kinetic parameter. This suggests that the pre-dialysis blood CysC level depends on whether the treatment is chronic, rather than on a single treatment.

In a previous study, we showed that the CysC reduction ratio ($(\text{predialysis CysC} - \text{postdialysis CysC})/\text{predialysis CysC}$) over the course of a single dialysis treatment (84%), mainly depended on the normalized liters of blood processed (L/kg), and was inversely related to by the ultrafiltration volume (L). We proposed that CysC has a volume of distribution equal to the extracellular fluid compartment volume and shows time-limited equilibration between the interstitial space and blood water.

As a result, we hypothesized that CysC levels may thus provide an additional index for assessing dialysis treatment adequacy. This is analogous to the use of hemoglobin A1C (CysC levels) and fasting glucose (single-pool $\text{Kt/V}_\text{urea}$) in diabetic monitoring. We hypothesized that CysC would be mostly cleared by convective clearance, unlike Ur. We, therefore, conducted this CysC kinetic study to calculate the diffusional and convectional clearances of CysC by hemodialysis, and to estimate its volume of distribution and intra-compartmental equilibration rate constants.

### 5.2. METHODS

**(1) Setting & Participants**

This study included a total of 9 patients from the home hemodialysis program at London Health Sciences Centre (London, Ontario, Canada). This study was approved by Western University’s Health Sciences Research Ethics Board (HSREB #: 16599E) and all patients gave signed informed consent prior to study
commencement. The patients were included if they had been on a stable hemodialysis regime for at least 3 months (30 patients eligible). The patients were excluded if they had known access recirculation or poor access flow (access blood flow < 400 mL/min) (0 patients), or they had recent hospital admissions (0 patients) or transportation issue/conflict with their schedule (6 patients). The remaining patients were contacted.

(2) Outcome and Measurements

We analyzed hemodialysis sessions from 9 patients. Each patient had at least 3-hours of hemodialysis using a high-efficiency high-flux polysulphone dialyzer (Optiflux® 160, Fresenius Medical Care North America, USA), one patient had two such sessions and one patient underwent a 4-hour session of hemodialysis. Each hemodialysis session consisted of 3 periods: one-hour was ultrafiltration alone (T_U), one-hour was dialysis only (T_D), and the last hour was combined ultrafiltration and dialysis (T_UD). The first two periods were in random order. One patient who had 4 hours of hemodialysis received 2 hours of T_UD. We divided a hemodialysis session into 3 different periods to minimize alteration of patients’ baseline dialysis treatment prescription.

For the T_D period, the blood flow rate on the hemodialysis machine (Q_b) was varied. Patients received dialysis at Q_b 200 mL/min (Q_b200) for ten minutes and at Q_b 400 mL/min (Q_b400) for 50 minutes in random order. The ultrafiltration volume for the individual patient was assessed by clinical examination (blood pressure and edema) and by the pre-hemodialysis weight. For each patient, half of the ultrafiltration volume was removed during the T_U and the other half was removed during the T_UD. No fluid was removed during the T_D.

Samples from the arterial lines were taken to measure the blood concentrations of CysC, creatinine (Cr) and Ur prior to and 30 minutes after each session. Additional blood samples were taken from both the arterial and the venous lines at the end of the treatment modalities (T_U, T_D and T_UD). During the T_D, the arterial and the venous samples were taken at Q_b200 and Q_b400. These samples were
sent immediately to the London Laboratory for measurements. With the pre-

hemodialysis blood samples, we also measured C-reactive protein (CRP) and

thyroid-stimulating hormone (TSH) levels.

Serum CysC levels were measured by immune nephelometry using an N-latex
cystatin C kit (Siemens Healthcare Diagnostics Ltd., Mississauga, ON) on a

Behring BN ProSpec analyzer (Dade Behring Marburg, Germany) at the

reference laboratory at the Children’s Hospital of Eastern Ontario in Ottawa. The

coefficient of variation (CV) of the CysC measurements has been previously

established at 3.1% at 1.06 mg/L; 3.5% at 2.04 mg/L and 6.7% at 5.26 mg/L. CRP

was measured by immunonephelometry (Dade Behring BN Prospec,

Mississauga, Canada) with CV of 4.02% at the level of 12.79 mg/L and 4.48% at

50.87 mg/L. TSH was measured by direct chemiluminescence assay (Bayer

Centaur Instrument, Germany). Ur and Cr were measured by enzymatic

photometric and enzymatic colorimetric methods, respectively, with reference

range <8.3-11.9 mmol/L and 55-120 µmol/L.

Although the machine blood pump $Q_b$ was set at either 200 or 400 mL/min during

$T_D$, the actual $Q_b$ may be lower especially at higher pump speeds. Furthermore,

the actual $Q_b$ was converted to a plasma flow rate (includes fluids inside the red

blood cells and plasma, $Q_p$) and a plasma water flow rate (includes only plasma,

$Q_{pw}$) in the calculation of dialysis clearances; to do so, we used the generic

correction factors of 0.85 for $Q_p$ and 0.59 for $Q_{pw}$, which is justified in this

population. These generic factors were based on our previous study that the

mean hematocrit (Hct) ± standard deviation (SD) and blood total protein (Tp) value ± SD were 0.36 ± 0.02 u and 66.5 ± 4.01 g/L.

\[ Q_p = Q_b (0.72 \text{ Hct} + [1 - \text{Hct}] [1-0.00107 \text{ Tp}]) \]  

\[ Q_{pw} = Q_b ([1 - \text{Hct}] [1-0.00107 \text{ Tp}]) \]

To prevent access recirculation, all patients who had arterial venous fistulae or

graft had access flow measured to ensure the blood flow rates were greater than

the dialysis blood flow rates and the arterial and venous needles were at least 2
inches apart. Although there were two patients who used central venous catheters, we ensured these patients had good catheter blood flow to minimize recirculation. In four of our patients, we also measured the true blood $Q_b$ to compare this with the dialyzer blood flow rate as assessed by the blood pump. Both $Q_a$ and $Q_b$ were measured by the Transonic HD01 monitor (Transonic Systems, Ithaca, NY). At machine $Q_b$ of 200 mL/min and 400 mL/min ($Q_{b200}$ and $Q_{b400}$) the actual blood flow rates were $179 \pm 2$ and $331 \pm 6$ mL/min, respectively. However, for consistency and clarity, the use of the parameters, “$Q_b$, $Q_{b200}$ and $Q_{b400}$”, will be continued throughout the chapter. These values were further corrected for $Q_p$ and $Q_{pw}$. The $Q_p$ values (152 mL/min and 281 mL/min for $Q_{b200}$ and $Q_{b400}$, respectively) were used for the Ur and Cr clearance calculation because Ur and Cr are distributed in plasma and intracellular space. The $Q_{pw}$ values (106 mL/min and 195 mL/min for $Q_{b200}$ and $Q_{b400}$, respectively) were used for CysC clearance calculation because CysC is likely distributed exclusively in the extracellular space.

**Dialyzer Clearance**

At the end of each dialysis period, dialyzer clearance ($K$) of CysC was calculated as follows:

$$K = \frac{Q_{pw} (C_{in} - C_{out})}{C_{in}}$$

Equation 5-3

where $Q_{pw}$ is the plasma water flow rate at the dialyzer inlet, and $C_{in}$ and $C_{out}$ are the concentrations of the solute at the inlet and outlet of the dialyzer. For Ur and Cr, we used $Q_p$ rather than $Q_{pw}$ to calculate the $K$ values.

In addition, the sieving coefficient ($S$) and mass transfer area coefficient ($K_0A$) of CysC were calculated as follows:$^{11}$

$$S = \frac{K_{uf}}{q_{uf}}$$

Equation 5-4
Equation 5-5

\[ K_0A = \frac{Q_{pw}Q_d}{Q_{pw}-Q_d} \ln \left[ \frac{1-K_d/Q_{pw}}{1-K_d/Q_d} \right] \]

where \( Q_{pw} \) and \( Q_d \) are the plasma flow rate and the dialysate flow rate. \( Q_{uf} \) is the ultrafiltration rate. \( K_{uf} \) is the convective clearance for the solute (i.e. CysC). \( K_d \) is the dialysate clearance.

**Multi-compartmental Model: Kinetics of CysC Distribution and Estimation of Volume of Distribution**

The CysC inter-compartmental (intravascular to and from extravascular space) clearance constants and CysC volume of distribution were estimated from blood CysC time course data using a compartmental model. Briefly, the model describes the body as two pools (two compartments defined as vascular and extravascular spaces) to which CysC distributes with mass transfer governed by inter-compartmental transfer constants. CysC generation provides input into the extravascular compartment while CysC elimination occurs through the vascular compartment by renal, non-renal pathways as well as through dialysis. While applying this model to characterize CysC kinetics, we made several assumptions. First, we set the CysC generation rate (117 ug/min/1.73m\(^2\)) based on data from the literature.\(^{12}\) From our previous study, we had shown that CysC pre-hemodialysis levels were not significantly different hemodialysis sessions.\(^4\) Therefore, we made the assumption that CysC level would reach the same pre-hemodialysis CysC level by the next hemodialysis session. We included the measured residual renal clearance in the model. The calculated amount of CysC removed from subject for each dialysis period was input into the model. Volume of the intravascular compartment (\( V_i \)) was assumed to be 40 mL/kg (plasma water volume). For each patient, the dialysis input plasma CysC concentrations, which reflect vascular compartment concentrations, prior to and throughout the dialysis periods, were used for least-squares fitting to the Model (Scientist, MicroMath Scientific Software, St. Louis, MO). Data fitting furnished estimates for three parameters: two CysC inter-compartment transfer constants (from intravascular space to extravascular space and from extravascular space to
intravascular space, $k_{IE}$ and $k_{EI}$), and the non-renal non-dialyzer CysC clearance. Inter-compartmental clearance ($K_C$) was calculated as the product of $V_I$ and $k_{IE}$. Volume of distribution at steady-state ($V_{SS}$) was calculated from the following relationship:

$$V_{SS} = V_I (1 + \frac{k_{IE}}{k_{EI}})$$  \hspace{1cm} \text{Equation 5-6}

Measuring Cystatin C Rebound 30 Minutes Post-hemodialysis

Finally, we measured CysC concentrations at the end of the hemodialysis treatment (0 minutes) and at 30-minutes post-hemodialysis session. We calculated the CysC rebound ratio at 30-minutes post-hemodialysis by:

$$\text{Rebound ratio} = \frac{\text{Cyst C at 30 min} - \text{Cyst C at 0 min}}{\text{Cyst C at 0 min}}$$  \hspace{1cm} \text{Equation 5-7}

(3) Statistical analysis

The statistical analysis was performed using SPSS V21.0. (IBM, SPSS Inc, \url{www.spss.com}) and GraphPad Prism software version 5 (GraphPad Software, San Diego, CA, U.S.A.). This study was designed as a pilot study with limited sample size ($n=9$). Baseline characteristics were described as mean (standard deviation (SD)) and median with interquartile range (IQR, 25th percentile, and 75th percentile). We have reported mean and SD of dialyzer clearance, and CysC mass transfer area coefficient, the sieving coefficient, distribution volume, whole body kinetics, and 30-minutes post-hemodialysis rebound ratio. However, the median and IQR can be found in tables. We compared the CysC, Ur, and Cr clearance values and 30-minutes post-hemodialysis rebound ratio using the Paired t-test. A $p$ value of $<0.05$ was considered significant.

5.3. RESULTS

We studied hemodialysis sessions in 9 patients (5 were female). The mean age $\pm$ SD of the patients was 57 $\pm$ 9.8 years. The mean dry-weight (kg) and
ultrafiltration volume per session (mL) were $80.8 \pm 27.82$ kg and $2.0 \pm 1.06$ L. Refer to Table 5-1 for patient data.

<table>
<thead>
<tr>
<th>Table 5-1. Patients’ Baseline Characteristics (N=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean or Percentage</td>
</tr>
<tr>
<td>Age (Years)</td>
</tr>
<tr>
<td>Gender (%; Male)</td>
</tr>
<tr>
<td>Dry-weight (kg)</td>
</tr>
<tr>
<td>Total ultrafiltration volume (L)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone (mIU/L)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
</tr>
<tr>
<td>Pre-hemodialysis cystatin C (mg/L)</td>
</tr>
<tr>
<td>Pre-hemodialysis creatinine (umol/L)</td>
</tr>
<tr>
<td>Pre-hemodialysis urea (mmol/L)</td>
</tr>
<tr>
<td>Hemodialysis Access (%)</td>
</tr>
<tr>
<td>Arterio-venous fistula</td>
</tr>
<tr>
<td>Arterio-venous graft</td>
</tr>
<tr>
<td>Central Venous Catheter</td>
</tr>
</tbody>
</table>

1) **Dialyzer Clearance of CysC under Conditions of Convection and/or Diffusion**

The mean CysC dialyzer clearances were $12 \pm 7.1$ mL/min, $19 \pm 5.9$ mL/min, $20 \pm 8.0$ mL/min and $26 \pm 8.6$ mL/min by ultrafiltration, dialysis at $Q_{b200}$, dialysis at $Q_{b400}$ and combination treatments, respectively. These clearance values were significantly lower than Cr and Ur clearance values ($p<0.05$) (Table 5-2).

2) **Mass Transfer Area Coefficient and the Sieving Coefficient**

During ultrafiltration, the clearances of Ur and Cr closely followed ultrafiltration rates (Table 5-2). However, clearance of CysC was less than ultrafiltration rate indicating lower efficiency of CysC removal with convection. Indeed, the sieving
The coefficient of CysC was 0.80 ± 0.266. The KoA of CysC was 22 ± 9.5 mL/min at $Q_{b400}$ ($Q_{pw} = 195$ mL/min) indicating relatively poor dialyzer membrane permeability of this middle molecule.

(3) CysC Distribution Volume and Whole Body Kinetics

Good fits to the two-pool Model were obtained with CysC concentrations over time for patients undergoing dialysis treatment periods in differing order. The CysC volume of distribution was estimated to be 204 ± 92.4 mL/kg. The CysC inter-compartmental clearance ($K_C$) was 2.3 ± 1.27 mL/min/kg. The CysC non-renal non-dialysis clearance was 25 ± 8.2 mL/min. Lastly, the CysC, Ur and Cr 30-minutes post-hemodialysis rebound ratio were 0.09 ± 0.059, 0.31 ± 0.250 and 0.31± 0.191. The CysC 30-minutes post hemodialysis rebound ratio was significant lower compared to Ur (-0.223, p=0.02) and Cr (-0.217, p<0.01). However, there was no significant difference between Ur and Cr (p>0.05). All the CysC values are summarized in Table 5-3.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cystatin C</th>
<th>Urea</th>
<th>Creatinine (Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>$K$ (mL/min): ultrafiltration only</td>
<td>12 (7.1)</td>
<td>10 (6.9, 19.4)</td>
<td>19 (8.7)</td>
</tr>
<tr>
<td>$K$ (mL/min): dialysis only at $Q_{b200}$</td>
<td>19 (5.9)</td>
<td>20 (16.0, 22.8)</td>
<td>141 (7.3)</td>
</tr>
<tr>
<td>$K$ (mL/min): dialysis only at $Q_{b400}$</td>
<td>20 (8.0)</td>
<td>19 (15.1, 24.5)</td>
<td>225 (13.2)</td>
</tr>
<tr>
<td>$K$ (mL/min): combined ultrafiltration and dialysis at $Q_{b400}$</td>
<td>26 (8.6)</td>
<td>23 (18.7, 34.9)</td>
<td>212 (35.0)</td>
</tr>
</tbody>
</table>

IQR: interquartile range; $K$: solute dialyzer clearance; $Q_{b}$ 200: Blood flow rate set at 200 mL/min on the hemodialysis machine; $Q_{b}$ 400: Blood flow rate set at 400 mL/min on the hemodialysis machine; SD: standard deviation
Table 5-3. **Assigned and Estimated Parameters for the Two-Pool Model of Cystatin C kinetics.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>Median (Interquartile Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CysC production rate (µg/min)</td>
<td>138</td>
<td>47.6</td>
<td>142 (96.0, 162.5)</td>
</tr>
<tr>
<td>Volume of the intravascular space (V_i, mL)</td>
<td>3300</td>
<td>1140</td>
<td>3400 (2300, 3890)</td>
</tr>
<tr>
<td>Volume of distribution at steady state, V_{SS} (mL/kg)</td>
<td>204</td>
<td>92.4</td>
<td>206 (98.5, 292.5)</td>
</tr>
<tr>
<td>Inter-compartmental clearance constant, K_{C} (mL/min/kg)</td>
<td>2.3</td>
<td>1.27</td>
<td>2.1 (1.59, 3.00)</td>
</tr>
<tr>
<td>Residual renal clearance (mL/min)</td>
<td>0.7</td>
<td>1.42</td>
<td>0 (0, 1.13)</td>
</tr>
<tr>
<td>Non-renal non-dialysis clearance (mL/min) 30-minutes post-hemodialysis rebound</td>
<td>25</td>
<td>8.2</td>
<td>22 (20.4, 28.7)</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>0.09</td>
<td>0.059</td>
<td>0.10 (0.061, 0.120)</td>
</tr>
<tr>
<td>Urea</td>
<td>0.31</td>
<td>0.250</td>
<td>0.24 (0.163, 0.416)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.31</td>
<td>0.191</td>
<td>0.22 (0.183, 0.402)</td>
</tr>
</tbody>
</table>

**5.4. DISCUSSION**

The study has two major findings: (i) that CysC is dialyzable and cleared by both convection and diffusion. Given its size, a significant proportion of the hemodialysis clearance should be through convection, which is compatible with our data; (ii) that the volume of distribution is approximately 200 mL/kg, which reflects the extracellular volume. There have been limited studies to assess volume of distribution of CysC.

There have been numerous studies assessing CysC as a marker for estimating GFR.\textsuperscript{13-20} By contrast, there are limited studies assessing the hemodialysis clearance of CysC and the potential use of this middle molecule for determining the adequacy of hemodialysis.\textsuperscript{4,5,21-23} Delaney et al.\textsuperscript{23} and Hoek et al.\textsuperscript{22} suggested that cystatin C levels mainly relate to the residual renal function, rather than peritoneal dialysis clearance. In this study, we demonstrate that CysC is cleared by hemodialysis, and by both diffusion and convection. This may explain the inaccuracy of the CysC residual renal function equations when
applied to other populations, as these equations do not take into account peritoneal dialysis clearance. The K by diffusion alone was higher than by convection; however, this of course would depend on the ultrafiltration rate. The mean ultrafiltration volume was 2.0 ± 1.06 L per session. The K by convection can also be expressed as 13 ± 4.4 mL/min per L of ultrafiltration.

During convectional treatment, the sieving coefficient of CysC was 0.80 ± 0.266. This is much lower than the sieving coefficients of small solutes, such as Ur and Cr, which are close to 1. The sieving coefficient of beta-2 microglobulin is close to 0.60. Furthermore, during diffusional dialysis, the K_{0A} of CysC was estimated as 22 ± 9.5 mL/min. Small solutes, such as Ur and Cr have much higher K_{0A} values (>700 mL/min) than CysC and reported with the same dialyzer. In comparison, lysozyme, a middle molecule with molecular weight of 14.8 KDalton, has a K_{0A} of 70 mL/min under normal clinical conditions. Differences between CysC and lysosome K_{0A} values may relate to differential protein interactions with the dialyzer membrane. It is also possible that there might be CysC dialyzer adsorption. This needs to be confirmed by staining the dialyzer and was not done in our study.

Although Thysell et al. showed that with low-flux hemodialysis, blood CysC concentrations rose after a single treatment, Lindström et al. demonstrated CysC levels were reduced with a dialysis session, and high-flux hemodiafiltration provided higher CysC clearances than did low flux hemodialysis. The study by Al Malki et al. demonstrated that pre-hemodialysis CysC levels correlated inversely with the standardized weekly Kt/V_{urea}, indicating that patients receiving more intensive dialysis have lower blood levels. We previously showed that the CysC reduction ratio over hemodialysis was 26% as compared to 70% and 65% reduction of Ur and Cr. We proposed that CysC had a slow inter-compartmental equilibration rate but was likely distributed throughout the extracellular space only. In this study, we confirmed that the inter-compartmental equilibration rates were slow, either from intravascular space to extravascular space or from extravascular space to intravascular space. This explained the observed CysC
Rebound 30-minutes post-hemodialysis of 8.5%. These rebound values (%) were lower than for Ur and Cr. Furthermore, the average $K_C$ of CysC was 2.3 mL/min/kg; a value considerably lower than that estimated for Ur of ~8 mL/min/kg.\(^\text{28}\) We did not perform serial CysC measurements post hemodialysis. Therefore, CysC rebound would likely be higher than 9%. Interestingly, we also observed that with all patients who received TD followed by TU, the CysC concentration increased in the blood during TU. This is likely due to the solute concentration effects of ultrafiltration together with relatively slow rebound. Indeed, dialyzer output CysC blood concentrations were greater than input blood concentrations (4.8 mg/L vs. 5.0 mg/L, respectively, $p=0.03$) during TU. Modeling of the blood CysC concentration time-course provided estimates for volume of distribution for CysC of $204 \pm 92.4$ mL/kg. This is approximately 1/3 of total body water and the volume of the extracellular space. Studies have also shown that CysC level is an independent predictor of cardiovascular outcomes, independent of residual renal function.\(^\text{29}\) Shlipak et al.\(^\text{30}\) demonstrated that CysC eGFR is a better predictor of overall mortality and cardiovascular mortality in patients with and without chronic kidney disease than Cr eGFR, and while Cr eGFR and CysC eGFR were no different in predicting end-stage renal disease. Perhaps, CysC could be a marker for extracellular volume and may be an attractive biomarker for dialysis adequacy and for volume assessment.\(^\text{7}\) Further understanding of CysC, its relationship to outcomes and how it may be removed by dialytic methods is warranted.

This study has some shortcomings including its small sample size. The study, however, was designed to be a pilot study of the kinetics of CysC removal during hemodialysis. In the estimation of total body CysC kinetics, we made several assumptions, including the CysC generation rate which we did not attempt to measure, but used values from the work of Sjostrom et al.\(^\text{12}\) Consequently, we estimate significant non-renal, non-dialytic clearance of CysC ($25 \pm 8.2$ mL/min) in this subject cohort. Moreover, the modeling results appear to correspond to what we and others have observed, especially with regard to the volume of distribution and inter-compartmental equilibration of CysC. Of relevance, beta-2
microglobulin, a commonly used middle molecule for assessing dialysis adequacy also has a volume of distribution approximately 1/3 of the urea distribution volume (total body water). Finally, we had demonstrated that there was significant CysC rebound post-hemodialysis. However, we only had one sample 30-minutes post-hemodialysis. Further studies are needed to better assess the duration and the extent of CysC rebound after 30 minutes of hemodialysis.

5.5. CONCLUSIONS

This study demonstrated that CysC is cleared by hemodialysis, both through convective and diffusive clearance. Its volume of distribution is 204 ± 92.4 kg/mL, a value indicating a distribution limited to the extracellular space. CysC has a slow equilibration rate with rebound of ~9 % at 30 minutes post-hemodialysis. These findings are important for understanding CysC kinetics during hemodialysis. The use of CysC as a dialysis adequacy marker deserves to be further assessed.
REFERENCE


6. CHAPTER 6. LIMITATIONS, FUTURE WORK AND SIGNIFICANCE

6.1. OVERVIEW AND SUMMARY

From our four studies, we have shown that cystatin C could be a marker for both dialysis and renal clearance, individually and/or combined.\textsuperscript{1-4} It is easy to measure and is stable between dialysis treatments, rather than being influenced by a single dialysis treatment. The cystatin C reduction ratio study showed that during a single hemodialysis treatment, the URR, the CRR and the CCRR were 70.2±9.0 %, 64.5±8.2 % and 26.1±11.8 % (p≤0.002). There was no correlation between the CCRR and the small molecule clearance, while the CCRR correlated positively with liters processed (normalized by weight), and negatively with ultrafiltration volume. Multiple regression analysis with these two parameters provided a model that explained 81% of the variance (r\textsuperscript{2}=0.811, p<0.001). The amount of cystatin C reduction was influenced positively by dialysis blood flow rate and treatment time, and negatively by ultrafiltration rate. Cystatin C reduction did not correlate with any urea removal parameters such as Sp K/V\textsubscript{urea}, URR and CRR. From this we hypothesized that cystatin C behaves as a middle molecule with its distribution in the extracellular compartment, with a slow equilibration rate between the interstitial and intravascular spaces.

Cystatin C level can also be used to predict residual renal function. As kidney function declined, there was a significant negative correlation between the error for the creatinine-based eGFR and the filtration fraction. Both cystatin C and beta-trace protein are not affected by differences in FF. This may be due to the changes in tubular creatinine secretion when the filtration fraction is altered. Previously, Hoek et al.\textsuperscript{5} developed an equation to estimate residual renal function in patients already on dialysis: RRF = 22/Cystatin C - 0.70. However, this equation does not take into account the dialysis clearance, which must have some influence on cystatin C concentration. Assuming a dialysis patient has no
kidney function remaining, the expected cystatin C level is based on the weekly Std Kt/Vurea equation derived from the Al-Malki study. The difference between the measured and estimated cystatin C levels had a significant correlation with the measured residual renal function, \( r^2 = 0.81 \) (\( p < 0.0001 \)). The equation we have developed had less bias when compared to Hoek’s. We found that the predialysis cystatin C levels incorporate both the Std Kt/Vurea and the RRF values.

Finally, we have estimated that the mean cystatin C dialyzer clearances were 12 ± 7.1 mL/min, 19 ± 5.9 mL/min, 20 ± 8.0 mL/min and 26 ± 8.6 mL/min by ultrafiltration, dialysis at \( Q_{b200} \), dialysis at \( Q_{b400} \) and combination treatments, respectively. The sieving coefficient of cystatin C was 0.80 ± 0.266. The KoA of cystatin C was 22 ± 9.5 mL/min at \( Q_{b400} \) and its volume of distribution was estimated to be 204 ± 92.4 mL/kg, with the cystatin C inter-compartmental clearance (\( K_C \)) of 2.3 ± 1.27 mL/min/kg. The cystatin C non-renal non-dialysis clearance was 25 ± 8.2 mL/min. The cystatin C rebound was 9 ± 59%. These results indicated that cystatin C is cleared by hemodialysis at a much slower rate than small solutes such as creatinine and urea. Cystatin C is dialyzable and cleared by both convection and diffusion. A significant clearance is through convection. The estimate of the cystatin C volume of distribution of 204 mL/kg, is approximately 1/3 of total body water and the volume of the extracellular space. The cystatin C inter-compartmental equilibration rates were slow. This explained the observed CysC rebound 30-minutes post-hemodialysis of only 9%.

Cystatin C levels are stable prior to hemodialysis sessions, and simple to measure. In the Al-Malki study, researchers have shown that cystatin C levels closely relate to dialysis intensity. Because cystatin C is distributed in extracellular space, it is also closely related to extracellular volume status. Therefore, indirectly, it is also a marker for monitoring volume status in clinical settings. In dialysis patients, most of the excessive volume is distributed in extracellular space. We have shown that cystatin C can also be a marker for residual renal function. Previous studies have shown that residual renal function is an important prognostic marker for dialysis patients. This is likely related to
both fluid removal and uremic toxin clearance. Intra-dialytic hypotension and episodes of dehydration are associated with a more rapid decline in residual renal function.\(^9\) Preserving residual renal function is an important goal.

### 6.2. LIMITATIONS OF CURRENT WORK

A number of limitations currently affect the potential clinical application of cystatin C in dialysis population. Firstly, our studies were relatively small in size. However, these were studies with the aims to understand the clearance and kinetics of cystatin C during dialysis treatments. As a result, sample size of the four studies was between 10-130 depending on the objectives of the studies.

Another limitation is that we have developed a residual renal function equation and have applied the Al-Malki equation to our studies. Both of these equations were only internally validated. We still need to validate both equations externally in a larger study with a different dialysis population. The applications of these two equations to general dialysis population need to be further assessed.

Although we have confirmed that cystatin C is removed by dialysis and its distribution in likely in extracellular space, we have not yet assessed its association with clinical outcome, such as cardiovascular mortality and/or fluid overload. By contrast, the NCDS showed that lower urea clearance is associated with higher hospitalization rate in hemodialysis patients.

The most common cause of death in hemodialysis patients is cardiovascular disease and the mortality rate of dialysis patients remains high at 5 years of dialysis treatment.\(^{10,11}\) The pathophysiology of cardiovascular disease in the dialysis population cannot be solely explained by traditional atherosclerosis coronary artery disease. In fact, improving traditional risk factors for cardiovascular disease have not resulted in a significant improvement in cardiovascular morbidity and mortality. The development of cardiovascular disease is multi-factorial (see Figure 6-1). relating to uremia, volume overload,
recurrent myocardial stunning and other factors resulting from ischemic perfusion injury.\textsuperscript{12,13}

Volume overload is common in dialysis patients and is a risk factor for increasing cardiovascular morbidity and mortality. Expanded ECV becomes the main driver for elevated blood pressure in dialysis patients. Volume overload is associated with left ventricular hypertrophy and poor cardiovascular survival rates.\textsuperscript{14,15} Left ventricular hypertrophy is associated with increased sudden cardiac death and cardiovascular mortality.\textsuperscript{16,17} Improving volume control can lead to the benefit of left ventricular mass regression.\textsuperscript{18} If a patient’s diet contains more sodium than is recommended, this will expand the extra-cellular space. Salt restriction resulted in better volume control and a reduction in left ventricular mass.\textsuperscript{19} By contrast, aggressive fluid removal and targeting euvolemia can have negative consequences. It can lead to intradialytic hypotension, and a decline in residual renal function.\textsuperscript{9} It can also lead to myocardial stunning involving muscle fiber survival but with poor contractile function. Therefore, accurate assessment of volume status is important. Unfortunately, despite advances in technology, there is currently no gold standard measurement for dry weight.

Because cystatin C is mainly distributed in extracellular space, and its level is influenced by extracellular volume, there is a need to assess the relationship between cystatin C levels and volume status, and cardiovascular outcome in dialysis population.
6.3. FUTURE WORK

At this point, the development of cystatin C as a dialysis adequacy biomarker (here, not a disease biomarker) would approximate stage two of the recommended guidelines (Table 6-1). We have been comparing results using this biomarker with the conventionally accepted measurements of dialysis adequacy using urea. In Chapter 1, we have briefly discussed the 5 recommended stages for developing and implementing biomarkers. In Stage three, retrospective studies establish whether the biomarker detects disease before a clinical diagnosis becomes evident. In stage four, the biomarker
undergoes prospective evaluation to determine performance characteristics of the test in a setting in which it will be clinically applied. Finally, in stage five, the focus is on the use of biomarkers to change the natural course of illness. When biomarkers are used for screening, it should be shown in randomized controlled trials that the application of interventions earlier in the process is indeed beneficial.

**Table 6-1. Recommended Stages for Biomarker Development and Testing**

<table>
<thead>
<tr>
<th>Preclinical Exploratory</th>
<th>Stage one</th>
<th>Promising marker identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Assay and Validation</td>
<td>Stage two</td>
<td>Clinical assay detects established disease</td>
</tr>
<tr>
<td>Retrospective Studies</td>
<td>Stage three</td>
<td>Biomarker can detect disease early before it becomes clinical.</td>
</tr>
<tr>
<td>Prospective Studies</td>
<td>Stage four</td>
<td>Extent and characteristics of disease detected by the test.</td>
</tr>
<tr>
<td>Disease Control</td>
<td>Stage five</td>
<td>Impact of screening on reducing the burden of disease is quantified</td>
</tr>
</tbody>
</table>

In our proposed future studies, we wish to confirm our findings, in a randomized prospective trial setting. At the same time, we wish to see whether a reduced cystatin C level is associated with improved clinical outcomes that reflect “renal health” and, eventually, survival. We plan to use the repository blood samples and data from the Frequent Hemodialysis Network (FHN) trials. The FHN trials are two multi-center North American sponsored randomized controlled trials designed to test the influence of increased frequency and/or time of dialysis treatment upon outcomes. The Daily Study compared six times per week treatments with the conventional three times, in an in-center situation. The Nocturnal Study compared six nights per week with three conventional treatments with the patients treated at home. There were 245 patients randomized in the Daily Study, 120 to three times per week and 125 to six times. On the other hand, only 87 patients were randomized in the Nocturnal Study, 42 to three times per week and 45 to six nights. Dialysis times (hours per week; all mean values ± standard deviation (SD)) for the treatment modalities were 10.4 ±
1.6 (Daily Study, 3x/week), 12.7 ± 2.2 (Daily Study, 6x/week), 12.5 ± 2.0 (Nocturnal Study, 3x/week), and 28.2 ± 11.4 (Nocturnal Study, 6x/week) hours. These treatments delivered weekly Std Kt/V_{urea} of 2.47 ± 0.27, 3.49 ± 0.63, 2.61 ± 0.44, and 4.47 ± 1.60, respectively. There were two co-primary composite outcomes for both trials: death or change in left ventricular mass in survivors (from baseline to 12 months), as assessed by cardiac magnetic resonance imaging, and death or change in the physical-health composite score of the RAND 36-item health survey. Enrolment for both studies started in 2006. Once randomized, the patients were followed for one year and the studies ended in 2010. The Daily Study showed that frequent hemodialysis, as compared with conventional hemodialysis, was associated with more favorable results with respect to the composite outcomes of death or change in left ventricular mass and death or change in physical-health composite score. The study details and results are now published in the New England Journal of Medicine.\textsuperscript{21} The Nocturnal Study did not show a significant benefit for either of the two co-primary outcomes. Possible explanations for the death or left ventricular mass results (with a Hazard Ratio of 0.68) include limited sample size and patient characteristics. The study details and results have been published.\textsuperscript{20}

In both studies, urea kinetic and residual renal function information were collected at baseline, and at four and twelve months for all trial participants. From these data 2 separate values for weekly Std Kt/V_{urea} have been calculated: a dialysis weekly Std Kt/V_{urea} and a total weekly Std Kt/V_{urea} which is adjusted for residual renal function clearance. Most trial participants also agreed to have blood collected at these times; these samples have been stored in a central biorepository. Our proposed research will be a supplementary study to these FHN trials. \textit{We have received approval from the FHN Executive committee} and will have access to all trial demographic and baseline data, as well as the urea kinetic and residual renal function information. We will have access to the biorepository blood samples, and thus we can make use of repeated measurements of cystatin C (baseline, 4 months, and 12 months). Most importantly, the blindly collected trial primary outcomes will be available to us.
6.4. SIGNIFICANCE

Urea clearance and kinetic modeling cannot be used as a sole method of assessing dialysis adequacy. The pre-dialysis urea level bears no constant relationship to adequacy outcomes; in fact a low urea gives a greater prediction of a poor, rather than good, outcome because of its relationship to protein malnutrition. While the correlation between cystatin C level and cardiovascular outcome may be speculative, it is nevertheless plausible, as previously discussed in Chapter 1.

The results of our studies are important and of clinical relevance in addressing the adequacy of cystatin C as a biomarker. Cystatin C may give a more stable indication of overall hemodialysis treatment efficacy, and it may be analogous to hemoglobin A1C in diabetic management. Furthermore, a single pre-dialysis cystatin C level will allow for an assessment of both residual renal function and dialytic clearance, without the inconvenience of urine collections, multiple blood samples and complicated mathematical computation. It is not affected by one poor dialysis run, as are urea kinetics.

The long-term goal will be to define the cystatin C threshold level that influences “hard outcomes” such as morbidity and mortality and to allow better dialysis prescriptions for patients with varying (and changing) residual renal function. The outcomes addressed by the FHN studies were surrogates for mortality in the dialysis population. If the FHN supplementary study demonstrates a significant positive correlation with FHN Trials outcomes, cystatin C will be the new standard for dialysis adequacy monitoring. Should cystatin C level correlate with FHN study outcomes, an important step in determining its value in dialysis care will have occurred. There is the potential for the first major advancement in assessing dialysis adequacy in thirty years. The simplicity of a single pre-dialysis, stable blood test as such an assessment will be appealing.
REFERENCES


17. Zoccali C, Benedetto FA, Mallamaci F, et al. Prognostic value of echocardiographic indicators of left ventricular systolic function in asymptomatic


Appendices

Appendix A: Human Sciences Research Ethics Board Approval

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General Info

FileNo: 6633
Title: Cystatin C as a marker for dialysis clearance - 16599E
Start Date: 01/12/2009
End Date: 30/06/2015
Keywords:
Appendix B. Copyright Material and Permission for the Four Publication Papers

| Order detail ID: | 65929377 |
| Order License Id: | 3505911373277 |
| ISSN: | 1555-905X |
| Publication Type: | Journal |
| Volume: | |
| Issue: | |
| Start page: | |
| Publisher: | AMERICAN SOCIETY OF NEPHROLOGY |
| Permission Status: | ✔ Granted |
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| Requestor type | Author of requested content |
| Format | Print |
| Portion | chapter/article |
| Title or numeric reference of the portion(s) | Renal function measurement is often......with other creatinine based formulae |
| Title of the article or chapter the portion is from | Hyperfiltration Affects Accuracy of Creatinine eGFR Measurement |
| Editor of portion(s) | N/A |
### Order Details

**Clinical Interventions in Aging**

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Residual Renal Function Calculated from Serum Cystatin C Measurements and Knowledge of the Weekly Standard Kt/V Urea

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Curriculum Vitae

Name: Shih-Han Susan Huang

Post-secondary Education and Degrees:

McMaster University
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1998-2001 B.Sc.

University of Ottawa
Ottawa, Ontario, Canada
2001-2005 M.D.

Western University
London, Ontario, Canada
2009-2015 Ph.D. Candidate

Honors and Awards:

G. and V. Simpson Memorial Scholarship
McMaster University
1999-2000

Gladys A. Young Scholarship
McMaster University
1999-2000

NSERC Scholarship for Summer Research
McMaster University
1994-1995

Merit Scholarship
University of Ottawa, Faculty of Medicine
2001-2002

Chinese Canadian Professionals Award - Highest Standing in Cardiology Block
University of Ottawa, Faculty of Medicine
2001-2002

Faculty of Medicine Award - Second Highest Standing in Year 1 Medicine
University of Ottawa, Faculty of Medicine
2001-2002

Ontario Graduate Scholarship
Western University
2010
CIHR Fellowship Health Professional Award
Western University
2012

Vanier CGS Award, CIHR
Western University
2013-2015

**Related Work Experience**

Post-Graduate Doctor of Medicine – Internal Medicine
Western University
2005-2008

Post-Graduate Sub-Specialty Doctor of Medicine -
Nephrology
Western University
2008-2010

Post-Graduate Research Fellowship Training - Candidate
Clinician Investigator Program
Western University
2010-2013

**Publications:**


2. Thomson BKA, Huang SH, Lindsay RM. The Choice of Dialysate Sodium is Influenced By Hemodialysis Frequency and Duration: What should it be and For What Mordality? Seminar in Dialysis. 2015 March;28(2):180-5. PMID: 25482159


22. Thomson BK, Dixon SN, Huang SH, Leitch RE, Suri RS, Chan CT, Lindsay RM. Modifiable variables affecting interdialytic weight gain include dialysis time, frequency, and dialysate sodium. Hemodial Int. 2013 Oct;17(4):576-585. PMID: 23782770


a. Poster Presentation - 2011 World Congress of Nephrology of the International Society of Nephrology

32. Filler G, Huang SH. Monitoring and Improving Renal Outcomes After Heart Transplantation. Pediatr Transplant. 2011 Nov;15(7):665-7. PMID: 21895904 (Citation:0)

33. Rothera C, MacCallum C, Huang SH, Heidenheim PA, and Lindsay RM. The Influence of Between Needle Cannulation Distance on the Efficacy of Hemodialysis Treatment. Hemodialysis International. 2011;15(4): 546-552. PMID: 22111824 (Citation:0)

34. Huang SH, Hildebrand A, Clark WF. Brief Review: Management of Lupus Nephritis – Randomized Controlled Trials: An Update. Open Journal of Internal Medicine, 2011, 1, 17-23. Published Online September 2011. (Citation:0)

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40. Filler G, Huang SH, Lindsay RM. Residual Renal Function Assessment with Cystatin C. Pediatric Nephrology. 2011 Mar;26(3):333-5. PMID: 21058022. (Citation: 0)


42. Huang SH, Sharma AP, Lindsay RM, Clark RF, and Filler G. Hyperfiltration Affects Accuracy of Creatinine but not Cystatin C or Beta Trace Protein eGFR Measurement. Clin J Am Soc Nephrol. 2011 Feb;6(2):274-80. PMID: 20966120. (Citation 1)


45. Lindsay RM, Huang SH, Filler G. Cystatin C Measurements in the Assessment of Residual Renal Function, Dialysis Adequacy, And Beyond. Perit Dial Int. 2010 Jul;30(4):437-9. PMID: 20628106 (Citation: 2)


47. Filler G, Huang SH. Progress in Pediatric Kidney Transplantation. Ther Drug Monit. 2010 June;32(3):250-2. PMID: 20431507 (Citation: 3)

48. Lepage N, Huang SH, Nieuwenhuys E, and Filler G. Pediatric Reference Intervals for Immunoglobulin G and Its Subclasses with Siemens
Immunonephelometric Assays. Clinical Biochemistry. 2010 May;43(7-8):694-6. PMID: 20153742. (Citation: 2)


50. Huang SH, Johnson K, and Pipe AL. The Use of Dietary Supplements and Medications by Canadian Athletes at the Atlanta and Sydney Olympic Games. Clin J Sport Med. 2006 Jan;16(1):27-33. PMID: 16377972 (Citation: 34)