RISK ASSESSMENT FOR RENAL INJURY POST AORTIC SURGERY USING NEW AND MORE SENSITIVE MARKERS OF RENAL INJURY

By

Woolagasen Ramalingham Pillay

Submitted in fulfillment of the requirements for the degree of

MASTER OF MEDICAL SCIENCE

in the

Department of Surgery

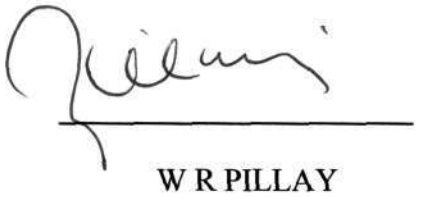
Nelson R Mandela School Medicine

University of Natal

2003

DECLARATION

This study represents original work by the author and has not been submitted in any form to another university.



W R PILLAY

To my wife,

Ger

for sacrificing

so much

Go raibh maith agat

ABSTRACT

Renal failure in patients undergoing Aortic surgery is associated with a poor outcome. The shortcomings of serum creatinine for measuring renal function are well documented. We examined the value of alternative markers in diagnosing and predicting renal damage in patients undergoing abdominal aortic surgery and those exposed to intravascular contrast media.

Cystatin C lacks some of the reservations associated with serum creatinine when used as a marker of glomerular filtration rate. The protease inhibitor alpha-glutathione S-transferase (α-GST) is recovered in urine after injury to proximal tubular cells. Urine microalbumin is a marker of glomerular permeability. Together we used all four assays to detect and characterize the nature of renal injury after surgery and contrast exposure.

Cystatin C had a marginally better sensitivity than serum creatinine at detecting baseline renal impairment. It also showed earlier changes in individual patients whose renal dysfunction deteriorated over time. The urinary markers showed an earlier significant rise after the onset of surgery when compared to serum markers, but only α-GST rose significantly after contrast exposure. Patients undergoing a supra-renal cross-clamp showed significantly higher α-GST levels (and not the other three markers) when compared to the infra-renal group. Cystatin C appears to have better sensitivity and specificity for predicting the need for dialysis in patients undergoing surgery. Peak serum

creatinine and cystatin C after contrast exposure show good correlation with peak values after surgery.

Cystatin C is equivalent to and may be better than serum creatinine in detecting pre-existing and deteriorating renal impairment. Although the urinary assays are earlier markers of renal injury, their clinical significance needs to be determined. Elevation in creatinine and cystatin C after contrast exposure parallel those after surgical intervention and may be helpful in selecting out high-risk patients prior to surgery.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to the following individuals and departments for their assistance in the preparation of this thesis:

- John Wolfe for his friendship and intellectual support and sponsorship toward the conduct of this study, and also for the opportunity to enjoy the academic and social privileges of living in London.
- David Newman (1959 – 2003) for his enthusiasm and direction, and for the use of the facilities of the South West Thames Institute for Renal Research, of which he was Scientific Director.
- The staff of Zachary Cope ward and Theatre 6 for their patience and assistance in the unenviable task of collecting blood and urine samples.
- Lucia D'Mello for performing the cystatin C and α-GST assays.
- The Academic Surgical Unit at St Mary's for use of the laboratory facilities.
- Teun Wilmink for his friendship and guidance in preparation of the thesis.
- Tonya Esterhuizen for her patience in dealing with my quest for that elusive 'p – value'.
- The support staff of the Department of Surgery, University of Natal, for tolerating my countless interruptions and requests for help.
- My teachers in surgery for leading by example.
- The patients from whom I have learnt the most.
- Professor JV Robbs for injecting the pulse of Vascular Surgery into my veins, and for allowing me the opportunity to expand my clinical exposure overseas.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Serum creatinine is a measure of glomerular filtration rate (GFR). It is freely filtered by the glomerulus and 10-40% may be secreted by the tubules.

Serum cystatin C is a new marker of GFR that is freely filtered by the glomerulus and undergoes no tubular secretion.

Urinary microalbumin is primarily a measure of glomerular permeability.Tubular reabsorption occurs.

Urinary alpha-glutathione S-transferase (α-GST) is a protein specific to the cells of the proximal tubule. It is readily released into the urine after cellular injury

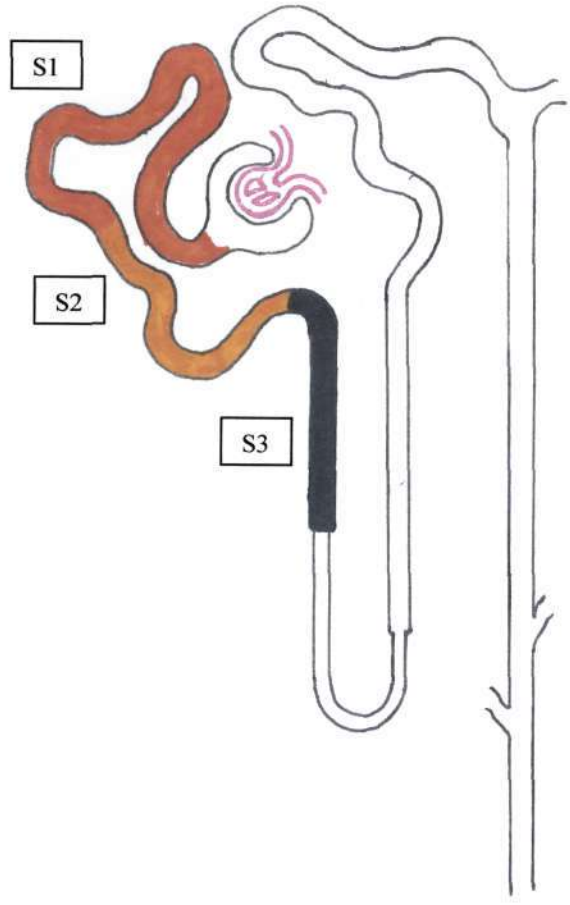


Plate 1. Diagrammatic representation of the different parts of the nephron. S1 and S2 segments are part of the proximal convoluted tubule. S2 and S3 segments form part of the proximal straight tubule. (After Venkatachalam et al. 1978)

AIMS

1. To determine the use of serum cystatin C, urine albumin and urine alpha-glutathione S-transferase (α-GST) in predicting and detecting renal failure in patients undergoing aortic surgery and those undergoing intravascular contrast exposure.
2. To determine the difference in the nature of renal injury in patients undergoing either a supra-renal or infra-renal aortic cross-clamp.
3. To determine the difference in the nature of renal injury following aortic surgery compared with intravascular contrast exposure.
4. To determine if the magnitude of renal injury after contrast exposure can predict the severity of post-operative renal dysfunction.

CHAPTER 1 BACKGROUND

1.1 RENAL FAILURE IN VASCULAR SURGERY

The prevalence of an elevated serum creatinine in a healthy subset of patients from the Framingham Study was 8.9% in men and 8.0% in women (Culleton et al. 1999). There are about 300 000 patients on renal replacement therapy in the USA (Luke 1998). About two thirds have diabetes or hypertension. There is also a higher prevalence of older age, dyslipidaemia and physical inactivity in this group. These are the typical traits of the vascular surgical patient.

Secondary hypertension occurs early in many progressive renal diseases and exacerbates the vasculopathy. In an angiographic study of vascular ward admissions, it was found that 34% had renal artery stenosis (Salmon et al. 1990). In the subgroup of patients with significant renal artery stenosis 93% had hypertension. Renovascular disease is thought to account for 12-14% of patients entering dialysis programmes (Mailloux et al. 1994).

Surgical intervention inflicts major stresses on the kidney. Contrast media, anaesthetic agents, hypovolaemia, cholesterol emboli and true ischaemic time can aggravate pre-existing disease or result in new organ dysfunction.

1.2 PRECIPITATING FACTORS FOR RENAL FAILURE

1.2.1 Renal cholesterol emboli

Microembolisation of atheroma or cholesterol crystals from aortic or renal plaques can occur spontaneously or secondary to surgical or endovascular intervention. Cases following thrombolytic therapy have been reported (Ridker et al. 1989; Shapiro 1989).

The kidney is the organ most frequently affected (Smith et al. 1981). Microscopic examination of the small arterioles reveal cholesterol clefts, the result of lipid dissolution during specimen preparation (Colt et al. 1988). Often the multitude of potential renal insults makes the diagnosis difficult unless the classical clinical signs are present. In a study of 52 patients with renal failure and histologically proven atheroembolism following angiography or cardiovascular surgery, only 50% had cutaneous signs of atheroembolism (Thadhani et al. 1995). Despite autopsy studies showing incidences of up to 77% of histological renal involvement in patients undergoing aortic aneurysm surgery, clinically significant disease is far less common (Smith, Ghose et al. 1981). The typical patient is a white male older than 60 years giving a history of hypertension, smoking and arterial disease, with a precipitating event followed by renal failure and signs of peripheral emboli (Scolari et al. 2000).

Patients who have significant renal emboli have a dismal outlook but aggressive supportive therapy may be rewarded.

1.2.2 Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDS) and angiotensin converting enzyme (ACE) inhibitors normally have little effect on the kidneys (Lee et al.; Fredman et al. 1999). When renal perfusion pressure is low, dilatation of the afferent glomerular arterioles by prostaglandins and constriction of the efferent glomerular arterioles by angiotensin II maintain intraglomerular pressures adequate for filtration (Abuelo 1995). Such situations are not uncommon in the vascular patient either due to a surgical insult or the presence of cardiac or renal artery disease. Glomerular filtration is subsequently impaired by the inhibition of prostaglandin synthesis by NSAIDS and of angiotensin II by ACE inhibitors. NSAIDS block the enzyme cyclooxygenase (COX), of which two isoforms exist, designated COX1 and COX2 (Breyer et al. 2001). The deleterious effects on the kidney are mediated by the blockage of COX2. COX2-selective NSAIDS, whilst they have gastrointestinal-sparing effects, require the same caution as non-selective NSAIDS with regard to the kidney. Similar caution has been recommended with angiotensin II receptor antagonists, due to a similar pharmacological result as the ACE inhibitors (Saine et al. 1996). 38% of patients with newly diagnosed renal artery stenosis (RAS) were found to be on ACE inhibitors on presentation (Scoble et al. 1993).

Potentially nephrotoxic antibiotics such as the anti-staphylococcal vancomycin and the aminoglycosides require dose adjustments in renal impairment. Frequent monitoring of blood levels is needed to avoid renal damage.

Volatile halogenated inhalational anaesthetic agents have been investigated for renal toxicity. Sevoflurane degrades into inorganic fluoride and 'Compound A' and has been shown to produce transient post-anaesthetic increase in urinary α-GST (Eger et al. 1997). A dose related response was demonstrated (Eger et al. 1997). Desflurane did not produce any rise in the urinary marker. In other studies low –flow sevoflurane and isoflurane (1 l/min) did not produce any significant changes in urinary markers including α-GST (Kharasch et al. 1997) and high-flows (2 l/min) produced elevations in urinary markers but this was not considered clinically significant (Ebert et al. 1998). There is no consensus on the toxicity of sevoflurane, but desflurane and isoflurane (low-flow) appear not to have any significant effect on renal function. Both these agents undergo minimal metabolism.

1.2.3 Intravascular contrast media

Contrast media reduce renal function by altering renal haemodynamics and by direct toxic effects on tubular epithelial cells (Tepel et al. 2000). Initially there is a transient increase in renal blood flow, followed by a prolonged vasoconstriction (Bakris et al. 1999). The role of dopamine receptor agonists, adenosine agonists, saline, mannitol, frusemide, calcium channel blockers, atrial natriuretic factor, acetylcysteine and ACE inhibitors have been studied with varying success (Albert et al. 1994; Solomon et al. 1994; Kapoor et al. 1996; Bakris, Lass et al. 1999; Gupta et al. 1999; Tepel, van der Giet et al. 2000).

High osmolality ionic media were associated with a high incidence of renal injury and since the introduction of low osmolality non-ionic media there has considerable interest in its renal effects (Golman et al. 1985). Interpretation of the literature is made difficult by the numerous definitions of post contrast renal failure, either in the use of different markers, the magnitude of change in the markers and also the timing of the assays (Kinnison et al. 1989; Lautin et al. 1991; Barrett et al. 1993). The various media, dosages, routes of administration and different populations studied add to the difficulty.

In patients with renal impairment, the incidence of acute renal failure after non-ionic iohexol ranges from 5.5% (increase in creatinine >50% or >1.0mg/dl) and 11.8% (increase in creatinine >0.5mg/dl) to 15% (increase in creatinine >1.0mg/dl) (Gomes et al. 1989; Taliercio et al. 1989; Rudnick et al. 1995). In patients with a normal serum creatinine a 4 - 10% incidence of acute renal failure has been quoted (increase in

creatinine >25%) (Jakobsen et al. 1994; Rosovsky et al. 1996). However, in a similar patient group, there was no change in GFR measured by Cr-51-EDTA after iohexol (Tornquist et al. 1984). The frequency of nephrotoxicity between low- and high-osmolality contrast media has been shown to be similar (Gomes, Lois et al. 1989; Kinnison, Powe et al. 1989; Moore et al. 1992) unless renal impairment with or without diabetes mellitus is present (Harris et al. 1991; Barrett and Carlisle 1993; Rudnick, Goldfarb et al. 1995).

Peritoneal and haemodialysis has been shown to be effective methods of eliminating the non-ionic contrast medium iohexol after angiography (Moon et al. 1995; Furukawa et al. 1996), however in patients with moderate renal impairment this has not been shown to reduce the incidence of nephrotoxicity (Lehnert et al. 1998; Sterner et al. 2000).

Carbon dioxide digital angiography has the advantages of being low cost, producing minimal discomfort, using smaller catheters, having no allergy risk and as it is excreted by the lungs in one circulation, allowing unlimited total volumes to be used without any apparent nephrotoxicity. Potential neurotoxicity, poor image quality and the phenomenon of 'vapour lock' (where blood flow is effectively prevented by too rapid injection of CO_2) are some disadvantages (Hawkins et al. 1994).

The use of the oral antihyperglycaemic agent metformin carries the risk of lactic acidosis. It undergoes renal excretion and therefore its use in renal disease is cautioned (Scheen 1996). It is also contra-indicated in pregnancy, peri-operatively and in liver, respiratory

and severe cardiac disease (Chan et al. 1999). Recently, there have been concerns about lactic acidosis after the use of intravascular iodinated contrast agents and in 1996 the Royal College of Radiologists published advice supporting the manufacturer that metformin should not be used in the 48 hours before or after the administration of intravenous contrast medium. Two subsequent studies reviewed the literature and concluded that in almost all cases of lactic acidosis there was pre-existing renal impairment or other contraindications to the use of metformin (Nawaz et al. 1998; McCartney et al. 1999). There has been only one reported case of lactic acidosis following intravenous contrast in a patient with normal renal function. Both studies conclude that it is safe to give intravascular contrast to patients on metformin, provided their renal function is normal.

1.3 POST OPERATIVE RENAL FAILURE

1.3.1 Introduction

In a prospective study of 1566 patients performed by the Joint Vascular Research Group in the United Kingdom (JVRG), the incidence of post operative renal failure in vascular patients varied from 45.5% (thoracoabdominal aneurysm) to 0.82% (carotid endarterectomy) (unpublished data). The definition of acute renal failure varies considerably in the literature. Definitions include

- > 1 mg/dl increase in serum creatinine
- creatinine level > 2.0mg/dl
- creatinine level > 3.0 mg/dl
- doubling of the serum creatinine and
- the need for dialysis (Novis et al. 1994; Kashyap et al. 1997)

The interpretation is confounded by the reporting of creatinine in units of either mg/dl or μmol/L (44 μmol/L is equivalent to 0.5 mg/dl). In general, a rise of ≥ 20% in serum creatinine will identify most patients whose creatinine clearance falls by more than 50% (Charlson et al. 1989). In the present study, we have used a rise > 25% to be significant.

1.3.2 Thoracoabdominal aneurysms

The mortality following renal failure complicating thoracoabdominal aneurysm surgery is 50%(unpublished data). In a review of their series of thoraco abdominal aneurysms, Safi et al. found a post operative acute renal failure rate of 17.5% and a mortality rate in this group of 49% (Safi et al. 1996). Other studies show renal failure incidences of 14%

(mortality 42%) (Schepens et al. 1994) and 25% (mortality 44%) (Godet et al. 1997) and 10% (Cambria et al. 1997). The development of post-operative renal failure is a significant risk factor for death. Furthermore peri-operative factors associated with post-operative renal failure were preoperative creatinine ≥ 2.8 mg/dl, visceral perfusion, left renal artery reattachment and simple cross clamp technique (Safi, Harlin et al. 1996), age (increased risk 1.2 per year) and pre-operative creatinine (increased risk 1.01 per 1 μmol/L) (Schepens, Defauw et al. 1994), age > 50 years, preoperative creatinine > 120 μmol/L, left kidney ischaemia > 30 minutes, transfusion of packed cells or cell saved blood > 5 units (Godet, Fleron et al. 1997) , pre-operative creatinine >1.5 g/dl (Kashyap, Cambria et al. 1997). Pre-operative renal impairment is also a significant predictor of mortality (Gilling-Smith et al. 1995; Cambria, Davison et al. 1997).

1.3.3 Abdominal Aortic Aneurysms

In patients undergoing repair for non-ruptured abdominal aortic aneurysms, the incidence of post-operative renal failure is much lower. The Canadian aneurysm study showed a renal failure rate of 5.4% and a dialysis rate of 0.6% (Johnston 1989). The mortality associated with renal failure was 28%. Other authors quote renal failure rates of 7% up to 18% (Diehl et al. 1983, Joseph et al. 1989). The rates of patients requiring post-operative dialysis has been reported at 1.2% up to 7% (O'Donnell et al. 1989; Holland et al. 1998). Again, elevation of the pre-operative creatinine is associated with the development of post-operative renal failure and is also a factor linked to peri-operative mortality. In our institution only age and pre-operative creatinine were independent pre-operative risk

factors for mortality on multivariate analysis (unpublished data). Schepens et al. found similar results for thoracoabdominal aneurysms (Schepens, Defauw et al. 1994).

Emergency surgical repair of a ruptured abdominal aortic aneurysm carries an overall mortality of 50% (Drott et al. 1992). In a single centre study, the mortality rate of 65 repairs complicated by acute renal failure was 75%. Of the survivors, the majority had irreversible renal impairment. Only 1 patient required long term dialysis. The results of 18 patients requiring renal support after ruptured aortic aneurysm showed eleven survivors (Gordon et al. 1994). By 3 months eight were independent of dialysis.

In our tertiary vascular practice, only 3.9 % of patients overall required renal replacement therapy (unpublished data). The in-hospital mortality associated with post-operative renal failure was 60%. Only 1 of the 1253 patients studied required long-term renal replacement therapy, affirming that complicated vascular surgery does not appear to place a significant long-term burden on renal services.

Patients with established end stage renal disease (ESRD) on haemodialysis also present for surgical management of their aortic aneurysms. A French study of 33 such patients operated on from six to eight hours after dialysis had a postoperative mortality of 9% (Lacombe 1998). However the long-term survival was 43% at five years and 11% at ten years.

Aortic reconstruction after renal transplantation places the renal allograft at risk for ischaemic damage. Intraoperative manoeuvers to maintain renal perfusion include extracorporeal pump oxygenation, temporary axillofemoral bypass, aortofemoral shunt and an indwelling shunt through the graft (Kashyap et al. 1999). For thoracoabdominal aneurysm repair we have used left atrio-femoral bypass to maintain flow to the allograft during aortic cross-clamp.

1.3.4 Endovascular Aneurysm Repair

Despite attempts to avoid the mortality and morbidity associated with open aneurysm repair, endovascular repair has not eradicated the postoperative complications. Indeed a whole new set of complications specific to this technology has now arisen. With regard to renal dysfunction, patients with normal preoperative renal function have a 6% risk of postoperative renal dysfunction after abdominal aortic aneurysm repair (Walker et al. 1998). About 44% of this subgroup will die. For patients with pre-operative renal failure, the peri-operative mortality is 27%.

1.3.5 Carotid Endarterectomy

In an analysis of our experience over a two-year period of 176 patients undergoing carotid endarterectomy, none had preoperative renal impairment and none required post-operative renal support (unpublished data). Nationally in the UK, the incidence of post-operative renal failure following carotid endarterectomy was 0.18% (unpublished JVRG data). In a multicentre risk assessment study of 9795 carotid endarterectomies, renal failure was significantly associated with increased operative stroke and mortality rates

(Plecha et al. 1993). In a smaller study the incidence of postoperative stroke and death was significantly higher (43%) in patients with severe chronic renal insufficiency (creatinine > 2.9 mg/dl) than in those with normal renal function (6% and 1%) (Rigdon et al. 1997). Complications in patients with mild renal insufficiency (creatinine 1.5-2.9 mg/dl) were not significantly different from the control group. Another report looking at patients with mild renal impairment (creatinine > 1.5 mg/dl) showed significantly higher stroke and death rates compared to those with normal renal function(Hamdan et al. 1999).

A recent publication challenges this difference in outcome for renal patients. In a study of a mixed group of chronic renal insufficiency (32) and end stage renal disease (ESRD) (19), the perioperative stroke-mortality rate was similar to the control group (2.0% vs. 2.6%). (Sternbergh et al. 1999). Operations were performed for asymptomatic as well as symptomatic disease. However the 4-year survival rate was 54% for chronic renal insufficiency and only 12% in ESRD. The poor long-term survival may well negate any stroke prevention benefits accrued through surgery, which dictate that the patients be expected to survive at least 2 years (Cina et al. 2000).

1.3.6 Infra-inguinal Vascular Disease

In our experience, infra-inguinal bypass is associated with a 5% incidence of renal failure. Other studies showed that 16% of patients with peripheral vascular disease had severe renal artery stenosis and about a third of this subgroup died from cardiac or renal complications post operation (Salmon and Brown 1990). Reperfusion syndrome

following revascularisation of the acutely ischaemic leg carries a high incidence of renal failure (Defraigne et al. 1998).

It is not uncommon for patients with ESRD to present to the vascular surgeon with a critically ischaemic leg. Cardiovascular disease is the leading cause of death in ESRD and peripheral vascular disease is a leading cause of morbidity (Holley 2000). The overall mortality is 41-48% at 2 to 3 year follow up (Marcelli et al. 1996; Peltonen et al. 1998). The shortened life expectancy of this group of patients affects the benefits gained from screening for vascular disease.

Results of intervention show satisfactory limb salvage rates in survivors. The mean limb salvage rate at 2 years was 72% (range 52-94%) (Isiklar et al. 1997). Infrageniculate reconstruction led to a subsequent higher quality of life. Perioperative mortality rates vary from 6-10% (Harrington et al. 1990; Johnson et al. 1995). At age 59 the expected remaining lifetime for dialysis patients is 4.3 years (3 years if diabetic as well) (Mailloux, Napolitano et al. 1994). Intervention for claudication should be carefully weighed against the poor long-term survival.

Primary amputation should be considered for forefoot gangrene. The amputation rate in diabetics with ESRD was 10 times that of the diabetic population at large (Eggers et al. 1999). Two thirds died within 2 years following amputation.

Interpretation of results relating to infrainguinal bypass surgery in general is made difficult by the failure to stratify patients according to symptoms, extent of bypass and conduit. The small numbers of patients in individual series with ESRD makes this subgroup even more difficult to interpret.

1.3.7 Renal Artery Stenosis

The true prevalence of Renal Artery Stenosis (RAS) in the general population is not known. Numerous studies have defined the prevalence in selected populations. Early autopsy studies found rates of significant (>50%) stenosis of 5% in patients < 64years old, 18% in patients 65 to 74 years old and 42% in patients >75 years old (Schwartz et al. 1964). About 50% were bilateral. During aortography for concurrent vascular disease the incidence of significant RAS in various studies was 16-28% for aortic disease, 14-30% for coronary artery disease and 22-45% for peripheral artery disease (Conlon et al. 2000).

The natural history of severe stenosis has been assessed, looking at both progression of stenosis and the reduction in renal size. Rates of progression of stenosis for patients undergoing coronary angiography at one and six years were 6.5% and 25% respectively (Crowley et al. 1998). Overall about 50% of patients will progress and 16% will occlude (Schreiber et al. 1984). About 40% of patients with stenosis > 75% will progress to occlusion. Reduction in renal size >10% occurred in 37% of patients with renovascular hypertension (Dean et al. 1981) and 26% of patients with significant RAS had a reduction in renal size >1cm after a mean of 14 months follow up (Strandness 1994). Progressive deterioration in renal function has also been shown, with a creatinine rise from a mean of

1.4 mg/dl to 2.0 mg/dl over 39 months (Chabova et al. 2000). In their prospective study at 44 months Dean et al. showed that 19 of 41 patients had a more than 25% increase in serum creatinine (Dean, Kieffer et al. 1981).

The long-term survival for patients with ischaemic nephropathy is poor. Scoble et al. found a 2-year mortality rate of around 25% with intervention and 30% without (Scoble, Sweny et al. 1993). Harden and colleagues had a mortality rate around 55% two years after renal artery stenting (Harden et al. 1997). Work done by the author show, after a mean follow up of 2.4 years, mortality rates of 33% with intervention and 29% without (Pillay et al. 2002). After a mean of 39 month follow up, the Mayo clinic found a mortality of 28% (unrelated causes) in patients with stenosis > 70% (Chabova, Schirger et al. 2000).

Overall outcome after intervention (surgery, angioplasty and stenting) reveal a distribution between improvement, no change and deterioration in renal function. Renal function improves in 22- 65% and deteriorates in 6 – 48% (Alcazar et al. 2000; Safian et al. 2001).

More important than the static effect on creatinine or GFR is the effect on the rate of decline in GFR or increment in creatinine. It is against this statistic that the success or failure of surgical or minimally invasive intervention should be judged. The mean slope of the reciprocal creatinine plot over time in 23 patients with RAS was -4.34 l/μmol/day

(Harden, MacLeod et al. 1997) and –0.0079 dl/mg/month in another 33 patients (Watson et al. 2000).

Large trials are required to identify those patients who will benefit from intervention i.e. whose rate of deterioration in renal function will improve. At the same time we need to be able to identify patients with poor long-term survival prospects, who do not survive long enough to reap the benefits of any intervention.

CHAPTER 2 ASSESSING RENAL FAILURE

2.1 INTRODUCTION

The glomerular filtration rate (GFR) is the standard measure of renal function. It is defined as the volume of plasma that can be completely cleared of a particular substance by the kidney in a unit of time. The most accurate method of measuring GFR is by inulin clearance. It is freely filtered in the kidney and does not undergo any metabolism, secretion or absorption. Inulin is expensive and the laboratory assay is difficult and expensive.

Other exogenous substances such as iohexol, ^{51}Cr-EDTA, ^{99m}Tc-labeled DTPA or ^{125}I-labeled iothalamate are equally labour-intensive. Thus, the measurement of endogenous substances to estimate GFR is common. The properties of an ideal substance should include release into the blood stream at a constant rate, free filtration by the glomerulus, no reabsorption or secretion by the renal tubules, and exclusive elimination via the kidneys (Laterza et al. 2002).

Initially blood urea was used but due to the large proportion that undergoes passive reabsorption, serum creatinine has become the most commonly used serum marker of renal function. Other endogenous markers include the low molecular weight proteins α_1-microglobulin, β_2-microglobulin and retinol binding protein. These have largely been abandoned due to the influence of non-renal factors on their circulating concentrations (Grubb et al. 1985; Grubb 1992).

Recovery of enzymes and other proteins in the urine was recognised early on as a marker of renal disease. The availability of newer assays to determine renal dysfunction allows discrimination between falling GFR and tubular dysfunction (Porter 1994; Scherberich et al. 1994). Further assessment of various urinary enzymes and low molecular weight proteins allows localisation of injury to different parts of the nephron.

Criteria were outlined for selecting potentially useful markers (Gonick et al. 1973). N-Acetyl-β-D-glucosaminidase (NAG) is a tubular enzyme that has been studied as a marker of tubular injury (Kunin et al. 1978). It has a high molecular weight to not be ordinarily filtered and is thought to arise from damage to the S3 segment and the distal nephron. Alanine aminopeptidase (AAP) appears to originate from the proximal tubule (Porter 1994). The wide variability of results and the influence of non-renal factors on some of these markers have limited their use (Jung 1994).

2.2 CREATININE

Because creatinine is freely filtered by the glomerulus, the serum creatinine has been used to estimate GFR. Creatinine levels are proportional to muscle mass (which is determined by age, body weight and sex), dietary intake, extrarenal clearance and tubular secretion. Furthermore, creatinine levels only start to rise after GFR has dropped by 50%, mainly because of the effects of tubular secretion (Watson, Hadjipetrou et al. 2000). There is now an increasing realisation that serum creatinine is an inadequate measure of the absolute level of renal function in the individual patient.

Despite this, many authors believe that the rate of decline in the reciprocal of serum creatinine accurately reflects the rate of loss of renal function. Furthermore it has been noted that there is a linear decline over time of the reciprocal of serum creatinine. Therefore, any change in slope of this linear decline over time has been interpreted to indicate a change in the rate of deterioration. However the change in slope is also affected by the rate of creatinine generation and extrarenal excretion. Studies demonstrating the linear decline had carefully selected their patients and had low correlation coefficients for their linear estimates (Levey et al. 1988). Nevertheless, the effect of intervention on the slope of the reciprocal of creatinine over time will continue to be used as a measure of success in clinical studies(Watson, Hadjipetrou et al. 2000). The ASTRAL trial, for example, has as a measure of its primary endpoint, the change in the slope of the reciprocal creatinine plot (2000).

24-hour creatinine clearances are fraught with errors of collection and because of the contribution of tubular secretion, generally overestimate GFR. Theoretically, because cimetidine competes with creatinine for tubular secretion, it decreases the secretion of creatinine by tubular cells. Its oral administration improves the accuracy of creatinine clearance measurements by decreasing the error caused by tubular secretion (Toto 1995).

Formulae have been developed to overcome the error from the contribution of the extrarenal determinants of serum creatinine. The Cockcroft-Gault formula, correcting serum creatinine for sex, age and weight, is the most consistently used and widely applicable (Cockcroft et al. 1976; Toto 1995) (see APPENDIX A). The estimates of GFR are most applicable in the range of 10-100 ml/min.

Studies looking at the biological variation in serum creatinine have calculated a value of 14% of the mean value in health (86 μmol/l) (Keevil et al. 1998). Hence a change of only 12 μmol/l is highly significant in the same individual.

The definition of renal failure varies considerably and we have used cut-off values of a more than 25% rise over baseline or a more than 44 μmol/l rise. These are well above the biological variation described.

2.3 CYSTATIN C

A promising new marker for estimating GFR is Cystatin C. It is a non-glycosylated 13 kD basic protein that is a member of the cystatin superfamily of cystein protease inhibitors (Grubb et al. 1982; Barrett et al. 1984). It is produced by all investigated nucleated cells (Lofberg et al. 1979). The structure of its gene and promoter has been determined. The gene is of the housekeeping type, which is compatible with a stable production rate by most cells (Abrahamson et al. 1990). The production rate is unaltered in inflammatory conditions. It is freely filtered in the renal glomeruli and almost completely reabsorbed and catabolised by the proximal tubular cells (Grubb 1992; Tenstad et al. 1996). The low molecular weight and its stable production rate strongly indicate that the blood serum concentration of this protein is mainly determined by the glomerular filtration rate (GFR). The serum concentration of cystatin C has been shown to be at least as good an indicator of GFR as the serum concentration of creatinine (Grubb, Simonsen et al. 1985; Simonsen et al. 1985).

Most studies suggest that serum cystatin C is independent of gender, however one study suggested that concentrations were lower in women than in men (Pergande et al. 1993). This study using the ELISA technique reported significantly higher serum concentrations than others. Some studies had utilised the technique of enzyme amplified single radial immunodiffusion (SRID). A rapid and automated procedure for the quantification of serum cystatin C based upon the latex particle enhanced immunoturbidmetric assay (PETIA) technique has been produced (Newman et al. 1995).

There was no significant difference in values between males and females. This was confirmed by other investigators (Randers et al. 1998). In 1997 Finney *et al.* described a particle-enhanced immunonephelometric immunoassay (PENIA) (Finney et al. 1997). This fully automated assay showed good intra- and interassay precision, as well as agreement with the previously described PETIA. No difference was found between samples analysed immediately and those stored at –20°C. 95% Reference intervals for serum cystatin C concentration were determined to be 0.51-0.98 mg/l. For women the interval was 0.49-0.94 mg/l and men 0.56-0.98 mg/dl. Because of the small difference it was suggested that a single range for all adults less than 50 years, without adjustment for body surface area, be used. (This version of the latex immunoassay was used for this study)

These enable a shorter reaction time and provide a result in 15 minutes. In addition the assay can be performed on the same instruments routinely used for assessment of serum creatinine by the Jaffe method(Newman, Thakkar et al. 1995; Keevil, Kilpatrick et al. 1998).

Cystatin C offers greater sensitivity in detecting an abnormal GFR with equivalent specificity and overall better diagnostic efficiency (Newman et al. 1994; Newman, Thakkar et al. 1995). With renal disease there was significantly better correlation of Cystatin C to GFR (^{51}Cr-EDTA) ($r = 0.80$) than serum creatinine ($r = 0.5$). It has also been demonstrated that Cystatin C is an earlier indicator of mild renal failure and is more likely to be abnormal with a decreased GFR than serum creatinine (Fliser et al. 2001).

Using a lower reference range of GFR to be 72 ml/min/1.73m^2, it was demonstrated that a cystatin C result of greater than or equal to 1.25mg/l had a sensitivity and specificity for diagnosing renal impairment of 71.4% and 95.1% respectively. A serum creatinine of greater than 110 μmol/l demonstrated results of only 52.4% and 91.8% respectively (Newman, Thakkar et al. 1995). Besides rising earlier, Cystatin C has been shown to also rise to a greater extent than serum creatinine for lower GFR. This makes cystatin C a potentially better marker for detecting renal impairment than serum creatinine.

We used a baseline cystatin C > 1.24 mg/l to denote pre-existing renal impairment.

The biological variation of cystatin C has been calculated at 37% of the mean reference value and would require a difference of 0.24 mg/l to be regarded as significantly different (Keevil, Kilpatrick et al. 1998). This suggests that cystatin C may not be as sensitive as serum creatinine in for detecting changes within the same individual.

2.4 ALPHA GLUTATHIONE S-TRANSFERASE

Alpha glutathione S-transferase (α-GST) is a cytosolic protein highly specific for the cells of the proximal renal tubules (Campbell et al. 1991). It is part of the family of glutathione S-transferases and plays a role in protecting cells by using reduced glutathione to conjugate or reduce many different reactive electrophiles. This usually inactivates these electrophiles and facilitates their excretion in urine or bile (Seidegard et al. 1997). It is found in high concentrations and is readily released from injured cells into the urine.

Alpha GST is found in the proximal renal tubule while pi GST is found in the distal tubule (Harrison et al. 1989). Studies have shown that the straight portion of the proximal tubule (the S3 segment) is the most susceptible to ischaemic and toxic injury (Venkatachalam et al. 1978; Torhorst et al. 1982). Alpha GST release has been shown to follow the time course of proximal tubular necrosis as proven by histological changes (Bass et al. 1979). Urinary alpha GST has been used as a sensitive marker of proximal tubular damage after exposure to nephrotoxic insults (Sherman et al. 1984; Eger, Koblin et al. 1997) Increased recovery of alpha GST has been documented after exposure to iodinated contrast media despite no rise in serum creatinine (Sherman, Feinfeld et al. 1984). This was dose related and occurred within 36 hours of contrast exposure.

Recently published data from a pilot study of 8 patients in Newcastle, UK showed that alpha GST provided an early indication of postoperative renal injury (Cressey et al. 2002). In patients undergoing infra-renal aortic aneurysm repair all patients with

postoperative renal dysfunction (using creatinine) had elevations in alpha GST:creatinine at one and three hours after removal of the aortic cross clamp. Peak α-GST:creatinine ratios were significantly associated with the peak % increase in serum creatinine.

Alpha GST is measured by a quantitative enzyme immunoassay and at a 1/2 dilution has a recordable range of 0-80 μg/l. The manufacturer's reference range is < 11.1 μg/l and interassay variation is up to 9.8% (Biotrin International, Dublin, Ireland).

No reference range is available for urine GST:creatinine ratio, though this correction allows spot samples to be taken, avoiding the problems associated with timed samples. This correction has been used before (Cressey, Roberts et al. 2002).

2.5 URINE ALBUMIN

Microalbuminuria describes pathological albuminuria in the 30-200 mg/l range, which is undetectable by chemical dip sticks (Watts et al. 1988).

Increased urine albumin may be due to increased glomerular filtration or to decreased tubular absorption. Increased glomerular permeability is best assessed by selectivity studies measuring different molecular weight protein markers in the urine. Urine albumin reflects primarily glomerular permeability, whereas total protein reflects a combination of permeability, tubular leakage, tubular secretion, and normal protein shed in the urine (Newman et al. 2000). Dividing by urine creatinine not only improves the intra-individual variation in albumin but also corrects for variations in urine flow.

Surgery-induced microalbuminuria occurs within 30 minutes of operation (Fleck et al. 1985). A relationship between high urinary albumin to creatinine ratios and subsequent pulmonary dysfunction has been shown in infra-renal aortic aneurysm repair (Smith et al. 1994). Changes in glomerular permeability probably reflect changes in systemic vascular permeability. Urine albumin measurement alone has been used as an assessment of increased permeability (Gosling et al. 1994; Pallister et al. 1997). Reperfusion injury with increased vascular permeability would therefore result in a significant increase in microalbuminuria.

Proteinuria has been studied in assessing potential nephrotoxicity of contrast agents. Massive albuminuria has been reported with the older media and very much less with the

LOM (Tornquist et al. 1985). The phenomenon is transient but must reflect glomerular and/or tubular damage.

Reference values for daytime ambulatory urine albumin range between 0.9-29.6 mg/l and for albumin:creatinine ratio between 0.1-2.3 mg/mmol (Watts, Morris et al. 1988). Intra-individual variation of urinary albumin: creatinine ratios in healthy subjects range between 52% and 111% (Watts, Morris et al. 1988; Newman, Pugia et al. 2000).

CHAPTER 3 MATERIALS AND METHODS

This was a prospective longitudinal observational study.

3.1 STUDY SETTING

Patients presenting for Vascular Surgical management at the Regional Vascular Unit at St Mary's Hospital were recruited for the study between September 2000 to August 2001. St Mary's Hospital NHS Trust is a tertiary referral centre for Vascular Surgery in the United Kingdom. Ethical approval to conduct the study was obtained from the St Mary's Local Research Ethics Committee and the University of Natal Research Ethics Committee.

Serum Cystatin C and all the urinary assays (urine creatinine, albumin and α-GST) were performed at the South West Thames Institute for Renal Research, which is affiliated to the St Helier NHS Trust.

3.2 PATIENT SELECTION

Patients were recruited at the Vascular Surgery Outpatient Clinic or on admission to the Vascular Surgery Ward. Informed consent (see Appendix B) was obtained after patient counselling (see Appendix C and D). No honorarium was paid for participation in the study.

Inclusion criteria:

- All patients undergoing elective intravascular-enhanced contrast radiology for vascular disease.
- All patients undergoing infra-renal and juxta-renal aortic aneurysm repair.
- All patients undergoing aorta-bifemoral bypass for aorto-iliac occlusive disease.

Exclusion criteria:

- Emergency surgery.
- Patients undergoing endovascular procedures.
- Patients in end stage renal failure.

3.3 DATA COLLECTION

There was no deviation from the routine pre-operative and post-operative management of patients except that additional blood specimens were taken at the time of routine venepuncture and from existing arterial lines, and mid-stream urine was collected pre-operatively and from indwelling urinary catheters post-operatively.

Demographic details were recorded on a pro forma data sheet and stored electronically on FileMaker Pro 4.0 (Claris) (see Appendix E). Further note was made of Angiography and CAT scan details including type and volume of contrast and operative details including operative time, renal ischaemia time, the use of cardiac bypass, volume of blood transfusion and type of renal revascularisation. The study data was only accessible to the primary investigators in the study.

3.4 SPECIMEN PROCESSING

Five millilitres of blood and 10 ml of urine specimens were taken at the following time points:

Contrast exposure (C):

- Pre contrast **(C1)**
- Day-1 post contrast **(C2)**
- Day-7 post contrast **(C3)**

Surgery (T):

- Pre-operation **(T1)**
- Prior to aortic cross-clamp **(T2)**
- Prior to lower limb reperfusion **(T3)**
- 2-hours after lower limb reperfusion **(T4)**
- Day-1 post-operation **(T5)**
- Day-4 post-operation **(T6)**
- Day-7 post-operation **(T7)**

Serum creatinine assays were performed as part of the routine intensive care management.

Clotted blood was centrifuged, separated and then 2 x 1ml aliquots stored at –20 degrees Celsius. Two 1 ml urine specimens were frozen at –20 degrees Celsius. After addition of a stabilizing buffer (4:1) a further 2 x 1 ml urine specimens were frozen at –20 degrees

Celsius. Samples were initially processed and stored in the Academic Surgical Unit Laboratory at St Mary's Hospital.

Samples were transported in batches to be processed at the South West Thames Institute for Renal Research, Surrey. The four anylates assessed were

- Serum cystatin C (PENIA)
- Urinary alpha glutathione S-transferase (alpha-GST) (Biotrin NEPHKIT™-ALPHA Human GST-Alpha, Biotrin Int. Ltd., Ireland)
- Urinary microalbumin and urinary creatinine.

Serum creatinine results were obtained by routine processing through the St Mary's Hospital biochemistry laboratory.

3.5 RESULTS CORRECTION

Due to the variation in urine flow and hence the concentration of the urinary markers, urinary GST and urinary albumin were corrected for by dividing the assay result by the result of urine creatinine assayed at the same point.

3.6 PROCEDURE DESCRIPTIONS

3.6.1 Intravascular contrast-enhanced radiological investigation

All patients had baseline serum creatinine and coagulation studies done. Patients with serum creatinine > 120 μmol/l had an intravenous line placed the night before and a litre of 0.9% saline infused over 12 hours. All studies were performed using Iohexol, a low-osmolar, non-ionic agent. Urine output was monitored for the 24 hours post contrast exposure.

3.6.2 Open aortic surgery

All operations were done under general anaesthesia with an epidural catheter and indwelling Foley catheter placed in theatre. Anaesthesia was maintained by desflurane or isoflurane inhalation. All patients were given an intravenous dose of vancomycin of 500mg on induction of anaesthesia. This was repeated for three days thereafter. Trough levels were checked prior to administering the subsequent doses.

'Renal' dopamine was defined as the use of a dopamine infusion at 3 μg/kg/min. Frusemide was infused at a rate of 0.5 – 1.0 mg/min in some patients. Significant intra-operative hypotension was defined as a sustained systolic blood pressure less than 70 mmHg requiring either the infusion of colloid or an inotrope (dobutamine). The decision in the use of anaesthetic agent, diuretic or inotropic agent was made individually by one of three consultant vascular anaesthetists.

Post-operatively patients were nursed in a high-dependency unit or ICU. Management was dictated by the individual patient responses and was along standard protocols for post-operative care.

CHAPTER 4 RESULTS FOR PATIENTS UNDERGOING VASCULAR SURGERY WITH AN AORTIC CROSS CLAMP

4.1 PATIENT DEMOGRAPHIC DATA

BACKGROUND

Thirty-five patients underwent aortic surgery; 29 for aortic aneurysms and 6 for aortic occlusive disease. There were 7 females and the median age was 71 years (Table I). The median Cockcroft-Gault calculated creatinine clearance at baseline was 58.7 ml/min. The median baseline creatinine clearance was not significantly different between the patients with aortic aneurysmal and occlusive disease.

INTRA-OPERATIVE DATA

Twenty-six operations involved an infra-renal aortic cross-clamp and 9 were performed with a supra-renal cross-clamp (Table II). Statistical significance between the supra-renal and infra-renal group was examined using the Fisher's Exact Test for the nominal variables and Mann-Whitney Test for the continuous variables. The proportion of patients with ischaemic heart disease was significantly higher in the group undergoing an infra-renal aortic cross-clamp ($P = 0.036$). All patients undergoing supra-renal aortic cross-clamp had an intra-operative infusion of dopamine ($P = 0.015$).

Two patients underwent left renal vein ligation to improve surgical access for the proximal anastomosis. Their calculated creatinine clearances were 70 and 61 ml/min respectively. The former patient had a rise in Cystatin C greater than 37%. No other adverse outcome (as defined for this study) was recorded in either patient.

DIALYSIS

A total of five patients needed dialysis after surgery. Three patients had aneurysmal disease and 4 underwent infra-renal aortic cross-clamp. Two patients requiring dialysis died. A more detailed analysis of this group is described in Section 4.3.

DEATHS

Overall 3 patients died. Two had undergone a supra-renal procedure for aneurysmal disease and had calculated creatinine clearances of 47 and 40 ml/min respectively. The former died of multi-system organ failure after a prolonged period of dialysis-dependence, and the latter died intra-operatively from massive intra-operative haemorrhage. The third patient underwent surgery for aortic occlusive disease. She died from complications after developing colonic ischaemia, necessitating a re-look laparotomy and a left hemi-colectomy (calculated creatinine clearance of 24 ml/min).

Preoperative variable	Supra-renal clamp n=9	Infra-renal clamp n=26	Overall n=35
Males	5	23	28
Age	69	73	71
Calculated creatinine clearance (ml/min)	62.3	54.6	58.7
Diabetes	2	2	4
Hypertension	3	16	19
Hyperlipidaemia	2	11	13
Smoking	4	10	14
ACE inhibitor drugs	0	6	6
Ischaemic Heart Disease*	0	10	10
Stroke	0	8	8

Table I. Pre-operative data for 35 patients undergoing aortic surgery and stratified by supra- or infra-renal aortic cross-clamp. Continuous variables are median values.

(* P = 0.036, Fisher's Exact Test)

Intra-operative variables	Supra-renal clamp n=9	Infra-renal clamp n=26	Overall n=35
Anaesthetic: Isoflurane	4	11	15
Anaesthetic: Desflurane	5	15	20
Mannitol	3	5	8
'Renal' dopamine*	9	14	23
Frusemide	5	12	17
Hypotension	3	8	11
Inotrope	2	4	6
Blood loss (ml)	3579	1800	2000
Units Packed Cells transfused	5.5	4.0	4.0
Intraoperative urine flow (ml/min)	3.0	2.5	2.7
Anaesthetic time (min)	255	202.5	220
Surgery time (min)	180	145	150
Lower limb ischaemic time (min)	80	67	69
True renal ischaemic time (min)	20	Not applicable	20

Table II. Intra-operative data for 35 patients undergoing aortic surgery and stratified by supra- or infra-renal aortic cross-clamp. Continuous variables are median values. (* P = 0.015, Fisher's Exact Test)

4.2 RENAL FAILURE DIAGNOSIS

4.2.1 Sensitivity and specificity of baseline serum creatinine and serum cystatin C in diagnosing pre-existing renal dysfunction.

INTRODUCTION

Serum creatinine values above 120 μmol/l are considered abnormal. It is well described that creatinine is an insensitive marker for renal dysfunction as it only starts to rise after a 50% drop in GFR (Section 2.2).

Reference values serum cystatin C range from 0.51 – 0.98 mg/l (Section 2.3). The sensitivity and specificity for diagnosing renal failure using a definition of GFR < 72 ml/min/1.73 m^2 (Cr-EDTA) has been shown to be better using cut-off levels of cystatin C >= 1.25 mg/l (71.4% and 95.1% respectively) versus serum creatinine >= 110 μmol/l (52.4% and 91.9% respectively).

For this study we used cut-off points of serum creatinine > 120 μmol/l and a serum cystatin C > 1.24 mg/l.

We investigated the sensitivity and specificity of the two assays at different cut-off points using a definition of calculated GFR (Cockcroft-Gault) < 72 ml/min.

METHODS

Receiver operating characteristic (ROC) curves were constructed using baseline serum creatinine and baseline serum cystatin values with the outcome of calculated creatinine clearance < 72 ml/min. Cut-off points to maximize the associations were determined. Furthermore, cut-off points previously described in the literature were used and their associations calculated.

RESULTS

The significance of the area under the curve and the coordinate points of the ROC curves are included in Appendix F

Figure 1. ROC Curve. Serum creatinine before surgery

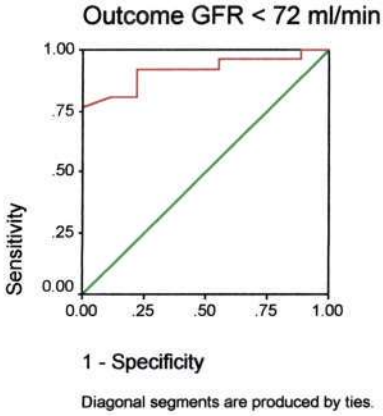
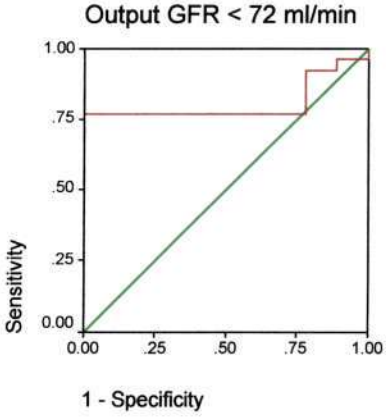


Figure 2. ROC Curve. Serum cystatin C before surgery



From the coordinate points the following data was extracted:

	Sensitivity %	Specificity %
Creatinine >= 111	46.2	100
>=120.5	26.9	100
>=99	76.9	100
Cystatin C >= 1.25	38.5	100
>=0.98	76.9	100

Table III. Sensitivity and specificity data for baseline serum creatinine in μmol/l and serum cystatin C in mg/l in diagnosing a calculated GFR < 72 ml/min

4.2 RENAL FAILURE DIAGNOSIS

4.2.2 Does an overall 37% increase in serum cystatin C diagnose more patients with renal dysfunction than an overall 25% or 44 μmol/l increase in serum creatinine?

INTRODUCTION

We set out to determine if cystatin C was a better test than serum creatinine in detecting patients with renal failure. The definition of a significant rise in creatinine varies in the literature (Section 2.2). The biological variability is 15%. We used an increase of more than 25% as denoting a significant rise in serum creatinine. Other papers have used definitions of a greater than 44 μmol/l or 88 μmol/l rise in serum creatinine. We have used a cut-off point as a rise greater than 44 μmol/l. From the reports on cystatin C, the biological variation is known to be 37% (Section 2.3). Increases more than 37% are considered significant.

METHODS

Values for baseline (T1) were compared to the subsequent six time points (T2 – T7) to calculate the number of patients with a positive diagnosis using the definitions of renal dysfunction explained above. McNemar's Chi Squared Test for related samples was used to determine if the overall proportions of positive results with the different diagnostic tests were similar.

RESULTS

(For each variable 0 = no, 1 = yes)

2X2 Table for rise in serum Creatinine > 25% vs rise in Cystatin C > 37%

			Rise in serum cystatin C >37%		
			0	1	Total
Rise in serum creatinine >25%	0	Count	17	4	21
		% within Creatinine rise>25%	81.0%	19.0%	100.0%
		% within Cystatin C rise> 37%)	77.3%	30.8%	60.0%
	1	Count	5	9	14
		% within Creatinine rise>25%	35.7%	64.3%	100.0%
		% within Cystatin C rise> 37%)	22.7%	69.2%	40.0%
Total		Count	22	13	35
		% within Creatinine rise>25%	62.9%	37.1%	100.0%
		% within Cystatin C rise> 37%)	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Exact Sig. (2-sided)
McNemar Test		1.000[a]
N of Valid Cases	35	

a. Binomial distribution used.

There is no significant difference between the proportions of positive diagnoses using the outcomes of a rise in serum creatinine >25% vs. a rise in serum cystatin C >37%.

2X2 Table for rise in serum Creatinine > 44 micromol/l vs rise in Cystatin C > 37%

			Rise in serum cystatin C >37%		
			0	1	Total
Rise in serum creatinine >44 micromol/l	0	Count	20	5	25
		% within Creatinine rise >44mmol	80.0%	20.0%	100.0%
		% within Cystatin C rise > 37%	90.9%	38.5%	71.4%
	1	Count	2	8	10
		% within Creatinine rise >44mmol	20.0%	80.0%	100.0%
		% within Cystatin C rise > 37%	9.1%	61.5%	28.6%
Total		Count	22	13	35
		% within Creatinine rise >44mmol	62.9%	37.1%	100.0%
		% within Cystatin C rise > 37%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Exact Sig. (2-sided)
McNemar Test		.453[a]
N of Valid Cases	35	

a. Binomial distribution used.

There is no significant difference between the proportions of positive diagnoses using the outcomes of a rise in serum creatinine > 44 μmol/l vs. a rise in serum cystatin C >37%.

4.2 RENAL FAILURE DIAGNOSIS

4.2.3 Does a 37% increase in serum Cystatin C diagnose patients with renal dysfunction earlier than a 25% or 44 μmol/l increase in serum creatinine?

INTRODUCTION

We set out to assess if a rise in serum cystatin C diagnosed more patients than a rise serum creatinine at each time point (T2 – T7). This is similar to the analysis in 4.2.2 but involves comparing the proportions of positive diagnoses at each individual time point rather that overall. This was done to see if a rise in serum cystatin C occurred earlier than a rise in serum creatinine for each individual patient.

METHODS

Values for baseline (T1) were compared to each of the subsequent six time points (T2 – T7) to calculate the number of patients with a positive diagnosis using the definitions of renal dysfunction explained above. McNemar's Chi Squared Test for related samples was used for each time point, to determine if the proportions of positive results with the different diagnostic tests were similar.

The data is presented first for:

A) a rise in creatinine > 25% versus a rise of serum cystatin C>37%

Each 2x2 table represents the proportions for each time point: T2, T3, T4, T5, T6, T7.

B) a rise in creatinine > 44 μmol/l versus a rise of serum cystatin C>37%

Each 2x2 table represents the proportions for each time point : T2, T3, T4, T5,

T6, T7.

RESULTS

A) A rise in serum creatinine > 25% verses a rise of serum cystatin C > 37%.

(For each variable 0 = no, 1 = yes)

2x2 Table for a rise in serum Creatinine > 25% vs a rise in Cystatin c > 37% (T2)

Count

		Rise in serum cystatin C > 37%		
		0	1	Total
Rise in serum creatinine > 25%	0	30	3	33
Total		30	3	33

2x2 Table for a rise in serum Creatinine > 25% vs a rise in Cystatin c > 37% (T3)

Count

		Rise in serum cystatin C > 37%		
		0	1	Total
Rise in serum creatinine > 25%	0	30	3	33
Total		30	3	33

2x2 Table for a rise in serum Creatinine > 25% vs a rise in Cystatin c > 37% (T4)

Count

		Rise in serum cystatin C > 37%		
		0	1	Total
Rise in serum creatinine > 25%	0	31	4	35
Total		31	4	35

For time points T2, T3 and T4 there were no patients diagnosed with renal dysfunction using rise in serum creatinine > 25%, despite positive results using a rise of serum cystatin C > 37%.

2x2 Table for a rise in serum Creatinine > 25% vs a rise in Cystatin c > 37% (T5)

			Rise in serum cystatin C > 37%		
			0	1	Total
Rise in serum creatinine > 25%	0	Count	20	3	23
		% within SCR525YE	87.0%	13.0%	100.0%
		% within T537%riseCys	83.3%	30.0%	67.6%
	1	Count	4	7	11
		% within SCR525YE	36.4%	63.6%	100.0%
		% within T537%riseCys	16.7%	70.0%	32.4%
Total		Count	24	10	34
		% within SCR525YE	70.6%	29.4%	100.0%
		% within T537%riseCys	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Exact Sig. (2-sided)
McNemar Test		1.000[a]
N of Valid Cases	34	

a. Binomial distribution used.

2x2 Table for a rise in serum Creatinine > 25% vs a rise in Cystatin c > 37% (T6)

			Rise in serum cystatin C > 37%		
			0	1	Total
Rise in serum creatinine > 25%	0	Count	22	5	27
		% within SCR625YE	81.5%	18.5%	100.0%
		% within T637%riseCys	84.6%	71.4%	81.8%
	1	Count	4	2	6
		% within SCR625YE	66.7%	33.3%	100.0%
		% within T637%riseCys	15.4%	28.6%	18.2%
Total		Count	26	7	33
		% within SCR625YE	78.8%	21.2%	100.0%
		% within T637%riseCys	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Exact Sig. (2-sided)
McNemar Test		1.000[a]
N of Valid Cases	33	

a. Binomial distribution used.

2x2 Table for a rise in serum Creatinine > 25% vs a rise in Cystatin c > 37% (T7)

			Rise in serum cystatin C > 37%		
			0	1	Total
Rise in serum creatinine > 25%	0	Count	22	4	26
		% within SCR725YE	84.6%	15.4%	100.0%
		% within T737%riseCys	95.7%	57.1%	86.7%
	1	Count	1	3	4
		% within SCR725YE	25.0%	75.0%	100.0%
		% within T737%riseCys	4.3%	42.9%	13.3%
Total		Count	23	7	30
		% within SCR725YE	76.7%	23.3%	100.0%
		% within T737%riseCys	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Exact Sig. (2-sided)
McNemar Test		.375[a]
N of Valid Cases	30	

a. Binomial distribution used.

For time points T5, T6 and T7 there was no statistical difference between the proportions of patients diagnosed with renal dysfunction using a rise in serum creatinine > 25% or using a rise of serum cystatin C > 37%.

B) A rise in serum creatinine > 44 μmol/l verses a rise of serum cystatin C > 37%.

(For each variable 0 = no, 1 = yes)

2x2 Table for a rise in serum Creatinine > 44 micromol/l vs a rise in Cystatin c > 37% (T2)

Count

		Rise in serum cystatin C > 37%		
		0	1	Total
Rise in serum creatinine > 44 micromol/l	0	31	3	34
Total		31	3	34

2x2 Table for a rise in serum Creatinine > 44 micromol/l vs a rise in Cystatin c > 37% (T3)

Count

		Rise in serum cystatin C > 37%		
		0	1	Total
Rise in serum creatinine > 44 micromol/l	0	31	3	34
Total		31	3	34

2x2 Table for a rise in serum Creatinine > 44 micromol/l vs a rise in Cystatin c > 37% (T4)

Count

		Rise in serum cystatin C > 37%		
		0	1	Total
Rise in serum creatinine > 44 micromol/l	0	31	4	35
Total		31	4	35

For time points T2, T3 and T4 there were no patients diagnosed with renal dysfunction using rise in serum creatinine > 44 μmol/l, despite positive results using a rise of serum cystatin C > 37%.

2x2 Table for a rise in serum Creatinine > 44 micromol/l vs a rise in Cystatin c > 37% (T5)

			Rise in serum cystatin C > 37%		
			0	1	Total
Rise in serum creatinine > 44 micromol/l	0	Count	23	4	27
		% within T544riseCr	85.2%	14.8%	100.0%
		% within T537%riseCys	95.8%	40.0%	79.4%
	1	Count	1	6	7
		% within T544riseCr	14.3%	85.7%	100.0%
		% within T537%riseCys	4.2%	60.0%	20.6%
Total		Count	24	10	34
		% within T544riseCr	70.6%	29.4%	100.0%
		% within T537%riseCys	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Exact Sig. (2-sided)
McNemar Test		.375[a]
N of Valid Cases	34	

a. Binomial distribution used.

2x2 Table for a rise in serum Creatinine > 44 micromol/l vs a rise in Cystatin c > 37% (T6)

			Rise in serum cystatin C > 37%		
			0	1	Total
Rise in serum creatinine > 44 micromol/l	0	Count	22	6	28
		% within T644riseCr	78.6%	21.4%	100.0%
		% within T637%riseCys	84.6%	85.7%	84.8%
	1	Count	4	1	5
		% within T644riseCr	80.0%	20.0%	100.0%
		% within T637%riseCys	15.4%	14.3%	15.2%
Total		Count	26	7	33
		% within T644riseCr	78.8%	21.2%	100.0%
		% within T637%riseCys	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Exact Sig. (2-sided)
McNemar Test		.754[a]
N of Valid Cases	33	

a. Binomial distribution used.

2x2 Table for a rise in serum Creatinine > 44 micromol/l vs a rise in Cystatin c > 37% (T7)

			Rise in serum cystatin C > 37%		
			0	1	Total
Rise in serum creatinine > 44 micromol/l	0	Count	22	5	27
		% within T744riseCr	81.5%	18.5%	100.0%
		% within T737%riseCys	95.7%	71.4%	90.0%
	1	Count	1	2	3
		% within T744riseCr	33.3%	66.7%	100.0%
		% within T737%riseCys	4.3%	28.6%	10.0%
Total		Count	23	7	30
		% within T744riseCr	76.7%	23.3%	100.0%
		% within T737%riseCys	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Exact Sig. (2-sided)
McNemar Test		.219[a]
N of Valid Cases	30	

a. Binomial distribution used.

For time points T5, T6 and T7 there was no statistical difference between the proportions of patients diagnosed with renal dysfunction using a rise in serum creatinine > 25% or using a rise of serum cystatin C > 37%.

DISCUSSION

The last three subsections have compared serum creatinine with serum cystatin C as tools for diagnosing renal failure.

Cystatin C at the upper reference limit of 0.98 mg/l had a higher sensitivity (76.9%) than creatinine at 111 μmol/l (46.2%) or at 120.5 μmol/l (26.9%) in diagnosing pre-existing renal dysfunction (Table III). This is in keeping with the literature. To achieve a similar sensitivity with creatinine one would need to use a cut-off value of 99 μmol/l, which is well within the reference range for serum creatinine. The more conservative upper limit of cystatin C of 1.25 mg/l reveals a sensitivity of 38.5% (not dissimilar to creatinine between 111 – 120.5 μmol/l).

Cystatin C appears to be a better marker for detecting baseline renal dysfunction.

Temporal changes (rise) in serum creatinine and cystatin C were then examined. In section 4.2.2 the overall proportions of diagnoses of renal failure was no different with cystatin C or creatinine at either the 25% or 44 μmol/l cut-off level.

When examined at each time point, a cystatin C rise > 37% appeared to be better at detecting changes at pre-aortic cross-clamp (T2) and prior to lower limb reperfusion (T3). No statistical significance could be determined, as the two cut-off points for creatinine did not pick up any positives.

The biological variability of cystatin C (37%) is far greater than creatinine (15%). One would expect that creatinine would be more sensitive in detecting (temporal) changes within an individual patient based on the variability. A smaller rise in creatinine is necessary to be considered significant. Other definitions of renal dysfunction e.g. creatinine rise > 88 μmol/l or a doubling of serum creatinine would be expected to yield even lower sensitivities.

Cystatin C is a better marker for diagnosing pre-existing renal dysfunction in a population and furthermore appears to be sensitive in picking up changes in renal function over time.

4.3 EARLIER MARKERS OF RENAL DYSFUNCTION

INTRODUCTION

The last section investigated changes in the assays based on biological variability. A comparison of the number of positive diagnoses at each individual time point was done to determine which assay yielded a higher proportion of positive diagnoses. We examined for the earliest time point that showed a difference and sought the assay responsible.

Here we examined the mean values of data at each time point (T2 – T7) to see which assays demonstrated a statistically significant rise compared to the baseline (T1). We then examined for the earliest time point that showed a statistical difference and sought the assays responsible.

The aim was to determine the value of the alternative assays to serum creatinine as earlier markers of renal damage in patients undergoing aortic surgery.

METHODS

Analysis revealed the data was not normally distributed and hence a logarithmic transformation of all the data for the four assays at the seven time points was done. Thereafter plots for each of the four assays were constructed for the mean natural log value at each time point.

Repeated measures ANOVA was then performed using a simple contrast of each time point versus the baseline value (T1). Univariate tests of the overall within-subjects effects of time were analysed (Lower-bound). The specific within-subjects contrast for each of the time points (T2 – T6) versus the baseline (T1) was then analysed to detect the first time point (Tx) at which there was a significant rise in mean value. This was done in turn for each of the 4 assays.

Comparison was then made between the Tx values of the 4 assays to determine which test/s were the most sensitive in detecting early changes in renal function deterioration.

RESULTS

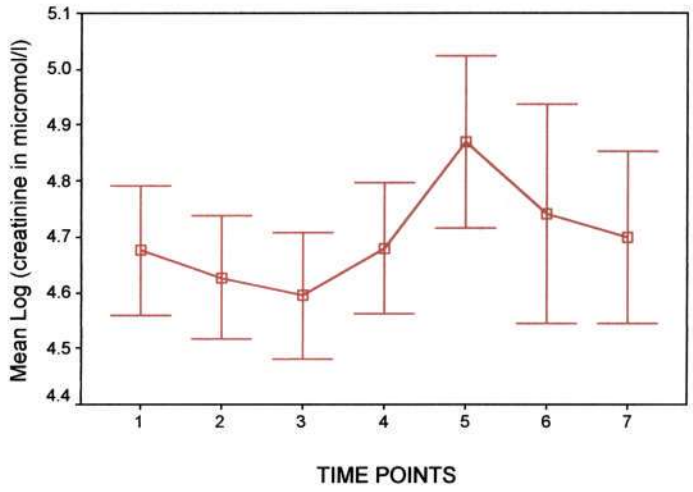


Figure 3. Change in mean log serum creatinine at 7 sample points in patients undergoing aortic surgery. Error bars are 95% CI

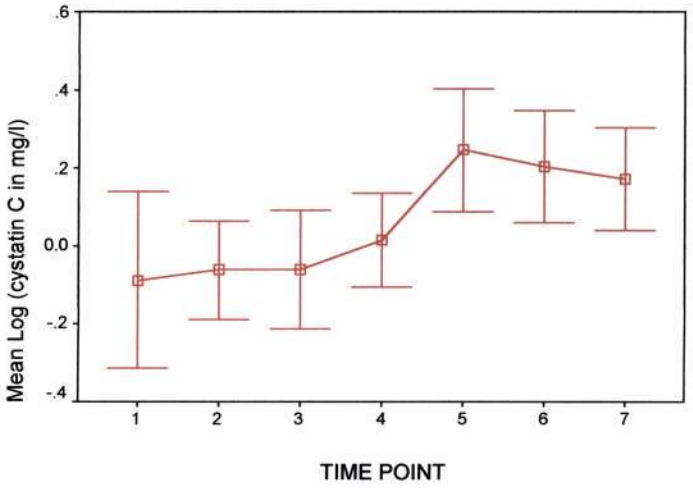


Figure 4. Change in mean log serum cystatin C at 7 sample points in patients undergoing aortic surgery. Error bars are 95% CI

Creatinine: The effect of time overall on mean serum creatinine was significant (Lower-bound F = 10.136, P = 0.004).

For the specific time points, there was a significant difference in mean serum creatinine at T2 (P = 0.006) and T3 (P = 0.01) versus the baseline (T10. This was not clinically significant as the mean creatinine values are lower than T1 (Figure 3). The first significant rise in mean creatinine occurs at T5 (F = 13.091, P = 0.001).

Cystatin C: The effect of time overall on mean serum cystatin C was significant (Lower-bound F = 10.944, P = 0.003).

For the specific time points, the first significant change in mean Cystatin C occurred with the rise at T5 (F = 13.020, P = 0.001) (Figure 4).

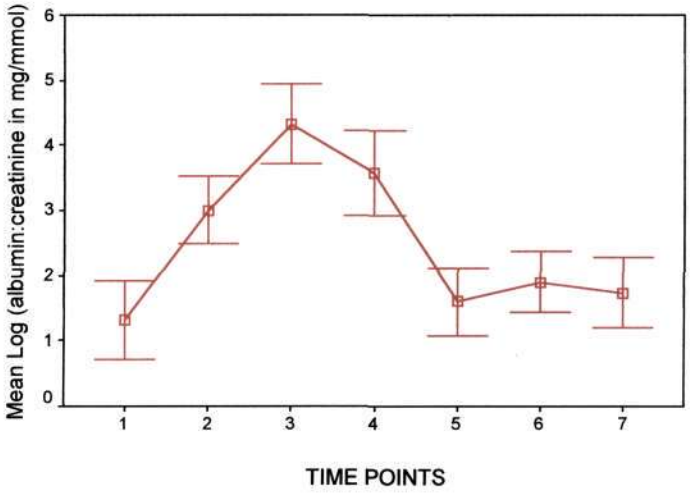


Figure 5. Change in mean log urine albumin:creatinine at 7 sample points in patients undergoing aortic surgery. Error bars are 95% CI

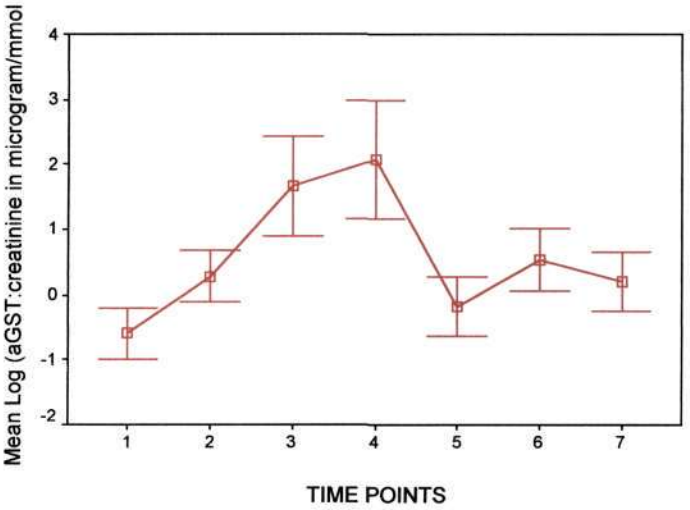


Figure 6. Change in mean log urine aGST:creatinine at 7 sample points in patients undergoing aortic surgery. Error bars are 95% CI

Urine albumin:creatinine: The effect of time overall on mean urine albumin:creatinine was significant (Lower-bound F = 26.870, P = 0.000).

For the specific time points, at T2 there was a significant rise in mean urine albumin:creatinine (F = 27.693, P = 0.00) (Figure 5).

Urine αGST:creatinine: The effect of time overall on mean serum αGST:creatinine was significant (Lower-bound F = 15.977, P = 0.001)

For the specific time points, at T2 there was a significant rise in urine αGST:creatinine (F = 31.345, P = 0.00) (Figure 6).

DISCUSSION

Serum creatinine, a measure of glomerular filtration rate (GFR) appears to decrease during the intra-operative period. The other marker of GFR, cystatin C, does not follow this pattern. Creatinine levels may fall in cases of decreased muscle mass or protein intake but these are long-term effects (see Section 2.2). Tubular secretion of creatinine may be affected by drugs and reabsorption of creatinine may occur in states of low urine flow rate. In any event, both of these mechanisms would result in increased levels of serum creatinine. Increased tubular secretion of creatinine is possible but increased recovery of urinary α-GST suggests tubular cell damage. If the drop in serum creatinine is reflective of an improvement in GFR then it is difficult to explain the absence of a similar drop in cystatin C.

Both markers first rise significantly only on Day-1 post operation. Cystatin C is therefore no better than serum creatinine in detecting early renal dysfunction, specifically a fall in the GFR. Furthermore, these findings suggest that GFR only significantly deteriorates on the first post-operative day but that the deterioration begins immediately after lower limb reperfusion (T4). Factors implicated in reperfusion injury may be implicated in the onset of deterioration of GFR. Intensive intra-operative anaesthetic management is evidenced by the maintenance of high urine flow rates (Table II). This may further delay the onset of deterioration in GFR.

Both urine albumin:creatinine and urine alpha GST:creatinine show an almost immediate significant rise prior to aortic cross-clamp (T2) and remain elevated until the first post-

operative day. Changes in glomerular permeability and proximal tubular cellular damage start to occur on induction of anaesthesia, despite maintenance of high urinary flow rates.

All patients were given a dose of vancomycin on induction of anaesthesia and this has been implicated in renal damage.

Furthermore inhalational anaesthetic agents have also been shown to affect the kidneys. Desflurane has been investigated and found to have no effect on the recovery of αGST in the urine, unlike Sevoflurane. Low-flow isoflurane show similar results to desflurane. The present population was exposed to high-flow desflurane and isoflurane (A comparison of the median peak assay values stratified by inhalational anaesthetic agent is discussed in Section 4.5)

The question that may be posed here is whether the degree of significant rise in the urinary markers albumin and αGST at T2 is predictive of eventual renal dysfunction. ROC Curve analysis of the T2 values for urine albumin:creatinine and urine alpha GST:creatinine as predictors for dialysis are discussed in Section 4.5 and the tables and figures can be found in Appendix I.

4.4 INFRA-RENAL VERSUS SUPRA-RENAL CROSS-CLAMP

INTRODUCTION

In the previous 2 sections an analysis was performed on the entire group of patients undergoing aortic surgery (n=35) looking at the value of the different assays as earlier markers of renal damage. In this section we analyse the group of patients by whether or not a supra-renal (SR) or infra-renal cross clamp (IR) was applied.

Previous analysis (Table 4.2) showed only 'renal' dopamine and the presence of ischaemic heart disease to be significantly different variables between the 2 groups. 'Renal' dopamine was infused in all SR group and just over half of IR group. Neither 'renal' dopamine nor the presence of ischaemic heart disease was positively or negatively related to the outcome of dialysis dependence (see Results section 4.5). We have therefore assumed, for interpretation of the results for this section, the difference between the 2 groups is primarily the period of true renal ischaemic time, resultant from the application of the supra-renal cross clamp (median 20 minutes).

We wished to assess the mechanism of injury to the nephron induced by true ischaemia.

METHODS

Analysis revealed that the data was not normally distributed and therefore a logarithmic transformation of all the data for the four assays at the seven time points was done.

Thereafter plots for the four assays were constructed using the means of the natural log value at each time point, by supra- or infra-renal cross clamp.

T-Test: Initially T-Tests were performed to determine if there was any significant difference at T1 (baseline) between the supra-renal (SR) group and infra-renal (IR) group mean log values for each assay. This was done to test for any possible bias between the two groups in terms of pre-existing renal dysfunction as measured by each of the four assays.

Similar analyses at the next six time points were also performed.

ANOVA: Repeated measures ANOVA were performed on the whole group of patients. We used a simple contrast of each time of the six point times (T2 – T7) versus the baseline value (T1), testing for within-subjects and between-subjects effects of the factor of a supra-renal aortic cross-clamp. This is similar to the analysis performed in Section 4.4 but with the addition of the factor SR or IR.

Patients were then stratified into two groups by whether they underwent a supra-renal (SR) or infra-renal (IR) aortic cross-clamp. Repeated measures ANOVA was then performed separately on each group using a simple contrast of each of the six time points (T2 – T7) versus the baseline value (T1). The specific within-subjects contrast for each of the time points (T2 – T7) versus the baseline (T1) was then analysed to detect the first

RESULTS

T-Test: Analysis for equal variance (Levine's Test) at T1 between SR and IR showed only serum albumin to have a significant value (F = 5.156, P = 0.03). For the other 3 assays equal variance was assumed in applying the t-test for equality of means.

2-Tailed t-tests showed no significant difference between supra-renal (SR) and infra-renal (IR) at baseline (T1) for all four assays.

For the subsequent five time points only urine GST showed a significantly higher mean for the supra-renal (SR) versus the infra-renal (IR) group (Figure 8). The results were:

- T2 P = 0.001 (95% CI 0.71 – 2.29)
- T3 P = 0.004 (95% CI 0.75 – 3.55)
- T4 P = 0.000 (95% CI 2.06 – 5.14)
- +T5 P = 0.012 (95% CI 0.27 – 2.01)
- T6 P = 0.08 (95% CI 0.34 – 2.06)

values are lower than T1 (see figure 5). The first significant rise in creatinine occurs at T5 ($F = 13.407$, $P = 0.001$).

SR: For patients undergoing surgery with a supra-renal aortic cross-clamp (SR) there was no significant difference in mean log serum creatinine from the baseline. However, at T5 ($F = 6.625$, $P = 0.05$) and T6 ($F = 6.076$, $P = 0.57$) the difference (increment) approaches statistical significance.

Serum cystatin C

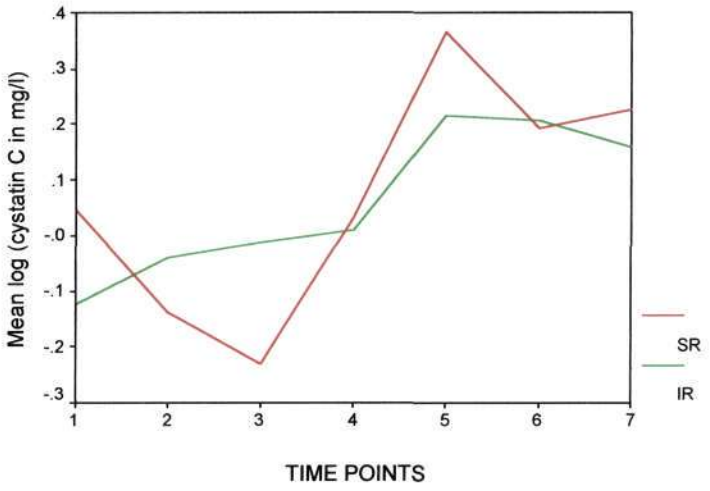


Figure 8. Change in mean serum cystatin C at 7 sample points stratified by supra-(SR) or infra-renal (IR) aortic cross-clamp

The Lower-bound test of the within-subjects interaction of time and supra-renal or infra-renal clamp was not significant (F = 1.910, P = 0.179). The between-subjects effect of a supra-renal or infra-renal clamp was also not significant (F = 0.005, P = 0.946).

IR: For patients undergoing surgery with an infra-renal (IR) aortic cross-clamp the mean log of serum cystatin C was significantly different (elevated) from baseline (T1) at T5 (F = 9.403, P = 0.006).

SR: For patients undergoing supra-renal aortic cross-clamp (SR) the value at T2 (F = 8.248, P = 0.035) was significantly different but inspection of figure 6 reveals this not to

be clinically significant. The first significant rise in the assay occurred at T6 (F = 9.143, P = 0.029), but approached significance at T5 (F = 4.946, P = 0.077).

Urine albumin:creatinine

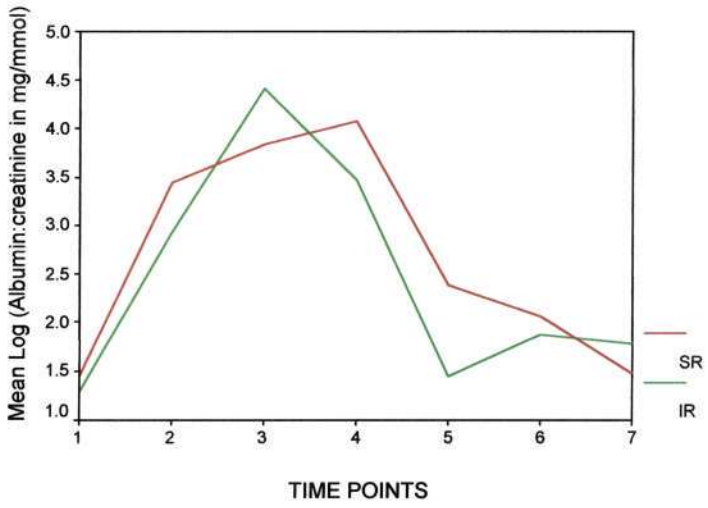


Figure 9. Change in mean log urine albumin:creatinine at 7 sample points stratified by supra- (SR) or infra-renal (IR) aortic cross-clamp

The Lower-bound test of the within-subjects interaction of time and supra-renal or infra-renal clamp was not significant (F = 0.750, P = .395). The between-subjects effect of a supra-renal or infra-renal clamp was also not significant (F = 0.203, P = 0.656).

The value at T2 was significantly elevated from baseline in both groups **IR** (F = 18.927, P = 0.000) and **SR** (F = 21.029, P = 0.019).

Urine αGST: creatinine

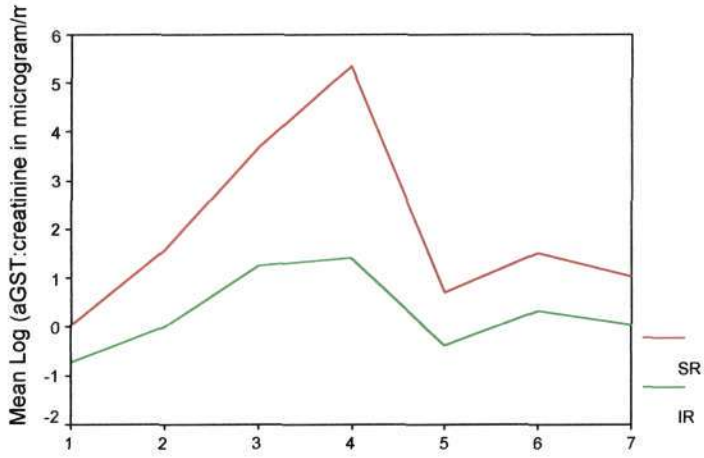


Figure 10. Change in mean log urine aGST:creatinine at 7 sample points stratified by supra- (SR) or infra-renal (IR) aortic cross-clamp

The Lower-bound test of the within-subjects interaction of time and supra-renal or infra-renal clamp was not significant (F = 3.289, P = 0,083). The between-subjects effect of a supra-renal or infra-renal clamp was found to be highly significant (F = 35.242, P < 0.001).

IR: For patients undergoing an infra-renal aortic cross-clamp (IR) there was a sustained significant rise in urine αGST: creatinine at T2, T3 and T4 (F = 28.625, P < 0.001).

SR: The patients undergoing a supra-renal (SR) aortic cross-clamp showed a statistically significant rise in urine αGST: creatinine at T4 (F = 32.550, P = 0.011).

DISCUSSION

There was no difference between supra-renal (SR) and infra-renal (IR) mean values for all four assays at the baseline (t-Test). This is not unexpected as there was also no significant difference between calculated creatinine clearances at baseline (Table I).

We can then assume that baseline renal function in both groups SR and IR were similar. Subsequent change in renal function measurement is therefore due to any factors affecting function during the perioperative period. We have shown that the only 'positive' intervention significantly in favour of the group undergoing supra-renal cross-clamp is the infusion of Dopamine at 'renal' doses. The only obviously significant negative difference is the supra-renal clamp itself. This group was therefore subjected to a period of true renal ischaemia of 20 minutes.

T-test analysis showed that only urinary alpha GST:creatinine was significantly higher in the group undergoing supra-renal aortic cross-clamp. This assay is a marker of proximal renal tubular cell injury. The markers of GFR (serum creatinine and serum cystatin C) and of glomerular permeability (urine albumin) rose with time but there was no significant difference between the two groups SR and IR.

This was similarly shown by the repeated measures ANOVA where the between-subjects effects of time and a supra-renal or infra-renal clamp were found only to be significant for the assay urinary GST:creatinine.

The nephrotoxic effect of true renal ischaemia appears to be directed at the proximal tubular cell rather than at sustained deterioration in GFR or glomerular permeability. The S_3 segment of the proximal tubule has been shown in the rat model to be the most sensitive to ischaemia. Alpha-GST is highly specific for the cells of the proximal tubule (Section 2.4).

However, the multifactorial nature of the renal insults that occur during the perioperative period of aortic surgery result in global deterioration in renal function measurements. As shown in the previous chapter the urinary assays for albumin and GST are earlier markers for deteriorating renal function. Infra-renal aortic cross-clamp is likely to also cause renal ischaemia by distortion of the peri-renal aorta and changes in the renal blood flow haemodynamics and autoregulation.

Comparison of values for T2 to T7 versus the baseline for each group SR (n=9) and IR (n= 26) is a similar analysis to that of the previous chapter where this was performed for the whole group of 35 patients (ANOVA). Similar trends are seen in that elevations in the urinary assays appear to be earlier markers of deterioration in renal function versus serum creatinine and serum cystatin C.

The failure to conclusively demonstrate this trend for the supra-renal group is probably a type II error. The limitations of small numbers of patients with a supra-renal clamp were further exacerbated by the listwise exclusion of patients with missing values, when performing the statistical analysis (SPSS 11.0 for Windows). The large variance in

measured values also contributed to the inability to demonstrate any significant change though the graphical representations in figures 7 - 10 suggest similar trends to those in the IR group.

The significant drop in Cystatin C at T3 in the supra-renal group when compared to the infra-renal group (figure 8) might be explained by the use of 'renal' dopamine in all these patients and the slightly higher urine flow rates that were achieved (Table II). The other marker of GFR, serum creatinine, demonstrates a similar trend. (Figure 7).

4.5 RENAL FAILURE PREDICTION

INTRODUCTION

An ability to predict post-operative renal dysfunction is useful not only for purposes of prognosis but also for deciding on strategies to minimise renal dysfunction. We defined renal dysfunction as either one of four outcomes (Section 4.2):

- Dialysis dependence
- Overall rise in serum creatinine > 37%
- Overall rise in serum creatinine > 44 μmol/l
- Overall rise in serum cystatin C > 37%

The predictors used were:

- Baseline serum creatinine > 120 μmol/l
- Baseline serum cystatin C > 1.24 mg/l

METHODS

A) In order to exclude bias we initially investigated for an association between the variables shown in Table 4.1 (pre-operative) and Table 4.2 (intra-operative) and the hard outcome of dialysis dependence

- Sex, Age, Supra- or Infra-renal aortic cross-clamp
- Diabetes, Hypertension, Hyperlipidaemia, Smoking, ACE inhibitor drugs, History of ischaemic heart disease and History of stroke
- Anaesthetic agent, Mannitol, 'Renal' Dopamine, Frusemide, Hypotension and Inotrope
- Blood loss, Units packed cells transfused, Intraoperative urine flow, Anaesthetic time, Surgery time and Lower limb ischaemic time

Fisher's Exact Test was performed on all the nominal variables from Tables I and II and Mann-Whitney Test on the continuous variables to determine if there was any difference between those patients that eventually needed dialysis and those that did not.

Furthermore, as suggested in Section 4.3, we also investigated the outcome of peak urine albumin: creatinine and αGST:creatinine (continuous variables) to see if there was a significant difference in the median values in the group who had isoflurane and those who had desflurane as an inhalational anaesthetic agent (Mann-Whitney test)

B) We used the previously defined predictors of renal dysfunction to stratify patients:

- Baseline serum creatinine > 120 μmol/l
- Baseline serum cystatin C > 1.24 mg/l

The sensitivity and specificity for diagnosing renal failure by each of the 4 outcomes was calculated after the construction of 2 x 2 Tables.

C) Receiver operating characteristic (ROC) curves were then calculated for the outcome 'dialysis' and the continuous **baseline** variables

- Serum creatinine
- Calculated creatinine clearance
- Serum cystatin C
- Urine albumin:creatinine

As suggested in Section 4.3 ROC Curves were also plotted for the **T2-values** (rather than baseline) of urine albumin:creatinine and αGST:creatinine using 'dialysis' as an outcome. We showed earlier that the urinary markers rose significantly at T2 and here we have looked at whether there was an association between this and dialysis i.e. could the magnitude of the rise at T2 predict outcome.

RESULTS

A)

Outcome Dialysis: For each of the variables described under Method A there was no significant difference between those patients that required dialysis and those that did not.

'Anaesthetic agent' and Peak urinary markers: Median peak urine albumin:creatinine was higher (but not significantly) in the isoflurane group (220.24 mg/mmol) versus the desflurane group (86.34 mg/mmol) (P = 0.077).

Median peak urine αGST:creatinine was higher (but not significantly) in the isoflurane group (31.97 μg/mmol) versus the desflurane group (6.00 μg/mmol) (P = 0.271)

B) Sensitivities and specificities. (2x2 Tables are included in Appendix G.)

Baseline creatinine > 120 μmol/l

Baseline serum creatinine > 120 μmol/l vs. outcome serum creatinine rise > 25%

%	Association	95% Confidence Interval
Sensitivity	35.7	16.3 – 61.2
Specificity	90.5	71.1 – 97.3
Positive predictive value	71.4	35.9 – 91.8
Negative predictive value	67.9	49.3 – 82.1

Baseline serum creatinine>120 μmol/l vs. outcome serum creatinine rise > 44 μmol/l

%	Association	95% Confidence Interval
Sensitivity	50.0	23.7 – 76. 3
Specificity	92.0	75.0 – 97.8
Positive predictive value	71.5	35.9 – 91.8
Negative predictive value	82.1	64.4 – 92.1

Baseline serum creatinine > 120 μmol/l vs. outcome serum cystatin c rise > 37%

%	Association	95% Confidence Interval
Sensitivity	38.5	17.7 – 64.5
Specificity	90.9	72.2 – 97.5
Positive predictive value	71.4	35.9 – 91.8
Negative predictive value	71.4	52.9 – 84.7

Baseline serum creatinine > 120 μmol/l vs. outcome dialysis

%	Association	95% Confidence Interval
Sensitivity	60	23.1 – 88.2
Specificity	86.7	70.3 – 94.7
Positive predictive value	42.9	15.8 – 75.0
Negative predictive value	92.9	77.4 – 98.0

Baseline cystatin C > 1.24 mg/l

Baseline serum cystatin C > 1.24 mg/l * outcome serum creatinine rise > 25%

%	Association	95% Confidence Interval
Sensitivity	42.9	21.4 – 67.4
Specificity	81.0	60.0 – 92.3
Positive predictive value	60.0	31.3 – 83.2
Negative predictive value	68.0	48.4 – 82.8

Baseline serum cystatin C > 1.24 mg/l * outcome serum creatinine rise > 44 μmol/l

%	Association	95% Confidence Interval
Sensitivity	50.0	23.7 – 76.3
Specificity	80.0	60.9 – 91.1
Positive predictive value	50.0	23.7 – 76.3
Negative predictive value	80.0	60.9 – 91.1

Baseline serum cystatin C > 1.24 mg/l * serum cystatin C rise > 37%

%	Association	95% Confidence Interval
Sensitivity	38.5	17.7 – 64.5
Specificity	77.3	56.6 – 89.9
Positive predictive value	50.0	23.7 – 76.3
Negative predictive value	68.0	48.4 – 82.8

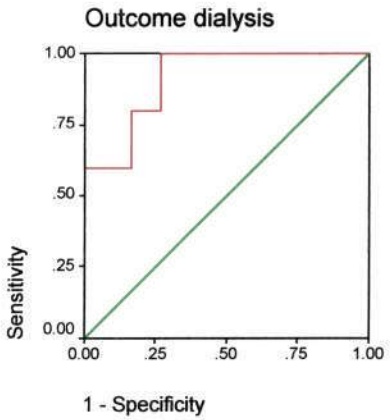
Baseline serum cystatin C > 1.24 mg/l * outcome Dialysis

	Association	95% Confidence Interval
Sensitivity	80.0	37.6 – 96.4
Specificity	80.8	62.7 – 90.5
Positive predictive value	40.0	16.8 – 68.7
Negative predictive value	96.0	80.5 – 99.3

C) ROC Curves for continuous baseline variables and outcome 'dialysis'

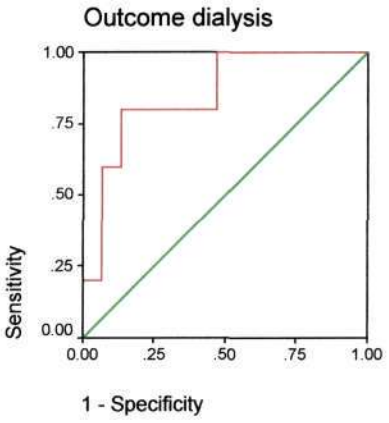
(significance of the area under the curve and coordinates of the curve are included in Appendix H)

Figure 11. ROC Curve. Baseline creatinine clearance before surgery



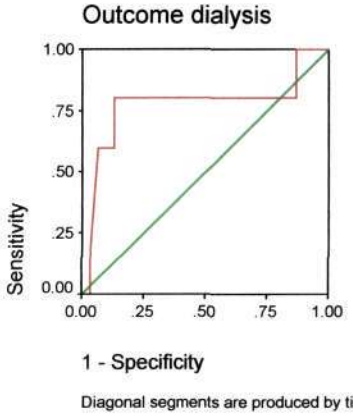
A calculated creatinine clearance <= 48.5 ml/min has a 100% sensitivity and 73.3%specificity for predicting dialysis. Using a cut-off <= 43.7 ml/min yields values of 80% and 83.3% respectively.

Figure 12. ROC Curve. Serum creatinine before surgery



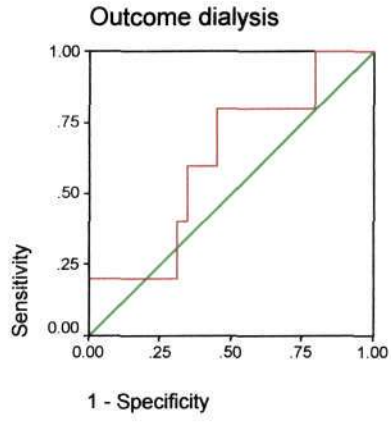
A cut-off serum creatinine >= 117.5 μmol/l improves the sensitivity from 60% (120 μmol/l) to 80% in predicting dialysis.

Figure 13. ROC Curve. Serum cystatin C before surgery



Using a cut-off of serum cystatin C >= 1.34 yields an unchanged sensitivity of 80% and an improved specificity of 86.7% for predicting dialysis.

Figure 14. ROC Curve. Urine albumin:creatinine before surgery



The area under the curve is not significantly different from 0.5. However, an estimate of the sensitivity and specificity for a value >= 3.97 mg/mmol is 80% and 55.5% respectively.

Continuous T2 variables for urinary albumin:creatinine and urinary αGST:creatinine and outcome 'dialysis'.

The area under the curve for both variables was not significantly different from 0.5.

The significance test results and ROC curves are included in Appendix I.

DISCUSSION

A) Although the median peak urinary assay values were not significantly different in those who had isoflurane anaesthesia, there was a trend towards higher means. Past studies have shown no significant rise in urinary αGST after desflurane and low-dose isoflurane anaesthesia. All patients in the present study had high-flow desflurane or isoflurane. It is possible that there exists a dose-related response of urinary markers to isoflurane. Further studies may help.

B) The sensitivity of baseline serum creatinine > 120 μmol/l and serum cystatin C > 1.24 mg/l in predicting renal dysfunction defined by biochemical changes is poor. Serum creatinine > 120 μmol/l appears to have overall better specificity.

For the hard outcome of 'dialysis' cystatin C has better sensitivity (80%) and specificity (80.8%) than creatinine. The low positive predictive values are in part due to the low prevalence of 'dialysis', which occurred in only 5 patients. The large confidence intervals may be explained by the small sample size.

C) The ROC curves for the baseline values show that similar sensitivities (80%) and specificities (83.3 – 86.7%) can be obtained for creatinine clearance, serum creatinine and serum cystatin C by manipulating the cut-off points. The change in cut-off in serum creatinine from 120μmol/l to 117.5 μmol/l and in serum cystatin C from 1.24 mg/l to 1.34 mg/l is within the biological variability of each assay and therefore may not be clinically relevant.

Using T2 urine albumin:creatinine does not yield improved sensitivities and specificities for predicting dialysis when compared to baseline ratios.

CHAPTER 5 RESULTS FOR PATIENTS UNDERGOING EXPOSURE TO INTRAVASCULAR CONTRAST

5.1 PATIENT DEMOGRAPHIC DATA

Thirty-seven patients underwent exposure to an intravenous contrast load (Table IV). The median age was 72.8 years and the median Cockcroft-Gault calculated creatinine clearance at baseline was 53.4 ml/min. Iohexol was used for all the patients and the median volume used was 145 ml. No patient required dialysis as a result of contrast exposure.

	Total n = 37	**Patients undergoing surgery n = 11**
Males	26	8
Females	11	3
Age (years)	72.8	73.3
Diabetes	7	1
Calculated baseline creatinine	53.4	56
Volume of contrast used (ml)	145	130
Hypertension	27	8
Hyperlipidaemia	14	4
Smoking	15	4
Ischaemic Heart Disease	11	3
Stroke	8	3
ACE inhibitor drugs	11	1

Table IV. Data for patients undergoing exposure to intravascular contrast (n=37). 11 patients subsequently underwent surgery. Continuous variables are median.

There was only 1 patient that had a serum creatinine rise > 25% or > 44 μmol/l. 3 patients developed a rise in cystatin C > 37%. There was no association between the volume of contrast used and the development of these outcomes.

Eleven patients subsequently underwent aortic surgery (C+S). There was no significant difference in any of the variables for these 11 patients versus the whole group of 37 patients (Fisher's Exact test for nominal data and Mann-Whitney test for continuous data). This group is discussed further in Chapter 6.

5.2 EARLIER MARKERS OF RENAL DYSFUNCTION

INTRODUCTION

Statistical analysis of the mean values of the four assays from time point 1 (C1) to time point 3 (C3) was performed. Results for time points 2 and 3 were compared in turn with the result for time point 1. We then examined for the earliest time point that showed a significant rise compared to baseline.

The aim was to determine the value of the alternative assays to serum creatinine as earlier markers of renal damage in patients undergoing intravascular contrast exposure. (This methodology is similar to that of Section 4.3)

METHODS

Initial analysis showed the data was not normally distributed. Logarithmic transformation of the values of the four assays at each of the three time points was performed. Plots for the four assays were constructed using the means of the natural log value at each time point.

Repeated measures ANOVA was performed using a simple contrast of each of the two time points (C2 and C3) versus the baseline (C1). Univariate tests of the overall within-subjects effects of time were analysed (Lower bound). The specific within-subjects

contrast for each of the two time points versus the baseline was then analysed to detect the first time (Cx) point at which there was a significant rise in each of the four assays.

Comparison was then made between the Cx values of the 4 assays to determine which test/s were the most sensitive in detecting early changes in renal function deterioration.

RESULTS

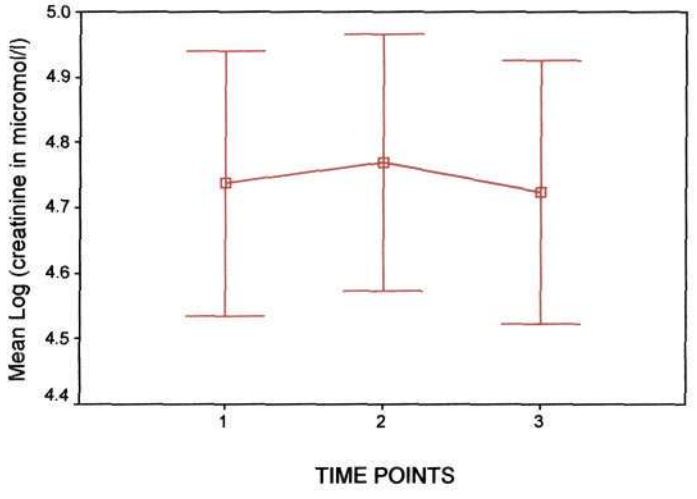


Figure 15. Change in mean log serum creatinine at 3 sample points in patients undergoing contrast exposure. Error bars are 95% CI

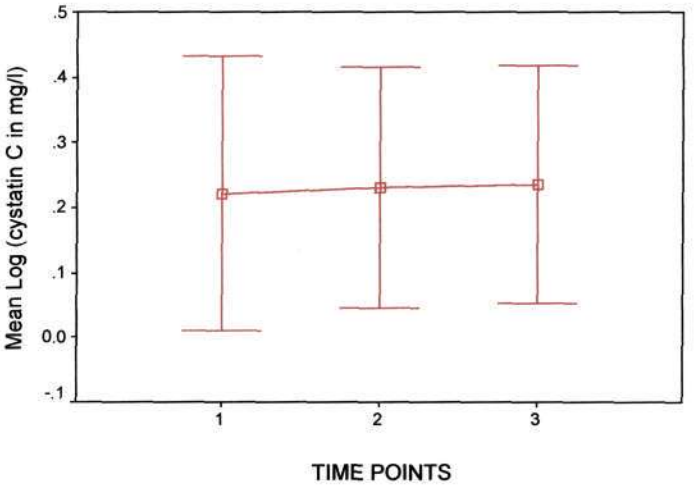


Figure 16. Change in mean log serum cystatin C at 3 sample points in patients undergoing contrast exposure. Error bars are 95% CI

Serum creatinine: The effect of time overall on mean serum creatinine was not significant (Lower-bound F = 0.872, P = 0.361).

For the specific time points there was no significant change in serum creatinine.

Cystatin C: The effect of time overall on mean serum cystatin C was not significant (Lower-bound F = 0.047, P = 0.830).

For the specific time points there was no significant change in serum cystatin C.

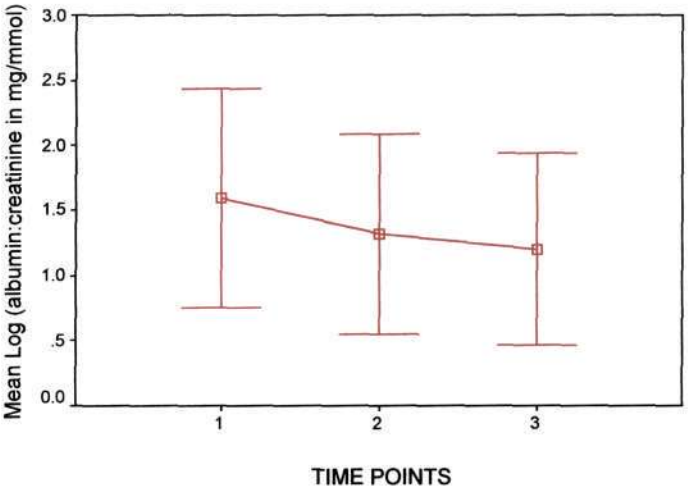


Figure 17. Change in mean urine albumin:creatinine at 3 sample points in patients undergoing exposure to contrast. Error bars are 95% CI

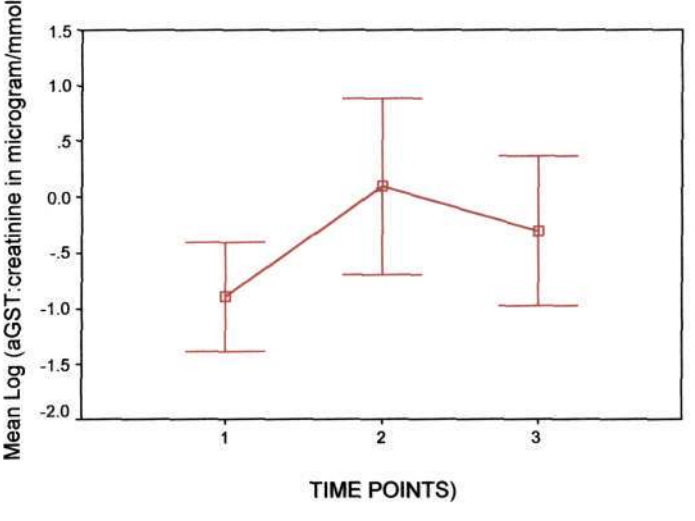


Figure 18. Change in mean log urine aGST:creatinine at 3 sample points in patients undergoing exposure to contrast. Error bars are 95% CI

Urine albumin:creatinine: The effect of time overall on mean urine albumin:creatinine was not significant (Lower-bound F = 1.927, P = 0.181).

For the specific time points, there was a significant difference in mean urine albumin:creatinine at C3 versus baseline (C1) (F = 5.537, P = 0.030) but examination of Figure 11 shows this to be lower than baseline.

Urine αGST:creatinine: The effect of time overall on mean urine serum αGST:creatinine was significant (Lower-bound F = 6.074, P = 0.024).

For the specific time points, there was statistically significant rise at C2 (F = 8.246, P = 0.010).

DISCUSSION

The use of Iohexol, a low-osmolar non-ionic agent, and the low incidence of diabetes in this population may explain the minimal morbidity seen after contrast exposure. From the literature one would have expected a renal failure incidence of 12% (4.4 patients) using a definition of creatinine rise > 44μmol/l for patients with renal impairment (Section 1.2.3).

The use of an intravenous saline infusion to maintain intravascular volume and promote diuresis may have affected the rate of renal impairment but the small sample size again, may be misleading. For purposes of the above analysis, no attempt was made to investigate for predictors of renal dysfunction, and hence the possible bias of the effect of a saline infusion is not entered into.

Urine GST:creatinine appears to be a more sensitive marker for renal injury after intravascular contrast exposure than the other three assays. Contrast therefore has toxic effects on the proximal tubular cell.

Unlike the data for patients undergoing surgery, the two urinary assays do not mirror each other. Urine albumin:creatinine drops significantly after contrast exposure while GST:creatinine rises significantly. Increased recovery of urine albumin can be due to increase glomerular permeability or decreased tubular absorption. It is unlikely that tubular absorption of albumin is enhanced by the exposure to contrast; in fact the significant rise in GST:creatinine suggests otherwise. Exposure may therefore result in

proximal tubular cell injury while its effect on the glomerulus is that of decreased permeability. This has not been described before. Glomerular filtration rate appears not to be affected.

Despite graphic representations suggesting the mean log of cystatin C continues to rise while that of creatinine drops at C3, the mean of the absolute cystatin c value at C3 (1.380 mg/l) is similar to the baseline T1 (1.378 mg/l) (figure 16). The mean of the absolute serum creatinine rose from 129.9 µmol/l (C1) to 134 µmol/l (C2). Intravascular contrast did not seem to have a significant deleterious effect on GFR in this study. In fact reports of a transient rise in renal blood flow are reported.

Further selective studies using urinary proteins of different molecular weight may help to elucidate the effects of contrast on glomerular function.

CHAPTER 6 RESULTS FOR PATIENTS UNDERGOING EXPOSURE TO INTRAVASCULAR CONTRAST AND SUBSEQUENT AORTIC SURGERY

6.1 COMPARISON OF OUTCOMES AFTER INTRAVASCULAR CONTRAST EXPOSURE AND SUBSEQUENT AORTIC SURGERY

INTRODUCTION

Many patients undergo preoperative radiological investigations necessitating the exposure to intravascular contrast. Contrast may precipitate renal failure but the risk with the newer low-osmolar isotonic agents is lower. Nephrotoxicity occurring after contrast exposure may be a marker for post-operative renal dysfunction. We investigated this possibility using serum creatinine as well as alternative markers of renal injury.

METHODS

Eleven patients underwent intravascular contrast exposure (CE) and subsequent aortic surgery (S)

Scatter plots were performed for data obtained after contrast exposure and after initiation of surgery. The following variables were used

- Peak serum creatinine (CE) vs. (S)
- Peak percentage rise in serum creatinine (CE) vs. (S)

- Peak serum cystatin C (CE) vs. (S)
- Peak percentage rise in serum cystatin C (CE) vs. (S)
- Peak urine αGST: creatinine (CE) vs. (S)
- Peak percentage rise in αGST:creatinine (CE) vs. (S)

In addition, for all three assays **Day-1** values after contrast exposure (CE) were compared respectively to the peak absolute and peak percentage rise after surgery (S).

- Creatinine Day-1 (CE) vs. Peak (S)
- Percentage rise creatinine Day-1 (CE) vs. Peak (S)
- Cystatin C Day-1 (CE) vs. Peak (S)
- Percentage rise cystatin C Day-1 vs. Peak (S)
- α-GST Day-1 (CE) vs. Peak (S)
- Percentage rise α-GST Day-1 (CE) vs. Peak (S)

Urine albumin:creatinine levels were shown in section 5.2 not to rise significantly after contrast exposure and hence were not used in this analysis.

A) Data was screened for outliers and then evidence of a linear relationship was sought. Non-parametric correlation using Spearman's rho was calculated.

B) For the variables that showed a linear relationship with significant correlation, the interpretation is that as the variable increases post contrast exposure, so too will it increase after undergoing surgery. Linear regression was used to estimate the coefficients of the linear equation $y = bx + a$. For the appropriate variable, the regression estimate of y

(the value resultant from undergoing surgery) can be obtained from the known value of x (value resultant from exposure to contrast).

The linear regression equation allows estimation of the second value post operation (S) based on the value post contrast exposure (CE).

C) For variables that showed a linear relationship, we investigated the relationship between the values after undergoing surgery and the outcome of dialysis (Mann-Whitney Test). The whole group of patients undergoing surgery was analysed (n = 35, Section 4.1). Only 1 patient had a positive outcome of dialysis in the subgroup of 11 patients and therefore use of this subgroup was unsuitable for statistical analysis.

Receiver operating characteristic (ROC) curves of the values were then plotted to determine the sensitivity and specificity at different cut-off points in predicting renal failure.

This would determine if there was any clinical significance attached to the statistical significance i.e. not only would it be useful to know that a value after contrast can help estimate the value after surgery, but if the latter value is associated with a negative outcome (e.g. dialysis), then knowing the value of a variable after exposure to contrast may help to predict the likelihood of needing dialysis after surgery.

RESULTS

Linear relationships were found between values after contrast (CE) and after surgery (S) for the following variables

- Peak serum creatinine after contrast and after surgery
- Day-1 serum creatinine after contrast and peak serum creatinine after surgery
- Peak serum cystatin C after contrast and after surgery
- Day-1 serum cystatin C after contrast and peak serum cystatin C after surgery

Urine αGST:creatinine showed no association between the two groups (neither peak/peak % rise nor Day-1/ Day-1 % rise). All correlation coefficients were less than 0.3. Exclusion of Patient 3, who was found to be an outlier, did not significantly improve the correlation. No further analysis was performed on this subgroup.

Inspection revealed Patient 1 to be an outlier for both serum creatinine and serum cystatin C measurement. The paired data were excluded from the correlation and regression analysis.

The results are presented first for:

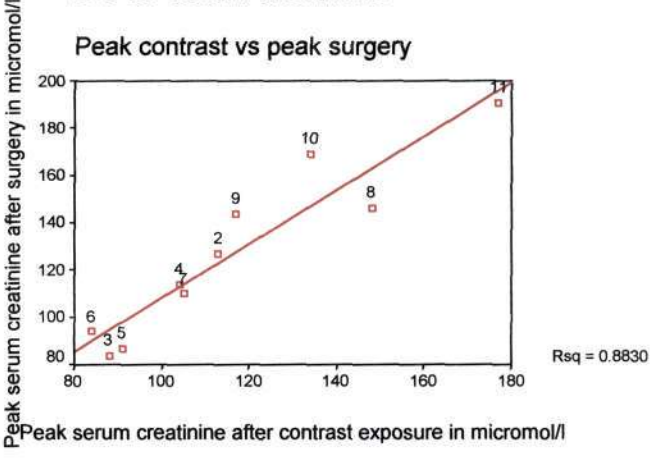
serum creatinine and

serum cystatin C.

Serum Creatinine:

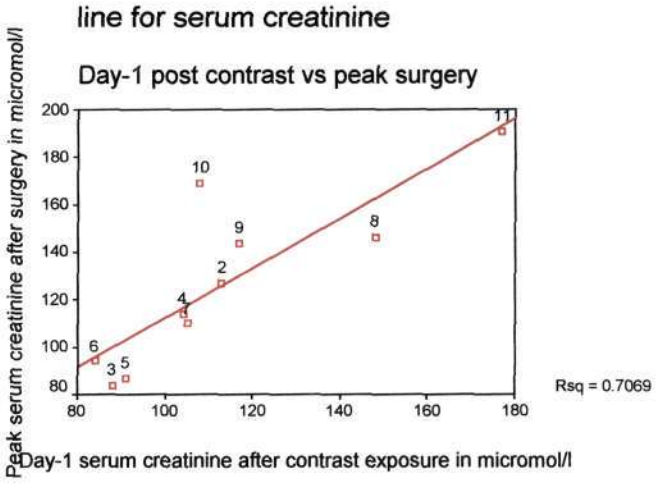
A)

Figure 19. Scatter Plot and regression line for serum creatinine



SPEARMAN'S RHO (PEAK CREATININE)	
Correlation coefficient	0.939
Sig. (2-tailed)	0.000
N	10
Correlation is significant at the 0.01 level (2-tailed).	

Figure 20. Scatter plot and regression line for serum creatinine



SPEARMAN'S RHO (D-1 vs. PEAK)	
Correlation coefficient	0.879
Sig. (2-tailed)	.001
N	10
Correlation is significant at the 0.01 level (2-tailed).	

Peak serum creatinine after contrast exposure yields a slightly higher correlation coefficient with peak serum creatinine after surgery (rho = 0.939), compared to Day-1 serum creatinine after contrast exposure (rho - 0.879). The former was used for the linear regression calculation.

B)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-6.093	17.560		-.347	.738	-46.587	34.400
	Peak serum creatinine after contrast	1.143	.147	.940	7.771	.000	.804	1.482

a. Dependent Variable: Peak serum creatinine after surgery

The equation for the regression line is therefore

$$Y = 1.143\,X - 6.093$$

Where Y is the estimated peak serum creatinine after surgery

X is the peak serum creatinine after contrast.

C) The Mann-Whitney test showed a significantly higher serum creatinine in patients requiring dialysis (366 μmol/l) than those who did not (117 μmol/l).

Ranks

	DIALYSIS	N	Mean Rank	Sum of Ranks
Peak serum creatinine after surgery	0	30	15.60	468.00
	1	5	32.40	162.00
	Total	35		

Test Statistics[b]

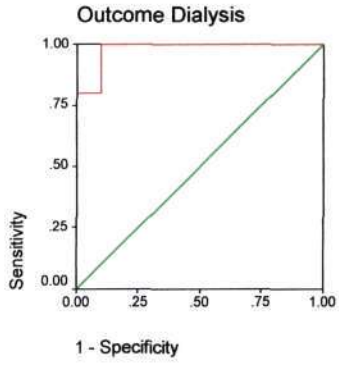
	Peak serum creatinine after surgery
Mann-Whitney U	3.000
Wilcoxon W	468.000
Z	-3.395
Asymp. Sig. (2-tailed)	.001
Exact Sig. [2*(1-tailed Sig.)]	.000[a]

a. Not corrected for ties.

b. Grouping Variable: DIALYSIS

ROC curves were then constructed.

Figure 21. ROC Curve. Peak serum creatinine after surgery

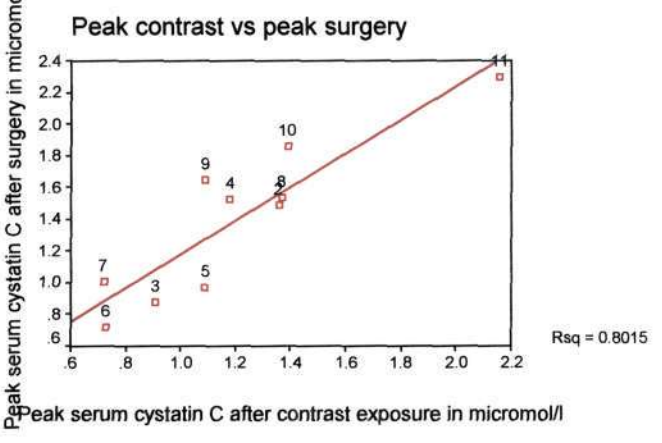


The area under the curve was significant and a peak serum creatinine >= 162μmol/l had a sensitivity of 100% and specificity 0f 90% (See Appendix J).

Serum cystatin C:

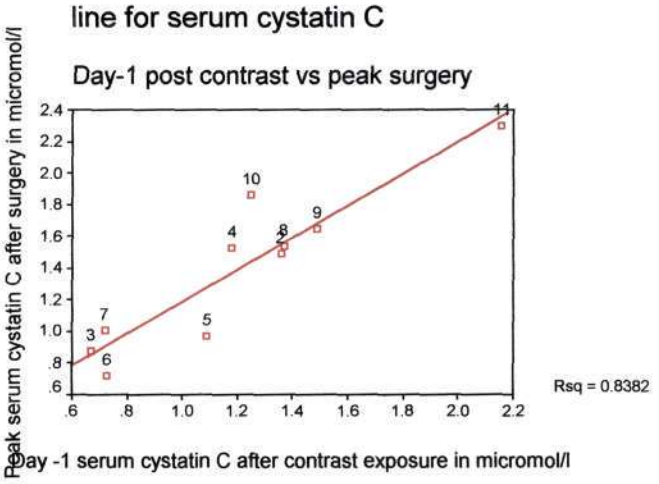
A)

Figure 22. Scatter plot and regression line for serum cystatin C



SPEARMAN'S RHO (PEAK CYSTATIN C)	
Correlation coefficient	0.815
Sig. (2-tailed)	0.004
N	10
Correlation is significant at the 0.01 level (2-tailed).	

Figure 23. Scatter plot and regression line for serum cystatin C



SPEARMAN'S RHO (PEAK CREATININE)	
Correlation coefficient	0.842
Sig. (2-tailed)	0.002
N	10
Correlation is significant at the 0.01 level (2-tailed).	

Day-1 serum cystatin after contrast exposure yields a slightly higher correlation coefficient with peak serum cystatin C after surgery (rho = 0.842), compared with peak serum cystatin C after contrast exposure (rho = 0.815). Serum cystatin C is not a routine clinical test. We chose the Day-1 sample for the linear regression analysis for practical purposes.

B)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	.181	.200		.907	.391	-.280	.642
	Day-1 serum cystatin c after contrast	1.010	.157	.916	6.438	.000	.648	1.371

a. Dependent Variable: Peak serum cystatin C after surgery

The equation for the regression line is therefore

$$Y = 1.010\,X + 0.181$$

Where Y is the estimated peak serum cystatin C after surgery

X is the Day-1 serum cystatin C after contrast

C) The Mann-Whitney showed a significantly higher serum cystatin C in patients requiring dialysis (2.93 mg/l) than those who did not (1.22 mg/l).

Ranks

	DIALYSIS	N	Mean Rank	Sum of Ranks
Peak serum cystatin C after surgery	0	30	15.78	473.50
	1	5	31.30	156.50
	Total	35		

Test Statistics[b]

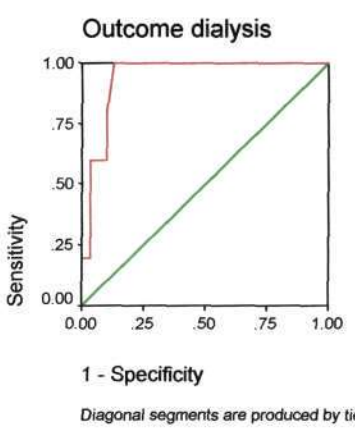
	Peak serum cystatin C after surgery
Mann-Whitney U	8.500
Wilcoxon W	473.500
Z	-3.136
Asymp. Sig. (2-tailed)	.002
Exact Sig. [2*(1-tailed Sig.)]	.000[a]

a. Not corrected for ties.

b. Grouping Variable: DIALYSIS

ROC curves were constructed.

Figure 24. ROC Curve. Peak serum cystatin C after surgery



The area under the curve was significant and a peak serum cystatin C > 1.67 mg/l had a sensitivity of 100% and a specificity of 86.7% in predicting dialysis (see Appendix J).

DISCUSSION

We showed that a significant linear association exists between peak or Day-1 values (CE) and the peak value after surgery (S) for both serum creatinine and cystatin C.

This relationship is important because the values at (S) show a significant association with the need for dialysis. It is not unexpected, because the initiation of dialysis is a clinical decision based, amongst other factors, on the serum creatinine. We have confirmed this statistically using the Mann-Whitney tests.

Day-1 cystatin C was chosen over peak cystatin C for the linear regression. Unlike creatinine, Cystatin C is not routinely performed in all laboratories and this would allow for a more repeatable sampling point.

Serum creatinine and serum cystatin C appear equally useful in predicting post-operative assay changes (S) based on the findings after contrast exposure (CE). The coefficients of the regression equations are both close to 1 and therefore one can expect a similar magnitude of rise after contrast and surgery.

The confidence intervals for the coefficients of the regression equation are wide. A larger sample size may have improved this.

SUMMARY

This study was conducted with the aim of evaluating the role of alternative markers of renal dysfunction in vascular surgical patients.

That renal dysfunction is indeed a significant clinical problem in vascular surgery, has been outlined in the earlier chapters of this report. Because of the well-described problems associated with the use of serum creatinine as a marker of renal failure, there has been a move toward finding better assays to diagnose and predict nephropathy.

David Newman was involved in much of the work in developing and validating the cystatin C assay. Urine α-GST was investigated early on for its use in predicting viability of donor kidneys. It has been also used to investigate the toxic effect of certain anaesthetic agents on the kidney and more recently in patients undergoing aortic surgery.

We have shown that cystatin C appears to be a better marker for diagnosing renal impairment than serum creatinine. Its apparent improvement over serum creatinine in detecting more patients with subsequent deterioration in renal function is unexpected given the generous definitions of renal dysfunction using a rise in serum creatinine.

The surgical insult is initially not associated with deterioration in GFR; in fact serum creatinine suggests an improvement in GFR before deterioration on Day-1. Intra-operative intensive anaesthetic management may explain this phenomenon. The

cumulative effects of anaesthesia, ischaemia and reperfusion appear to have maximal impact on GFR on Day-1.

The present study clearly demonstrates that urinary markers are more sensitive in detecting renal injury. This is true when one compares changes over time. We have also shown that true renal ischaemia results in much higher recovery of α-GST in the urine compared to infra-renal surgery. This is in keeping with reports of the S_3 segment of the renal tubule being the most sensitive to ischaemia.

Intravascular contrast was unimpressive in the induction of renal injury. Aggressive medical management and the use of newer contrast agents have decreased the incidence of renal dysfunction. The interesting outcome was non-concordance of the GST and albumin recovery in the urine. Intense vasoconstriction is described after contrast exposure and this leads to hypoxic damage to the nephron. This would explain the increased recovery of α-GST in the urine. The concomitant fall in urine albumin is unlikely to be due to increased tubular resorption of albumin. Our data suggest that glomerular permeability is decreased while the markers of worsening GFR are not markedly raised. Further study is needed before firm conclusions can be drawn.

A clinically important issue is to be able to predict patients that will develop subsequent renal dysfunction after intervention. Cystatin C cut-off values are marginally better than serum creatinine in predicting renal dysfunction after surgery.

It would be expected that there would be a relationship between response to contrast and response to surgery. We have shown a strong correlation between the two responses. Regression coefficients for both serum creatinine and serum cystatin C were calculated but they were both close to unity suggesting that the insult of contrast exposure is similar in renal terms to that of surgery. Surgical intervention would be expected to be more invasive and should cause a higher peak serum creatinine value. Our data suggest that either this is not so or that deterioration in renal function is determined by inherent patient factors modified by external factors. However, we were unable to show any difference in demographic data and negative outcomes.

CONCLUSIONS

1. Cystatin C is more sensitive than serum creatinine in detecting pre-existing and subsequent renal impairment in patients undergoing aortic vascular surgery.
2. Increased glomerular permeability and proximal renal tubular cell damage almost occur immediately after the onset of surgery, whereas significant deterioration glomerular filtration rate (GFR) only occurs at day-1 post-operation.
3. Low-osmolar non-ionic contrast exposure appears to cause proximal tubular cell damage without significant deterioration in GFR. Glomerular permeability appears to be decreased.
4. A supra-renal clamp (true renal ischaemia), when compared to an infra-renal clamp, appears to cause significantly more proximal tubular cell damage. Similar deterioration in GFR and glomerular permeability is seen.
5. Cystatin C appears to have better sensitivity and specificity for predicting the need for dialysis.
6. After contrast exposure, peak and day-1 serum values of cystatin C and creatinine show good correlation with the peak value post aortic surgery. The magnitude of response after contrast exposure is predictive of the response after undergoing aortic surgery.

COMMENTS

This study was conducted primarily to evaluate the value of alternative diagnostic tests and hence is littered with statistical methods and calculations. For maximum strength of the significance of the positive results a larger sample size would be needed.

It is likely that this has led to a type II error in some of the calculations i.e. not showing a statistically significant difference because of small sample size, when in fact one exists.

Lastly, interpretation of the results needs to be placed into clinical context. Although renal failure is a major problem in patients undergoing vascular surgery, the present study has demonstrated biochemical changes that are important in understanding mechanisms of renal damage. Not all patients with changes in biochemical markers developed clinically overt renal dysfunction. The earlier changes that were observed may well pre-empt significant later dysfunction, but minor changes may be transient and benign.

APPENDIX

APPENDIX A

COCKCROFT-GAULT EQUATION

Creatinine Clearance = 1.23 x (140 – age) x weight / serum creatinine

x 0.85, if female

APPENDIX B

CONSENT FORMS:

KENSINGTON & CHELSEA AND WESTMINSTER HEALTH AUTHORITY
ST MARY'S LOCAL RESEARCH ETHICS COMMITTEE

CONSENT FORM

AGREEMENT TO PARTICIPATE IN RESEARCH PROJECT

I, (name of subject) .....

Of (address) .....

Agree to take part (or agree that my child/ward may take part in the research project:

Risk assessment for renal injury post aortic surgery using new and more sensitive markers of renal injury.

I confirm that the nature and demands of the research have been explained to me and I understand and accept them. I understand that my consent is entirely voluntary and that I may withdraw from the research project if I find that I am unable to continue for any reason and this will not affect my medical care.

Signed: ..... Print Name: .....

Witness: ..... Print Name: .....

Date: .....

Investigator's Statement:

I have explained the nature, demands and foreseeable risks of the above research to the subject:

Signature: ..... Date: .....

2000/2001

IMPERIAL COLLEGE SCHOOL OF MEDICAL AT ST MARYS' HOSPITAL

AGREEMENT FOR THE DONATION OF BLOOD OR TISSUE SAMPLES

I, (Name of subject) .....

of (Address) .....

.....

agree to donate any blood or tissue samples taken during the course of my treatment under the direction of:

(Name of consultant): .....

and assign all right, title and interest in such samples to ICSM at St Mary's, London W2.

I confirm that it has been explained to me that the samples may also be used for research or teaching purposes and that components of the samples may be used to develop commercial diagnostic or therapeutic agents. I understand that, should I not wish my samples to be used for these purposes I am free not to sign this form, and that such a decision would have no adverse effect on my care.

Signed: ..... Date:

Witness: .... Date:

(Name) .....

Investigator's statement

I have explained to the subject the clinical reasons for taking samples, their possible uses, the assignment of ownership and that if the subject did not wish to donate his/her samples this would have no effect on his/her clinical care.

Signed: ..... Date: .....

(Name)

TO BE HELD IN PATIENT'S NOTES OR RETAINED BY THE INVESTIGATOR

APPENDIX C

COPY OF INFORMATION SHEET USED FOR THIS STUDY

INFORMATION FOR VOLUNTEERS

Dear Mr./ Mrs./Miss.....

Would you consider helping us in our study on kidney failure in patients with diseases of the arteries? The following information leaflet explains the details of the study. Please contact me should you have any questions.

Research project title

Risk assessment for renal injury post aortic surgery using new and more sensitive markers of renal injury.

What is the purpose of the study?

Ballooning or dilatation of the aorta is called an aortic aneurysm. As the aorta enlarges further the risk of the aorta bursting (rupture) is higher. In order to avoid this patients are offered an operation to replace the abnormal aorta with a man-made tube (prosthetic graft). In patients who have narrowing or blockage of the aorta a similar operation is done to increase the blood flow to the legs. In order to do this you will undergo X-rays (either a CAT scan or an angiogram) and then an operation under general anaesthetic. One of the risks of the operation is kidney failure, which occurs partly because the kidneys get their blood supply from the aorta near the aneurysm. This risk depends on many factors including whether you have kidney problems at the moment and how extensive the aneurysm is. The contrast (dye) given to outline the arteries during the X-ray may also contribute this.

We wish to study all the patients who have aortic surgery to see why some patients get kidney failure and others do not. We shall do this using new and better tests of kidney function.

In this way we can learn if there are future changes we can make to reduce the risk of kidney damage.

We also plan to study patients that have pain in their legs because of reduced blood flow. This is due to narrowed or blocked arteries in their legs and they undergo bypass operations in their legs. If this is your problem then the risk of kidney failure is much lower but we need to study you as well to compare your results with the other patients. You will serve as a comparison with the other patients. When doing a study it is a good idea to do this to avoid making false conclusions about the results.

What will I have to do?

If you consider helping us we will answer any questions you may have and ask you to sign a consent form. Your treatment during your hospital stay will not be different to those who choose not to take part in the study.

The study involves collecting urine and blood samples from you before, during and after the operation. This will be done when you have an injection to take bloods for other ward test to avoid you any discomfort.

The results of these tests will be studied to see if we can find out who gets kidney failure and why.

What if I do not want to participate?

We emphasise that participation in the study is voluntary. The tests do not form part of your routine care. You are free to decline. You will remain free to withdraw from the study at any stage without any further consequence or obligation. This will not affect your care in this hospital.

What are the risks?

Research studies may involve some risk, but in this study we do not anticipate any. No part of your care will be changed because you are part of the study.

What are the benefits?

It is unlikely that this study will benefit you as an individual. However the tests will provide information that may benefit patients that need to have the same operation in the future.

How much will it cost?

The tests are expensive but we will meet the cost. We have applied for a research grant. There will be NO additional expense to you.

What will be done with the results?

All the information gathered would be strictly confidential. Only the investigators will have access to the study information. Once all the information is gathered the results of the study will be published in a scientific research journals. At no stage will your personal details be given out.

Who can I contact for further information?

If you have any questions about this study, you may contact Mr. Pillay (0207 886 6188) or any member of the vascular team looking after you at St. Mary's Hospital.

APPENDIX D

PATIENT/VOLUNTEER CONSENT CHECKLIST

The participant or key carer should complete the whole of this sheet himself/herself.
(please cross out as necessary)

Have you been asked to consent for yourself or on behalf of someone else? Self/Other

If your answer to the above is "other" please give the name of the person form whom you are consenting:
.....

Have you read the Information sheet for patients and healthy volunteers? YES / NO

Have you had an opportunity to ask questions and discuss this study? YES / NO

Have you received satisfactory answers to all of your questions? YES / NO

Have you received enough information about the study? YES / NO

Have you been told whether you would be entitled to reimbursement of travel expenses? YES / NO

Who have you spoken to? Dr / Mrs / Ms / Mr .....

Do you understand that your decision to consent is entirely voluntary and that you are free to withdraw from the study at any time, without having to give a reason for withdrawing and without affecting your future medical care? YES / NO

Do you agree to take part in this study? YES / NO

Signed: ..... Date: .....

NAME IN BLOCK LETTERS: .....

2000/2001

CONSENT CHECKLIST FOR INVESTIGATORS

1. Have you given the Patient Information Sheet to the subject? YES / NO
2. Have you given an oral explanation to the subject, including:
   * this is a research project: YES / NO
   * participation is voluntary: YES / NO
   * the aims of the project: YES / NO
   * the likely duration of the subject's involvement: YES / NO
   * the expected benefits to the subject and/or others: YES / NO
   * the expected nature of the drug or device being tested: YES / NO
   * that the subject may instead, receive a reference treatment or placebo: YES / NO
   * what risks, inconvenience, discomfort of distress may reasonably be anticipated for this patient: YES / NO
   * that a refusal to participate may be given without reasons and will not affect the care which will be given to the subject: YES / NO
   * that personal information may be scrutinised during audit by competent authorities and properly authorised people, but all personal information will be treated as strictly confidential and will not be made publicly available: YES / NO
   * what compensation arrangements are available: YES / NO
   * whom to contact in an emergency and how: YES / NO

3. Have you asked the subject:
   - for authorisation to approach his/her GP and for permission for
   - the GP to disclose medical information? YES / NO
   * to tell you if he/she is or has been involved in any other research studies: YES / NO
   * to tell you if he/she is or has recently been taking any other medication or preparations? YES / NO

4. If you have answers NO or not answered any of the above questions in section 2 or 3, record why:

5. Have you allowed the subject sufficient time to consider the matter on his/her own, discuss with others if wishes, or ask you questions? YES / NO

6. In your opinion, has the subject understood and consented

APPENDIX E

DATA ENTRY FORM FOR RENAL FAILURE STUDY.

surname
name
dob
age
sex
hosp no
height
weight

diagnosis
type
outcome

alive
dot
died in hosp: day

diabetes
hypertension
ihd
cva
hyperlipidaemia
renal artery stenosis
renal split function

anaesthetic agent
heparin dose
protamine
trasolol

renal ischaemia time
lower limb ischaemia time
surgical time
anaesthetic time
dye time

cardio pulmonary bypass
left heart bypass
renal perfusion
left renal patch
renal jump grafts
blood loss
packed cells transfused
cell saved blood transfused

peak vancomicin level
renal support
peak creatinine
days in icu
days in hosp

ct scan	date
	access
	contrast
	volume

angiogram	date
	access
	contrast
	volume

CT SCAN 1:

1. preradiology	serum creatinine
	serum urea
	serum cystatin
	LFT
	urine GST
	urine albumin
	urine creatinine
2. day 1	serum creatinine
	serum urea
	serum cystatin
	LFT
	urine GST
	urine albumin
	urine creatinine
3. day 7	serum creatinine
	serum urea
	serum cystatin
	urine GST
	urine albumin
	urine creatinine
4. day 14	serum creatinine
	serum urea
	serum cystatin
	urine GST
	urine albumin
	urine creatinine

ANGIOGRAM 2:

1. preradiology	serum creatinine
	serum urea
	serum cystatin
	LFT
	urine GST
	urine albumin
	urine creatinine

2. day 1	serum creatinine serum urea serum cystatin LFT urine GST urine albumin urine creatinine
3. day 7	serum creatinine serum urea serum cystatin urine GST urine albumin urine creatinine
4. day 14	serum creatinine serum urea serum cystatin urine GST urine albumin urine creatinine
SURGERY	
1. preoperation	serum creatinine serum urea serum cystatin c urine GST urine albumin urine creatinine
2. pre clamp	serum creatinine serum urea serum cystatin c urine GST urine albumin urine creatinine
3. pre clamp removal serum creatinine	serum urea serum cystatin c urine GST urine albumin urine creatinine
4. 2hrs post clamp removal serum creatinine	serum urea serum cystatin c urine GST urine albumin urine creatinine
5. day 1	serum creatinine serum urea

	serum cystatin c
	LFT
	urine GST
	urine albumin
	urine creatinine
6. day 4	serum creatinine
	serum urea
	serum cystatin c
	urine GST
	urine albumin
	urine creatinine
7. day 7	serum creatinine
	serum urea
	serum cystatin c
	urine GST
	urine albumin
	urine creatinine

APPENDIX F

Data for patients undergoing surgery (n= 35). Coordinate points for the ROC Curve for baseline serum creatinine and cystatin C * baseline GFR < 72 ml/min

Area Under the Curve baseline serum creatinine * outcome GFR < 72ml/min

Test Result Variable(s): s1creats

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.917	.047	.000	.824	1.009

The test result variable(s): s1creats has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve baseline serum creatinine in micromol/l *outcome GFR < 72 ml/min

Test Result Variable(s): s1creats

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
62.0000	1.000	1.000
66.0000	1.000	.889
71.5000	.962	.889
76.5000	.962	.667
82.0000	.962	.556
86.5000	.923	.556
88.5000	.923	.444
90.0000	.923	.333
91.5000	.923	.222
93.0000	.885	.222
94.5000	.808	.222
96.5000	.808	.111
99.0000	.769	.000
100.5000	.731	.000
101.5000	.692	.000
103.0000	.654	.000
106.0000	.615	.000
108.5000	.538	.000
109.5000	.500	.000
111.0000	.462	.000
114.0000	.423	.000
116.5000	.346	.000
117.5000	.308	.000
120.5000	.269	.000
128.5000	.231	.000
138.0000	.192	.000
142.5000	.154	.000
153.5000	.115	.000
179.0000	.077	.000
250.5000	.038	.000
308.0000	.000	.000

The test result variable(s): s1creats has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve baseline serum cystatin C * outcome GFR < 72 ml/min

Test Result Variable(s): s1cystc

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.808	.074	.007	.662	.953

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve baseline serum creatinine in micromol/l * Outcome GFR < 72 ml/min

Test Result Variable(s): s1cystc

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
-.8300	1.000	1.000
.2150	.962	1.000
.2900	.962	.889
.4600	.923	.889
.6500	.923	.778
.7050	.885	.778
.7350	.846	.778
.7650	.808	.778
.7800	.769	.778
.8000	.769	.667
.8150	.769	.556
.8550	.769	.444
.8950	.769	.333
.9350	.769	.222
.9800	.769	.000
1.0100	.692	.000
1.0350	.654	.000
1.0500	.615	.000
1.1000	.577	.000
1.1450	.538	.000
1.1700	.462	.000
1.2100	.423	.000
1.2500	.385	.000
1.2950	.346	.000
1.3350	.308	.000
1.4350	.269	.000
1.5550	.231	.000
1.6500	.192	.000
1.9650	.077	.000
2.4800	.038	.000
3.7400	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

APPENDIX G

Data for patients undergoing surgery (n= 35). 2x2 Tables for baseline variables * outcomes (For each variable 0 = no, 1 = yes)

Baseline serum creatinine>120 micromol/l * Creatinine rise>25% Crosstabulation

Count

		serum creatinine rise >25%		
		0	1	Total
Baseline serum creatinine>120 micromol/l	0	19	9	28
	1	2	5	7
Total		21	14	35

Baseline serum creatinine > 120 micromol/l * serum creatinine rise > 44micromol/l Crosstabulation

Count

		serum creatinine rise > 44 micromol/l		
		0	1	Total
Baseline serum creatinine > 120 micromol/l	0	23	5	28
	1	2	5	7
Total		25	10	35

Baseline serum creatinine > 120 micromol/l * serum cystatin C rise > 37% Crosstabulation

Count

		Serum cystatin C rise > 37%		
		0	1	Total
Baseline serum creatinine > 120 micromol/l	0	20	8	28
	1	2	5	7
Total		22	13	35

Baseline serum creatinine > 120 micromol/l * Dialysis Crosstabulation

Count

		DIALYSIS		Total
		0	1	
Baseline serum creatinine > 120 micromol/l	0	26	2	28
	1	4	3	7
Total		30	5	35

Baseline serum cystatin C > 1.24 mg/l * serum creatinine rise > 25% Crosstabulation

Count

		Serum creatinine rise >25%		Total
		0	1	
Baseline serum cystatin C > 1.24 mg/l	0	17	8	25
	1	4	6	10
Total		21	14	35

Baseline serum cystatin C > 1.24 mg/l * serum creatinine rise > 44 micromol/l Crosstabulation

Count

		serum creatinine rise > 44 micromol/l		Total
		0	1	
Baseline serum cystatin C > 1.24 mg/l	0	20	5	25
	1	5	5	10
Total		25	10	35

Baseline serum cystatin C > 1.24 mg/l * serum cystatin C rise > 37% Crosstabulation

Count

		Serum cystatin C rise > 37%		Total
		0	1	
Baseline serum cystatin C > 1.24 mg/l	0	17	8	25
	1	5	5	10
Total		22	13	35

Baseline serum cystatin C > 1.24 mg/l * Dialysis Crosstabulation

Count

		DIALYSIS		
		0	1	Total
Baseline serum cystatin C > 1.24 mg/l	0	24	1	25
	1	6	4	10
Total		30	5	35

APPENDIX H

Data for patients undergoing surgery (n=35). Coordinate points for ROC Curve baseline calculated creatinine clearance/ serum creatinine/ serum cystatin C * outcome dialysis

Area Under the Curve Baseline Creatinine clearance

Test Result Variable(s): ccrs1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.913	.059	.003	.797	1.030

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve for Baseline Creatinine clearance in ml/mim * Outcome Dialysis

Test Result Variable(s): ccrs1

Positive if Less Than or Equal To[a]	Sensitivity	1 - Specificity
18.4024	.000	.000
21.3912	.200	.000
23.6868	.400	.000
28.2448	.600	.000
36.0606	.600	.033
39.7269	.600	.067
40.1686	.600	.100
40.5362	.600	.133
42.0585	.600	.167
43.7327	.800	.167
44.4718	.800	.200
45.6342	.800	.233
46.6168	.800	.267
48.5238	1.000	.267
50.7367	1.000	.300
52.2676	1.000	.333
54.6201	1.000	.367
57.3911	1.000	.400
59.8273	1.000	.433
61.6824	1.000	.467
62.5625	1.000	.500
63.0073	1.000	.533
63.9067	1.000	.567
64.6982	1.000	.600
67.0998	1.000	.633
69.8917	1.000	.667
72.1704	1.000	.700
77.9573	1.000	.733
85.9649	1.000	.767
91.2024	1.000	.800
93.4325	1.000	.833
95.2586	1.000	.867
97.3386	1.000	.900
107.7217	1.000	.933
126.5133	1.000	.967
137.1380	1.000	1.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve Baseline Serum Creatinine

Test Result Variable(s): s1creats

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.853	.086	.012	.686	1.021

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve for Baseline serum Creatinine in micromol/l * Outcome Dialysis

Test Result Variable(s): s1creats

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
62.0000	1.000	1.000
66.0000	1.000	.967
71.5000	1.000	.933
76.5000	1.000	.867
82.0000	1.000	.833
86.5000	1.000	.800
88.5000	1.000	.767
90.0000	1.000	.733
91.5000	1.000	.700
93.0000	1.000	.667
94.5000	1.000	.600
96.5000	1.000	.567
99.0000	1.000	.500
100.5000	1.000	.467
101.5000	.800	.467
103.0000	.800	.433
106.0000	.800	.400
108.5000	.800	.333
109.5000	.800	.300
111.0000	.800	.267
114.0000	.800	.233
116.5000	.800	.167
117.5000	.800	.133
120.5000	.600	.133
128.5000	.600	.100
138.0000	.600	.067
142.5000	.400	.067
153.5000	.200	.067
179.0000	.200	.033
250.5000	.200	.000
308.0000	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve Baseline Serum Cystatin C

Test Result Variable(s): s1cystc

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.773	.149	.053	.482	1.065

The test result variable(s): s1cystc has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve for Baseline serum cystatin C in mg/l * Outcome Dialysis

Test Result Variable(s): s1cystc

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
-.8300	1.000	1.000
.2150	1.000	.967
.2900	1.000	.933
.4600	1.000	.900
.6500	1.000	.867
.7050	.800	.867
.7350	.800	.833
.7650	.800	.800
.7800	.800	.767
.8000	.800	.733
.8150	.800	.700
.8550	.800	.667
.8950	.800	.633
.9350	.800	.600
.9800	.800	.533
1.0100	.800	.467
1.0350	.800	.433
1.0500	.800	.400
1.1000	.800	.367
1.1450	.800	.333
1.1700	.800	.267
1.2100	.800	.233
1.2500	.800	.200
1.2950	.800	.167
1.3350	.800	.133
1.4350	.600	.133
1.5550	.600	.100
1.6500	.600	.067
1.9650	.200	.033
2.4800	.000	.033
3.7400	.000	.000

The test result variable(s): s1cystc has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve Baseline Urine Albumin:Creatinine

Test Result Variable(s): s1albc

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.621	.130	.395	.366	.875

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the curve for Baseline Urine Albumin:creatinine in mg/mmol * Outcome Dialysis

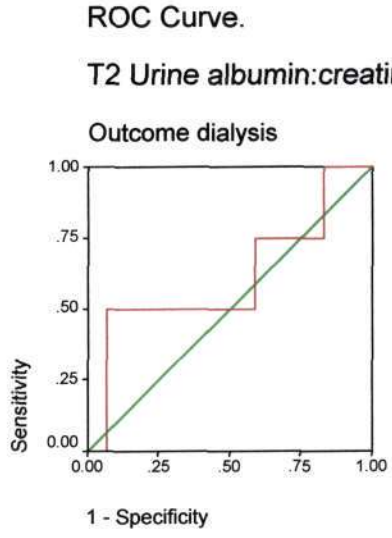
Test Result Variable(s): s1albc

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
-.8647	1.000	1.000
.4020	1.000	.966
.7102	1.000	.931
.7768	1.000	.897
.8340	1.000	.862
.9322	1.000	.828
1.1178	1.000	.793
1.3524	.800	.793
1.5358	.800	.759
1.6080	.800	.724
1.8802	.800	.690
2.2681	.800	.655
2.6163	.800	.621
2.9283	.800	.586
3.1859	.800	.552
3.4121	.800	.517
3.5833	.800	.483
3.9701	.800	.448
4.4029	.600	.448
4.7815	.600	.414
5.1002	.600	.379
5.3645	.600	.345
5.6132	.400	.345
5.8818	.400	.310
6.2756	.200	.310
7.6633	.200	.276
12.3248	.200	.241
19.4817	.200	.207
23.3031	.200	.172
25.4299	.200	.138
32.2149	.200	.103
38.3971	.200	.069
68.2535	.200	.034
197.2848	.200	.000
298.8304	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

APPENDIX I

Data for patients undergoing surgery (n=35). Coordinate points for ROC Curves for T2 urine albumin:creatinine and αGST:creatinine * outcome dialysis



Area Under the Curve for T2 Urine albumin:creatinine

Test Result Variable(s): s2albc

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.612	.173	.473	.273	.951

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve for T2 Urine albumin:creatinine in mg/mmol

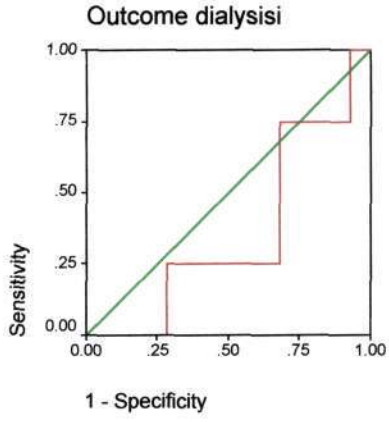
Test Result Variable(s): s2albc

Positive if *Greater Than* or Equal To[a]	Sensitivity	1 - Specificity
.0384	1.000	1.000
1.8587	1.000	.966
2.9656	1.000	.931
4.5658	1.000	.897
6.5672	1.000	.862
8.2167	1.000	.828
9.3776	.750	.828
10.5082	.750	.793
11.4942	.750	.759
12.2000	.750	.724
12.9090	.750	.690
13.6852	.750	.655
16.7857	.750	.621
19.8887	.750	.586
20.7389	.500	.586
22.3592	.500	.552
24.4936	.500	.517
26.0393	.500	.483
27.2825	.500	.448
28.3413	.500	.414
31.7082	.500	.379
35.6879	.500	.345
39.5968	.500	.310
50.3108	.500	.276
59.6446	.500	.241
62.2986	.500	.207
63.8280	.500	.172
66.5074	.500	.138
79.4903	.500	.103
127.2901	.500	.069
235.4089	.250	.069
527.3656	.000	.069
1359.1759	.000	.034
1970.7260	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

ROC Curve.

T2 Urine GST:creatinine



Area Under the Curve for T2 urine GST:creatinine

Test Result Variable(s): s2gstc

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.357	.133	.362	9.615E-02	.618

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve for T2 urine GST:creatinine in microgram/mmol

Test Result Variable(s): s2gstc

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
-.8578	1.000	1.000
.2702	1.000	.964
.4122	1.000	.929
.4316	.750	.929
.4675	.750	.893
.5041	.750	.857
.5102	.750	.821
.5247	.750	.786
.5770	.750	.750
.6508	.750	.714
.7644	.750	.679
.8462	.500	.679
.8661	.250	.679
.9821	.250	.643
1.1009	.250	.607
1.1380	.250	.571
1.1911	.250	.536
1.2882	.250	.500
1.5127	.250	.464
1.7756	.250	.429
1.9167	.250	.393
2.0269	.250	.357
2.1405	.250	.321
2.6540	.250	.286
3.3421	.000	.286
3.9297	.000	.250
5.0167	.000	.214
6.0049	.000	.179
6.4488	.000	.143
7.4006	.000	.107
10.1278	.000	.071
16.3124	.000	.036
21.5479	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

APPENDIX J

Data for patients undergoing surgery (n= 35). Coordinate points for RIC Curves for peak serum creatinine/ peak cystatin C post op * outcome dialysis

Area Under the Curve Peak serum creatinine * outcome dialysis

Test Result Variable(s): peak scr

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.980	.023	.001	.935	1.025

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve Peak serum creatinine (S) in micromol/l

Test Result Variable(s): peak scr

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
77.0000	1.000	1.000
81.0000	1.000	.967
85.5000	1.000	.933
88.0000	1.000	.900
90.5000	1.000	.867
93.0000	1.000	.833
96.0000	1.000	.767
98.5000	1.000	.733
102.5000	1.000	.700
107.0000	1.000	.667
108.5000	1.000	.633
109.5000	1.000	.600
112.0000	1.000	.567
115.0000	1.000	.533
117.0000	1.000	.500
119.0000	1.000	.467
120.5000	1.000	.433
122.5000	1.000	.400
125.5000	1.000	.367
128.5000	1.000	.333
130.5000	1.000	.300
137.5000	1.000	.267
145.0000	1.000	.233
149.0000	1.000	.200
153.5000	1.000	.167
162.0000	1.000	.100
180.0000	.800	.100
231.5000	.800	.033
287.5000	.800	.000
334.5000	.600	.000
397.5000	.400	.000
447.0000	.200	.000
466.0000	.000	.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Area Under the Curve Peak serum cystatin C * outcome dialysis

Test Result Variable(s): Peak S Cyst C

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.943	.039	.002	.867	1.020

The test result variable(s): Peak S Cyst C has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Coordinates of the Curve Peak serum cystatin C in mg/l

Test Result Variable(s): Peak S Cyst C

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
-.2800	1.000	1.000
.7900	1.000	.967
.8700	1.000	.933
.9050	1.000	.867
.9500	1.000	.833
.9900	1.000	.800
1.0150	1.000	.733
1.0300	1.000	.700
1.0500	1.000	.667
1.0950	1.000	.633
1.1450	1.000	.600
1.1800	1.000	.567
1.2050	1.000	.533
1.2200	1.000	.500
1.2500	1.000	.467
1.3200	1.000	.433
1.3850	1.000	.400
1.4400	1.000	.367
1.4850	1.000	.300
1.5100	1.000	.267
1.5350	1.000	.233
1.5500	1.000	.200
1.6050	1.000	.167
1.6700	1.000	.133
1.7750	.800	.100
1.9150	.600	.100
2.1350	.600	.067
2.6150	.600	.033
3.0700	.400	.033
3.4400	.200	.033
4.2150	.200	.000
5.7600	.000	.000

The test result variable(s): Peak S Cyst C has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

REFERENCES

(2000). Angioplasty and STent for Renal Artery Lesions Protocol Book, University of Birmingham Clinical Trials Unit.

Abrahamson, M., I. Olafsson, et al. (1990). "Structure and expression of the human cystatin C gene." Biochem J **268**(2): 287-94.

Abuelo, J. G. (1995). "Diagnosing vascular causes of renal failure." Ann Intern Med **123**(8): 601-14.

Albert, S. G., M. J. Shapiro, et al. (1994). "Analysis of radiocontrast-induced nephropathy by dual-labeled radionuclide clearance." Invest Radiol **29**(6): 618-23.

Alcazar, J. M. and J. L. Rodicio (2000). "Ischemic nephropathy: clinical characteristics and treatment [In Process Citation]." Am J Kidney Dis **36**(5): 883-93.

Bakris, G. L., N. A. Lass, et al. (1999). "Renal hemodynamics in radiocontrast medium-induced renal dysfunction: A role for dopamine-1 receptors." Kidney Int **56**(1): 206-10.

Barrett, A. J., M. E. Davies, et al. (1984). "The place of human gamma-trace (cystatin C) amongst the cysteine proteinase inhibitors." Biochem Biophys Res Commun **120**(2): 631-6.

Barrett, B. J. and E. J. Carlisle (1993). "Metaanalysis of the relative nephrotoxicity of high- and low-osmolality iodinated contrast media." Radiology **188**(1): 171-8.

Bass, N. M., R. E. Kirsch, et al. (1979). "Radioimmunoassay measurement of urinary ligandin excretion in nephrotoxin-treated rats." Clin Sci **56**(5): 419-26.

Breyer, M. D. and R. C. Harris (2001). "Cyclooxygenase 2 and the kidney." Curr Opin Nephrol Hypertens **10**(1): 89-98.

Cambria, R. P., J. K. Davison, et al. (1997). "Thoracoabdominal aneurysm repair: perspectives over a decade with the clamp-and-sew technique." Ann Surg **226**(3): 294-303; discussion 303-5.

Campbell, J. A., A. V. Corrigall, et al. (1991). "Immunohistologic localization of alpha, mu, and pi class glutathione S- transferases in human tissues." Cancer **67**(6): 1608-13.

Chabova, V., A. Schirger, et al. (2000). "Outcomes of atherosclerotic renal artery stenosis managed without revascularization [see comments]." Mayo Clin Proc **75**(5): 437-44.

Chan, N. N., H. P. Brain, et al. (1999). "Metformin-associated lactic acidosis: a rare or very rare clinical entity?" Diabet Med **16**(4): 273-81.

Charlson, M. E., C. R. MacKenzie, et al. (1989). "Postoperative changes in serum creatinine. When do they occur and how much is important?" Ann Surg **209**(3): 328-33.

Cina, C. S., C. M. Clase, et al. (2000). "Carotid endarterectomy for symptomatic carotid stenosis." Cochrane Database Syst Rev **2**.

Cockcroft, D. W. and M. H. Gault (1976). "Prediction of creatinine clearance from serum creatinine." Nephron **16**(1): 31-41.

Colt, H. G., R. J. Begg, et al. (1988). "Cholesterol emboli after cardiac catheterization. Eight cases and a review of the literature." Medicine (Baltimore) **67**(6): 389-400.

Conlon, P. J., E. O'Riordan, et al. (2000). "New insights into the epidemiologic and clinical manifestations of atherosclerotic renovascular disease." Am J Kidney Dis **35**(4): 573-87.

Cressey, G., D. R. Roberts, et al. (2002). "Renal tubular injury after infrarenal aortic aneurysm repair." J Cardiothorac Vasc Anesth **16**(3): 290-3.

Crowley, J. J., R. M. Santos, et al. (1998). "Progression of renal artery stenosis in patients undergoing cardiac catheterization." Am Heart J **136**(5): 913-8.

Culleton, B. F., M. G. Larson, et al. (1999). "Prevalence and correlates of elevated serum creatinine levels: the Framingham Heart Study." Arch Intern Med **159**(15): 1785-90.

Dean, R. H., R. W. Kieffer, et al. (1981). "Renovascular hypertension: anatomic and renal function changes during drug therapy." Arch Surg **116**(11): 1408-15.

Defraigne, J. O. and J. Pincemail (1998). "Local and systemic consequences of severe ischemia and reperfusion of the skeletal muscle. Physiopathology and prevention." Acta Chir Belg **98**(4): 176-86.

Diehl, J. T., R. F. Cali, et al. (1983). "Complications of abdominal aortic reconstruction. An analysis of perioperative risk factors in 557 patients." Ann Surg **197**(1): 49-56.

Drott, C., B. Arfvidsson, et al. (1992). "Age-standardized incidence of ruptured aortic aneurysm in a defined Swedish population between 1952 and 1988: mortality rate and operative results." Br J Surg **79**(2): 175-9.

Ebert, T. J., E. J. Frink, Jr., et al. (1998). "Absence of biochemical evidence for renal and hepatic dysfunction after 8 hours of 1.25 minimum alveolar concentration sevoflurane anesthesia in volunteers." Anesthesiology **88**(3): 601-10.

Eger, E. I., 2nd, D. Gong, et al. (1997). "Dose-related biochemical markers of renal injury after sevoflurane versus desflurane anesthesia in volunteers." Anesth Analg **85**(5): 1154-63.

Eger, E. I., 2nd, D. D. Koblin, et al. (1997). "Nephrotoxicity of sevoflurane versus desflurane anesthesia in volunteers [see comments]." Anesth Analg **84**(1): 160-8.

Eggers, P. W., D. Gohdes, et al. (1999). "Nontraumatic lower extremity amputations in the Medicare end-stage renal disease population [see comments]." Kidney Int **56**(4): 1524-33.

Finney, H., D. J. Newman, et al. (1997). "Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems (BNA, BN II)." Clin Chem **43**(6 Pt 1): 1016-22.

Fleck, A., G. Raines, et al. (1985). "Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury." Lancet **1**(8432): 781-4.

Fliser, D. and E. Ritz (2001). "Serum cystatin C concentration as a marker of renal dysfunction in the elderly." Am J Kidney Dis **37**(1): 79-83.

Fredman, B., E. Zohar, et al. (1999). "Diclofenac does not decrease renal blood flow or glomerular filtration in elderly patients undergoing orthopedic surgery." Anesth Analg **88**(1): 149-54.

Furukawa, T., J. Ueda, et al. (1996). "Elimination of low-osmolality contrast media by hemodialysis." Acta Radiol **37**(6): 966-71.

Gilling-Smith, G. L., L. Worswick, et al. (1995). "Surgical repair of thoracoabdominal aortic aneurysm: 10 years' experience." Br J Surg **82**(5): 624-9.

Godet, G., M. H. Fleron, et al. (1997). "Risk factors for acute postoperative renal failure in thoracic or thoracoabdominal aortic surgery: a prospective study." Anesth Analg **85**(6): 1227-32.

Golman, K. and T. Almen (1985). "Contrast media-induced nephrotoxicity. Survey and present state." Invest Radiol **20**(1 Suppl): S92-7.

Gomes, A. S., J. F. Lois, et al. (1989). "Acute renal dysfunction in high-risk patients after angiography: comparison of ionic and nonionic contrast media." Radiology **170**(1 Pt 1): 65-8.

Gonick, H. C., H. J. Kramer, et al. (1973). "Urinary -glucuronidase activity in renal disease." Arch Intern Med **132**(1): 63-9.

Gordon, A. C., S. Pryn, et al. (1994). "Outcome in patients who require renal support after surgery for ruptured abdominal aortic aneurysm." Br J Surg **81**(6): 836-8.

Gosling, P., K. Sanghera, et al. (1994). "Generalized vascular permeability and pulmonary function in patients following serious trauma." J Trauma **36**(4): 477-81.

Grubb, A. (1992). "Diagnostic value of analysis of cystatin C and protein HC in biological fluids." Clin Nephrol **38**(Suppl 1): S20-7.

Grubb, A. and H. Lofberg (1982). "Human gamma-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis." Proc Natl Acad Sci U S A **79**(9): 3024-7.

Grubb, A., O. Simonsen, et al. (1985). "Serum concentration of cystatin C, factor D and beta 2-microglobulin as a measure of glomerular filtration rate." Acta Med Scand **218**(5): 499-503.

Gupta, R. K., A. Kapoor, et al. (1999). "Captopril for prevention of contrast-induced nephropathy in diabetic patients: a randomised study." Indian Heart J **51**(5): 521-6.

Hamdan, A. D., F. B. Pomposelli, Jr., et al. (1999). "Renal insufficiency and altered postoperative risk in carotid endarterectomy." J Vasc Surg **29**(6): 1006-11.

Harden, P. N., M. J. MacLeod, et al. (1997). "Effect of renal-artery stenting on progression of renovascular renal failure [see comments]." Lancet **349**(9059): 1133-6.

Harrington, E. B., M. E. Harrington, et al. (1990). "End-stage renal disease--is infrainguinal limb revascularization justified?" J Vasc Surg **12**(6): 691-5; discussion 695-6.

Harris, K. G., T. P. Smith, et al. (1991). "Nephrotoxicity from contrast material in renal insufficiency: ionic versus nonionic agents." Radiology **179**(3): 849-52.

Harrison, D. J., R. Kharbanda, et al. (1989). "Distribution of glutathione S-transferase isoenzymes in human kidney: basis for possible markers of renal injury." J Clin Pathol **42**(6): 624-8.

Hawkins, I. F., C. S. Wilcox, et al. (1994). "CO2 digital angiography: a safer contrast agent for renal vascular imaging?" Am J Kidney Dis **24**(4): 685-94.

Holland, A. J., R. Bell, et al. (1998). "Prognostic factors in elective aortic reconstructive surgery." Aust N Z J Surg **68**(1): 16-20.

Holley, J. L. (2000). "Preventive medical screening is not appropriate for many chronic dialysis patients.[In Process Citation]." Semin Dial **13**(6): 369-71.

Isiklar, M. H., M. Kulbaski, et al. (1997). "Infrainguinal bypass in end-stage renal disease: when is it justified?" Semin Vasc Surg **10**(1): 42-8.

Jakobsen, J. A., K. J. Berg, et al. (1994). "Renal effects of nonionic contrast media after cardioangiography." Acta Radiol **35**(2): 191-6.

Johnson, B. L., M. H. Glickman, et al. (1995). "Failure of foot salvage in patients with end-stage renal disease after surgical revascularization." J Vasc Surg **22**(3): 280-5; discussion 285-6.

Johnston, K. W. (1989). "Multicenter prospective study of nonruptured abdominal aortic aneurysm. Part II. Variables predicting morbidity and mortality." J Vasc Surg **9**(3): 437-47.

Joseph, M. G., P. T. McCollum, et al. (1989). "Abnormal pre-operative creatinine levels and renal failure following abdominal aortic aneurysm repair." Aust N Z J Surg **59**(7): 539-41.

Jung, K. (1994). "Urinary enzymes and low molecular weight proteins as markers of tubular dysfunction." Kidney Int Suppl **47**: S29-33.

Kapoor, A., N. Sinha, et al. (1996). "Use of dopamine in prevention of contrast induced acute renal failure-- a randomised study." Int J Cardiol **53**(3): 233-6.

Kashyap, V. S., R. P. Cambria, et al. (1997). "Renal failure after thoracoabdominal aortic surgery." J Vasc Surg **26**(6): 949-55; discussion 955-7.

Kashyap, V. S. and W. J. Quinones-Baldrich (1999). "Abdominal aortic aneurysm repair in patients with renal allografts." Ann Vasc Surg **13**(2): 199-203.

Keevil, B. G., E. S. Kilpatrick, et al. (1998). "Biological variation of cystatin C: implications for the assessment of glomerular filtration rate." Clin Chem **44**(7): 1535-39.

Kharasch, E. D., E. J. Frink, Jr., et al. (1997). "Assessment of low-flow sevoflurane and isoflurane effects on renal function using sensitive markers of tubular toxicity." Anesthesiology **86**(6): 1238-53.

Kinnison, M. L., N. R. Powe, et al. (1989). "Results of randomized controlled trials of low-versus high-osmolality contrast media [see comments]." Radiology **170**(2): 381-9.

Kunin, C. M., R. W. Chesney, et al. (1978). "Enzymuria as a marker of renal injury and disease: studies of N-acetyl-beta-glucosaminidase in the general population and in patients with renal disease." Pediatrics **62**(5): 751-60.

Lacombe, M. (1998). "[Treatment of abdominal aortic aneurysms in chronic hemodialysis]." J Mal Vasc **23**(5): 349-53.

Laterza, O. F., C. P. Price, et al. (2002). "Cystatin C: an improved estimator of glomerular filtration rate." Clin Chem **48**(5): 699-707.

Lautin, E. M., N. J. Freeman, et al. (1991). "Radiocontrast-associated renal dysfunction: incidence and risk factors [see comments]." AJR Am J Roentgenol **157**(1): 49-58.

Lee, A., M. G. Cooper, et al. "Effects of nonsteroidal anti-inflammatory drugs on post-operative renal function in adults (Cochrane Review). In: The Cochrane Library, Issue 4, 2000. Oxford: Update software."

Lehnert, T., E. Keller, et al. (1998). "Effect of haemodialysis after contrast medium administration in patients with renal insufficiency." Nephrol Dial Transplant **13**(2): 358-62.

Levey, A. S., R. D. Perrone, et al. (1988). "Serum creatinine and renal function." Annu Rev Med **39**: 465-90.

Lofberg, H. and A. O. Grubb (1979). "Quantitation of gamma-trace in human biological fluids: indications for production in the central nervous system." Scand J Clin Lab Invest **39**(7): 619-26.

Luke, R. G. (1998). "Chronic renal failure--a vasculopathic state [editorial; comment]." N Engl J Med **339**(12): 841-3.

Mailloux, L. U., B. Napolitano, et al. (1994). "Renal vascular disease causing end-stage renal disease, incidence, clinical correlates, and outcomes: a 20-year clinical experience." Am J Kidney Dis **24**(4): 622-9.

Marcelli, D., D. Stannard, et al. (1996). "ESRD patient mortality with adjustment for comorbid conditions in Lombardy (Italy) versus the United States." Kidney Int **50**(3): 1013-8.

McCartney, M. M., F. J. Gilbert, et al. (1999). "Metformin and contrast media--a dangerous combination?" Clin Radiol **54**(1): 29-33.

Moon, S. S., S. E. Back, et al. (1995). "Hemodialysis for elimination of the nonionic contrast medium iohexol after angiography in patients with impaired renal function." Nephron **70**(4): 430-7.

Moore, R. D., E. P. Steinberg, et al. (1992). "Nephrotoxicity of high-osmolality versus low-osmolality contrast media: randomized clinical trial." Radiology **182**(3): 649-55.

Nawaz, S., T. Cleveland, et al. (1998). "Clinical risk associated with contrast angiography in metformin treated patients: a clinical review." Clin Radiol **53**(5): 342-4.

Newman, D. J., M. J. Pugia, et al. (2000). "Urinary protein and albumin excretion corrected by creatinine and specific gravity." Clin Chim Acta **294**(1-2): 139-55.

Newman, D. J., H. Thakkar, et al. (1994). "Serum cystatin C: a replacement for creatinine as a biochemical marker of GFR." Kidney Int Suppl **47**: S20-1.

Newman, D. J., H. Thakkar, et al. (1995). "Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine." Kidney Int **47**(1): 312-8.

Novis, B. K., M. F. Roizen, et al. (1994). "Association of preoperative risk factors with postoperative acute renal failure." Anesth Analg **78**(1): 143-9.

O'Donnell, D., G. Clarke, et al. (1989). "Acute renal failure following surgery for abdominal aortic aneurysm." Aust N Z J Surg **59**(5): 405-8.

Pallister, I., P. Gosling, et al. (1997). "Prediction of posttraumatic adult respiratory distress syndrome by albumin excretion rate eight hours after admission [see comments]." J Trauma **42**(6): 1056-61.

Peltonen, S., F. Biancari, et al. (1998). "Outcome of infrainguinal bypass surgery for critical leg ischaemia in patients with chronic renal failure." Eur J Vasc Endovasc Surg **15**(2): 122-7.

Pergande, M. and K. Jung (1993). "Sandwich enzyme immunoassay of cystatin C in serum with commercially available antibodies." Clin Chem **39**(9): 1885-90.

Pillay, W. R., Y. M. Kan, et al. (2002). "Prospective multicentre study of the natural history of atherosclerotic renal artery stenosis in patients with peripheral vascular disease." Br J Surg **89**(6): 737-40.

Plecha, E. J., T. A. King, et al. (1993). "Risk assessment in patients undergoing carotid endarterectomy." Cardiovasc Surg **1**(1): 30-2.

Porter, G. A. (1994). "Urinary biomarkers and nephrotoxicity." Miner Electrolyte Metab **20**(4): 181-6.

Randers, E., J. H. Kristensen, et al. (1998). "Serum cystatin C as a marker of the renal function." Scand J Clin Lab Invest **58**(7): 585-92.

Ridker, P. M. and T. Michel (1989). "Streptokinase therapy and cholesterol embolization." Am J Med **87**(3): 357-8.

Rigdon, E. E., N. Monajjem, et al. (1997). "Is carotid endarterectomy justified in patients with severe chronic renal insufficiency?" Ann Vasc Surg **11**(2): 115-9.

Rosovsky, M. A., H. Rusinek, et al. (1996). "High-dose administration of nonionic contrast media: a retrospective review [see comments]." Radiology **200**(1): 119-22.

Rudnick, M. R., S. Goldfarb, et al. (1995). "Nephrotoxicity of ionic and nonionic contrast media in 1196 patients: a randomized trial. The Iohexol Cooperative Study." Kidney Int **47**(1): 254-61.

Safi, H. J., S. A. Harlin, et al. (1996). "Predictive factors for acute renal failure in thoracic and thoracoabdominal aortic aneurysm surgery [published erratum appears in J Vasc Surg 1997 Jan;25(1):93]." J Vasc Surg **24**(3): 338-44; discussion 344-5.

Safian, R. D. and S. C. Textor (2001). "Renal-artery stenosis." N Engl J Med **344**(6): 431-42.

Saine, D. R. and E. R. Ahrens (1996). "Renal impairment associated with losartan." Ann Intern Med **124**(8): 775.

Salmon, P. and M. A. Brown (1990). "Renal artery stenosis and peripheral vascular disease: implications for ACE inhibitor therapy [letter] [see comments]." Lancet **336**(8710): 321.

Scheen, A. J. (1996). "Clinical pharmacokinetics of metformin." Clin Pharmacokinet **30**(5): 359-71.

Schepens, M. A., J. J. Defauw, et al. (1994). "Risk assessment of acute renal failure after thoracoabdominal aortic aneurysm surgery." Ann Surg **219**(4): 400-7.

Scherberich, J. E. and G. Wolf (1994). "Disintegration and recovery of kidney membrane proteins: consequence of acute and chronic renal failure." Kidney Int Suppl **47**: S52-7.

Schreiber, M. J., M. A. Pohl, et al. (1984). "The natural history of atherosclerotic and fibrous renal artery disease." Urol Clin North Am **11**(3): 383-92.

Schwartz, C. J. and T. A. White (1964). "Atenosis of renal artery: An unselected necropsy study." BMJ **2**: 1415-1421.

Scoble, J. E., P. Sweny, et al. (1993). "Patients with atherosclerotic renovascular disease presenting to a renal unit: an audit of outcome." Postgrad Med J **69**(812): 461-5.

Scolari, F., R. Tardanico, et al. (2000). "Cholesterol crystal embolism: A recognizable cause of renal disease [In Process Citation]." Am J Kidney Dis **36**(6): 1089-109.

Seidegard, J. and G. Ekstrom (1997). "The role of human glutathione transferases and epoxide hydrolases in the metabolism of xenobiotics." Environ Health Perspect **105 Suppl 4**: 791-9.

Shapiro, L. S. (1989). "Cholesterol embolization after treatment with tissue plasminogen activator." N Engl J Med **321**(18): 1270.

Sherman, R. A., D. A. Feinfeld, et al. (1984). "A prospective study of urinary ligandin in patients at risk of renal tubular injury." Uremia Invest **8**(2): 111-5.

Simonsen, O., A. Grubb, et al. (1985). "The blood serum concentration of cystatin C (gamma-trace) as a measure of the glomerular filtration rate." Scand J Clin Lab Invest **45**(2): 97-101.

Smith, F. C., P. Gosling, et al. (1994). "Microproteinuria predicts the severity of systemic effects of reperfusion injury following infrarenal aortic aneurysm surgery." Ann Vasc Surg **8**(1): 1-5.

Smith, M. C., M. K. Ghose, et al. (1981). "The clinical spectrum of renal cholesterol embolization." Am J Med **71**(1): 174-80.

Solomon, R., C. Werner, et al. (1994). "Effects of saline, mannitol, and furosemide to prevent acute decreases in renal function induced by radiocontrast agents." N Engl J Med **331**(21): 1416-20.

Sternbergh, W. C., 3rd, C. L. Garrard, et al. (1999). "Carotid endarterectomy in patients with significant renal dysfunction." J Vasc Surg **29**(4): 672-7.

Sterner, G., B. Frennby, et al. (2000). "Does post-angiographic hemodialysis reduce the risk of contrast-medium nephropathy?" Scand J Urol Nephrol **34**(5): 323-6.

Strandness, D. E., Jr. (1994). "Natural history of renal artery stenosis." Am J Kidney Dis **24**(4): 630-5.

Taliercio, C. P., S. H. McCallister, et al. (1989). "Nephrotoxicity of nonionic contrast media after cardiac angiography." Am J Cardiol **64**(12): 815-6.

Tenstad, O., A. B. Roald, et al. (1996). "Renal handling of radiolabelled human cystatin C in the rat." Scand J Clin Lab Invest **56**(5): 409-14.

Tepel, M., M. van der Giet, et al. (2000). "Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine." N Engl J Med **343**(3): 180-4.

Thadhani, R. I., C. A. Camargo, Jr., et al. (1995). "Atheroembolic renal failure after invasive procedures. Natural history based on 52 histologically proven cases." Medicine (Baltimore) **74**(6): 350-8.

Torhorst, J., D. de Rougemont, et al. (1982). "Morphology of the renal medulla in ischemic acute renal failure in the rat." Nephron **31**(4): 296-300.

Tornquist, C., T. Almen, et al. (1985). "Renal function following nephroangiography with metrizamide and iohexol. Effects on renal blood flow, glomerular permeability and filtration rate and diuresis in dogs." Acta Radiol [Diagn] (Stockh) **26**(4): 483-9.

Tornquist, C. and S. Holtas (1984). "Renal angiography with iohexol and metrizoate." Radiology **150**(2): 331-4.

Toto, R. D. (1995). "Conventional measurement of renal function utilizing serum creatinine, creatinine clearance, inulin and para-aminohippuric acid clearance." Curr Opin Nephrol Hypertens **4**(6): 505-9; discussion 503-4.

Venkatachalam, M. A., D. B. Bernard, et al. (1978). "Ischemic damage and repair in the rat proximal tubule: differences among the S1, S2, and S3 segments." Kidney Int **14**(1): 31-49.

Walker, S. R., S. W. Yusuf, et al. (1998). "Renal complications following endovascular repair of abdominal aortic aneurysms." J Endovasc Surg **5**(4): 318-22.

Watson, P. S., P. Hadjipetrou, et al. (2000). "Effect of renal artery stenting on renal function and size in patients with atherosclerotic renovascular disease." Circulation **102**(14): 1671-7.

Watts, G. F., R. W. Morris, et al. (1988). "Urinary albumin excretion in healthy adult subjects: reference values and some factors affecting their interpretation." Clin Chim Acta **172**(2-3): 191-8.