Assessment of a novel biomarker panel for the earlier prediction of acute kidney injury in patients with diabetes mellitus undergoing coronary angiography and intervention

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Assessment of a Novel Biomarker Panel for the Earlier Prediction of Acute Kidney Injury in Patients with Diabetes mellitus undergoing Coronary Angiography and Intervention

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Abstract

Diabetes mellitus triples the risk of developing coronary heart disease (CHD). The manifestations of CHD are more severe in patients with diabetes with both more extensive and more diffuse disease. Outcomes in patients with diabetes are significantly worse both in terms of Major Adverse Cardiovascular Events (MACE) overall and specifically following revascularisation procedures such as percutaneous coronary intervention (PCI). Furthermore patients with chronic kidney disease (CKD) are excluded from the vast majority of cardiology trials and therefore there is a lack of data for these patients. Contrast induced acute kidney injury (AKI) is an important complication following procedures in the cardiac catheterisation laboratory and patients with diabetes are at particular risk.

Patients with diabetes and CKD are a particular challenge. They have an extremely high incidence of co-morbidities such as CHD and peripheral vascular disease (PVD). When renal function is already impaired by other pathological processes, the kidney is much less capable of tolerating the stress of excreting a contrast load. If AKI develops, there is a risk of further loss of nephron units and irreversible reduction in residual renal function. Earlier identification of patients as risk of developing AKI will allow earlier therapeutic intervention which might translate into improved clinical outcomes for these patients.

The study aimed to evaluate the incidence of contrast induced AKI in a high risk population (diabetes and CKD) undergoing coronary angiography and PCI.

From a literature review Neutrophil Gelatinase Associated Lipocalin (NGAL) and Interleukin-18 (IL-18) were identified as promising candidates for inclusion in a 'renal injury' biomarker panel for development of AKI early post coronary angiography or PCI. It was then determined if concentrations of these markers changed significantly at various time intervals post procedure. The aim was to establish a panel of markers with the best predictive ability for identifying development of AKI.

208 patients with a known diagnosis of diabetes mellitus and CKD (eGFR < 60 ml/min) were recruited over a one year period at The London Chest Hospital. 39 patients (18.8%) developed contrast induced AKI. There were no significant differences between patients who did/did not develop AKI with respect to baseline demographics, cardiac risk factors and co-morbidities. There was a significant trend towards patients in the AKI group having a higher NYHA Class ($p=0.048$) and this was further supported by echocardiogram and other imaging data. Additional reinforcement came from the higher rate of loop diuretic prescription (59% vs 26.9%) in the AKI group, $p=0.012$.

59 patients underwent renal angiography at the time of their coronary procedure. There was no association between presence of structural renal disease and development of AKI. 7 patients (12.5%) had moderate or severe renal artery stenosis with 1 patient requiring further renal angioplasty due to the presence of a critical lesion.

There was no association between severity of CAD as assessed by the SYNTAX score and development of AKI. However the Clinical SYNTAX score (CSS) was significantly higher in patients who developed AKI (82.7 vs. 35.4, p=0.036). The CSS also had a reasonable accuracy for predicting AKI with a C statistic = 0.64 on ROC analysis. The Mehran risk score also performed well at identifying patients with AKI with a C statistic of 0.69. ROC curve analysis did not suggest that either NGAL or IL-18 in isolation were particularly beneficial markers of AKI in our patient cohort as our best C statistic was 0.634 for serum NGAL, 2h post procedure. However on exploratory analysis a 20% rise in urine NGAL at 2h, combined with a 20% rise in urine IL-18 at 2 hours and the Mehran risk score had a C statistic of 0.77. Simple scoring systems such as the Mehran score and CSS are useful tools for risk stratifying patients in both the clinical and research settings.

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Table of Contents

Index of Tables

Index of Figures

Abbreviations and acronyms

ACC	American College of Cardiology
ACCORD	The Action to Control Cardiovascular Risk in Diabetes Study Group
ACE	Angiotensin Converting Enzyme
ACE-I	Angiotensin Converting Enzyme Inhibitor
ACEF score	Age, serum Creatinine and Ejection Fraction
ACS	Acute Coronary Syndromes
ACT	Acetylcysteine for the prevention of Contrast induced nephropathy Trial
ADA	American Diabetes Association
ADP	Adenosine Diphosphate
ADVANCE	The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation Trial
AGEs	Advanced Glycation End-Products
AHA	American Heart Association
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMP	Adenosine Monophosphate
AP-1	Activator Protein-1
ARB	Angiotensin II Receptor Blocker
ARTS	Arterial Revascularisation Therapies Study
AT_1	Angiotensin II Type 1
AT_2	Angiotensin II Type 2
AUC	Area Under Curve
AURORA	A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Haemoodialysis
BARI	Bypass Angioplasty Revascularisation Investigation
BARI-2D	Bypass Angioplasty Revascularisation Investigation 2 in Diabetes
BCIS-CCAD	British Cardiovascular Intervention Society - Central Cardiac Audit Database
BMI	Body Mass Index
BP	Blood Pressure
CABG	Coronary Artery Bypass Grafting
CCB	Calcium Channel Blocker
CAPD	Continuous Ambulatory Peritoneal Dialysis
CCU	Coronary Care Unit
CHD	Coronary Heart Disease
CARE	Cardiac Angiography in Renally Impaired Patients
CCS	Canadian Cardiovascular Society

CCF	Congestive Cardiac Failure
CCU	Coronary Care Unit
CCS	Canadian Cardiovascular Society
CHD	Coronary Heart Disease
CIN	Contrast Induced Nephropathy
CK	Creatinine Kinase
CKD	Chronic Kidney Disease
CI	Confidence Interval
COX	Cyclo-oxygenase
CrCl	Creatinine Clearance
CRF	Case Report Form
CRP	C Reactive Protein
CSS	Clinical Syntax Score
CV	Cardiovascular
CVA	Cerebrovascular Accident
Cx	Circumflex coronary artery
DCCT	Diabetes Control and Complications Trial
4D	Die Deutsche Diabetes Dialyse Studie
DIGAMI	Diabetes Mellitus Insulin Glucose Infusion in Acute Myocardial Infarction
DM	Diabetes mellitus
DVD	Dialysis Versus Diuresis
EASD	European Association for the Study of Diabetes
ECM	Extracellular Matrix
eGFR	Estimated Glomerular Filtration Rate
EF	Ejection Fraction
ELISA	Enzyme Linked Immunosorbent Assay
ESC	European Society of Cardiology
ESRD	End Stage Renal Disease
ET-1	Endothelin-1
FBC	Full Blood Count
FFR	Fractional Flow Reserve
FGF-23	Fibroblast Growth Factor - 23
FPG	Fasting Plasma Glucose
GFR	Glomerular Filtration Rate
GP IIb/IIIa	Glycoprotein IIb IIIa
GTP	Guanosine Triphosphate
HbA1c	Haemoglobin A1c (glycated haemoglobin)
HCt	Haematocrit
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HOCM	High Osmolal Contrast Media
IABP	Intra-Aortic Balloon Pump

IFCC	International Federation of Clinical Chemistry
IFG	Impaired Fasting Glucose
IGF-1	Insulin-like Growth Factor 1
IGT	Impaired Glucose Tolerance
IL-6	Interleukin 6
IL-18	Interleukin 18
IOCM	Iso-osmolal contrast media
INR	International Normalised Ratio
IPC	Ischaemic Pre-Conditioning
IQR	Interquartile Range
ITT	Insulin Tolerance Test
IVUS	Intravascular Ultrasound
KIM-1	Kidney Injury Molecule-1
KDOQI	Kidney Disease Outcomes Quality Initiative
LAD	Left anterior descending
LDL	Low density Lipoprotein
L-FABP	Liver Type Fatty Acid Binding Protein
LFT	Liver Function Tests
LOCM	Low osmolal contrast media
LMS	Left Main Stem
LpA	Lipoprotein a
LV function	Left ventricular function
LVH	Left ventricular hypertrophy
MACCE	Major Adverse Cardiovascular and Cerebrovascular Events
MACE	Major Adverse Cardiac Events
MCP-1	Monocyte Chemoattractant Protein-1
M-CSF	Macrophage Colony Stimulating Factor
MDRD	Modification of Diet in Renal Disease
MI	Myocardial infarction
MICRO-HOPE	The Microalbuminuria, Cardiovascular, and Renal Outcomes in HOPE
MMP	Matrix Metallo-Proteinases
MMP-9	Matrix Metalloproteinase 9
Mφ	Macrophage
MRA	Magnetic Resonance Angiography
MRFIT	Multiple Risk Factor Intervention Trial
MREC	Multi-centre Research Ethics Committee
MVD	Multivessel Disease
NAG	N-acetyl-β-D-glucosaminidase
NGAL	Neutrophil Gelatinase Associated Lipocalin
NO	Nitric Oxide
NRI	Net Reclassification Index

NSTEMI	Non-ST Elevation Myocardial Infarction
NYHA	New York Heart Association
OGTT	Oral Glucose Tolerance Testing
OR	Odds Ratio
NAC	N-acetylcysteine
NF-κB	Nuclear Factor Kappa B
NGAL	Neutrophil Gelatinase Associated Lipocalin
NICE	National Institute for Clinical Excellence
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
PAI-1	Plasminogen Activator Inhibitor 1
PC	Prostacyclin
PCI	Percutaneous Coronary Intervention
PDGF	Platelet Derived Growth Factor
P_{gc}	Glomerular Capillary Hydraulic Pressure
PG	Prostaglandin
PKC	Protein Kinase C
PKC	Protein Kinase C
PROactive	PROspective pioglitazone Clinical Trial in macroVascular Events
PTH	Parathyroid Hormone
PVD	Peripheral vascular disease
QoL	Quality of Life
RAGE	Receptor for Advanced Glycation End Products
RAS	Renin Angiotensin System
RAAS	Renin Angiotensin Aldosterone System
RBF	Renal Blood Flow
RCA	Right Coronary Artery
RIFLE	Risk, Injury, Failure, Loss and End-stage renal disease
RIPC	Remote Ischaemic Pre-Conditioning
RNS	Reactive Nitrogen Species
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
RRT	Renal Replacement Therapy
SB	Side Branch
SD	Standard Deviation
SHARP	Study of Heart and Renal Protection
sICAM	Soluble Intercellular Adhesion Molecule
SP-1	Specificity Protein-1
STEMI	ST-Elevation Myocardial Infarction
sVCAM	Soluble Vascular Cell Adhesion Molecule
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

TB	Tuberculosis
TBXA-$_2$	Thromboxane A-2
TF	Tissue Factor
TGF-β	Transforming Growth Factor beta
TO	Total Occlusion
Tpa	Tissue Plasminogen Activator
TVD	Triple Vessel Disease
U and E	Urea and Electrolytes
UKPDS	United Kingdom Prospective Diabetes Study
UO	Urine Output
VALOR	Visipaque Angiography/Interventions with Laboratory Outcomes in Renal Insufficiency Trial
VCAM-1	Vascular Cell Adhesion Molecule-1
VEGF	Vascular Endothelial Growth Factor
VLDL	Very Low Density Lipoprotein
vWF	von Willebrand's Factor
WCC	White Cell Count

Publications Arising from this Research

Presentations to Learned Societies and Published Abstracts

A.C. Qureshi, R. Rampat, S.M. Harwood, M. Roughton, Yaqoob M.M. and Kapur A. **Serum NGAL Identifies Contrast Nephropathy Early in Patients with Diabetes Mellitus and Chronic Kidney Disease Undergoing Coronary Angiography or Angioplasty.** British Cardiovascular Society Annual Conference and Exhibition, Manchester, 13th-15th June 2011

A.C. Qureshi, R. Rampat, S.M. Harwood, M. Roughton, Yaqoob M.M. and Kapur A. **Serum NGAL Identifies Contrast Nephropathy Early in Patients with Diabetes Mellitus and Chronic Kidney Disease Undergoing Coronary Angiography or Angioplasty.** Euro PCR , Paris 17th-20th May 2011

A.C. Qureshi, R. Rampat, S.M. Harwood, M. Roughton, Yaqoob M.M. and Kapur A. **NGAL and Interleukin -18 Identify Contrast-Induced Acute Kidney Injury Early in Patients with Chronic Kidney Disease Undergoing Coronary Angiography and Percutaneous Coronary Intervention.** Finalist President's Medal Cardiology Section, Royal Society of Medicine July 6th 2011

A.C. Qureshi, R. Rampat, S.M. Harwood, M. Roughton, Yaqoob M.M. and Kapur A. **Urine NGAL and Interleukin -18 Identify Contrast-Induced Acute Kidney Injury Early in Patients with Chronic Kidney Disease Undergoing Coronary Angiography and Percutaneous Coronary Intervention**

European Society of Cardiology, Paris 27th-31st August 2011

A.C. Qureshi, R.P. Weerackody, M. Ozkor, M.Yaqoob and A. Kapur

Relationship between SYNTAX Score and Contrast Induced Acute Kidney Injury in Patients with CKD undergoing Coronary Angiography or PCI

Trans Catheter Therapeutics, San Francisco 2011

A.C. Qureshi, R. Rampat, S.M. Harwood, M. Roughton, Yaqoob M.M. and Kapur A.

Urine NGAL and Interleukin -18 Identify Contrast-Induced Acute Kidney Injury Early in Patients with Diabetes and Chronic Kidney Disease Undergoing Coronary Angiography and Percutaneous Coronary Intervention

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Chapter One

Introduction

Chapter One: Introduction

1.1 The diabetic coronary disease epidemic

The developed world is in the midst of a worldwide epidemic of diabetes mellitus, almost totally attributable to an increase in Type 2 diabetes mellitus (T2DM). It is estimated that approximately 8% of the adult population in the developed world have T2DM and it is predicted that the prevalence will at least double over the next 12 - 15 years to approximately 300 million affected by 2025 (1-3). Yet these projections are likely to underestimate the scale of the problem as it has been estimated that up to 50% of the general population may be affected but undiagnosed (4-6). Furthermore up to double the number of patients who have T2DM have insulin resistance syndrome, associated with an elevated risk of development of T2DM (7). Causative factors include increasing obesity, sedentary lifestyles with decreasing rates of physical activity, increasing dietary calorie intake and an aging population (8).

Atherosclerosis is often present in patients at the time of diagnosis of T2DM, a finding that suggests that the preceding 'pre-diabetic' period is a key stage in plaque development. Coronary heart disease (CHD) is the leading cause of death in patients with diabetes and additionally is associated with increased morbidity and increased rates of stroke and peripheral vascular disease; together these are defined as macrovascular disease (9-13). Overall the age adjusted mortality rates associated with CHD have declined over the last 20 years but this has not been reflected in patients with T2DM (14-16). Data from the MRFIT (Multiple Risk Factor Intervention Trial) trial data (13) shows that men with T2DM have a threefold higher absolute risk of

cardiovascular death than those without diabetes and this is present even after adjusting for age, race, cholesterol, blood pressure, smoking and socio-economic status.

T2DM trebles the risk of developing CHD and once this has developed the risk of acute coronary syndromes (ACS) doubles with an additional doubling of clinical risk once a coronary event has occurred (17-20). The Framingham study (10) found that even after adjustment for other factors, diabetic patients had increased mortality and a higher incidence of reinfarction and heart failure in both the acute and post-infarct setting. One year mortality post MI is double in diabetic compared to non-diabetic patients (21). Approximately 20% of patients in the United Kingdom undergoing cardiac catheterisation procedures have a diagnosis of diabetes (7;22) and in recent randomised trials diabetic patients accounted for over 20% of revascularisation procedures (23-26). At the London Chest Hospital, patients with diabetes account for approximately 30% of revascularisation procedures reflecting the demographics of our institution's referral base (22). Outcomes following coronary revascularisation (either by percutaneous coronary intervention, (PCI) or coronary artery bypass grafting (CABG) are significantly worse in patients with diabetes compared with non-diabetic patients (26-34).

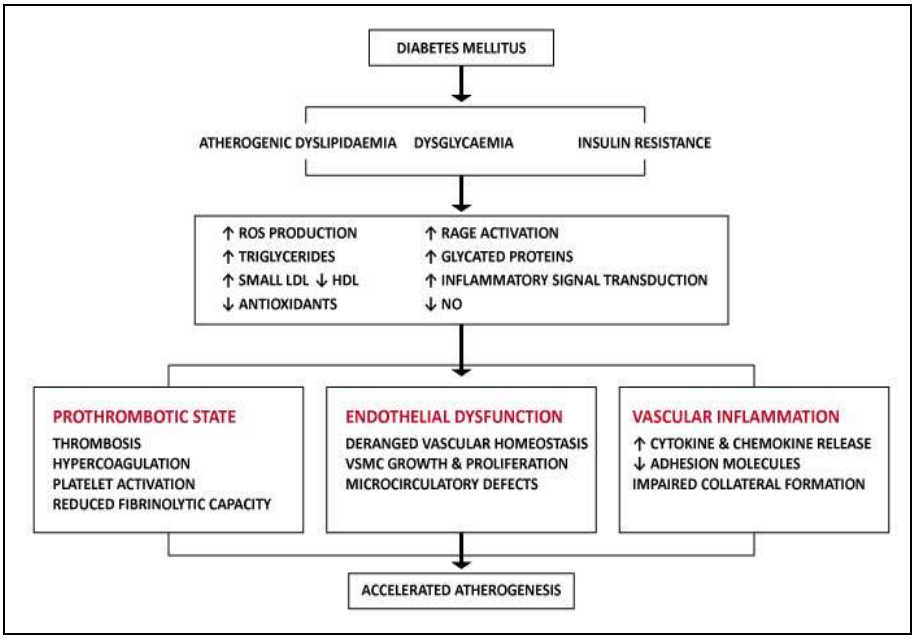
Patients with diabetes have a higher rate of repeat revascularisation procedures following the index procedure. In addition to the increased risk associated with diabetes itself, the manifestations of CHD in diabetic patients are more severe with both more extensive and diffuse disease of the coronary arteries (34;35). Data from the BARI (Bypass Angioplasty Revascularisation Investigation) Trial (35) showed that diabetic patients had a higher incidence than non-diabetic patients of triple vessel disease

(TVD) (46% vs. 40%, $p<0.05$) and left ventricular function (LV function) below 50% (31% vs. 20%, $p<0.001$) resulting in a significantly lower 5 year survival.

1.2 Why is diabetic coronary disease different?

The metabolic abnormalities that characterise diabetes are summarised in Figure 1.

Figure 1: Summary of key metabolic abnormalities in diabetes mellitus



(adapted from D'Souza *et al* Mol Cell Biochem 2009 (36)). The metabolic abnormalities which characterise diabetes, particularly hyperglycaemia, free fatty acids and insulin resistance stimulate molecular mechanisms causing alterations in the structure and function of blood vessels. These mechanisms include increased oxidative stress, alterations of intercellular signal transduction (e.g. activation of PKC) and activation of RAGE. The result is decreased availability of NO, increased production of ET-1, activation of transcription factors such as NF-κB and AP-1 and increased generation of pro-thrombotic factors such as TF and PAI-1. (Abbreviations: ROS: Reactive Oxygen Species, PKC: Protein Kinase C, RAGE: Receptor for Advanced Glycation End Products, NO: Nitric oxide, ET-1: Endothelin-1, NF-κB: Nuclear Factor Kappa B, AP-1: Activator Protein-1, TF: Tissue Factor, PAI-1: Plasminogen activator inhibitor-1).

Several biological mechanisms exist that account for the premature development of atherosclerosis and the increased risk of CHD in patients with diabetes. This is due to a combination of general risk factors such as hypertension and hyperlipidaemia which are more prevalent in diabetes (37), and specific risks resulting from the triad of hyperinsulinaemia, insulin resistance and hyperglycaemia (38). These risks are summarised in Table 1.

Table 1: Factors which increase risk of CHD in Diabetes

Factor	**Effect**
Dyslipidaemia	Increased LDL and triglyceride rich VLDL Reduced HDL
Endothelial dysfunction	Increased expression of cellular adhesion molecules Impaired vasomotor activity due to decreased availability of nitric oxide
Oxidative stress	Increased concentrations of markers such as oxidised LDLs and F2-isoprostanes
Inflammation	Increased expression of markers such as fibrinogen and CRP
Abnormalities of coagulation and fibrinolysis	Overproduction of fibrinogen Expression of PAI-1 Reduced t-PA
Glycation of proteins	Formation of proatherogenic advanced glycation end products (AGE) in LDL and collagen within the arterial wall

(Adapted from Kapur and Qureshi Chapter 21: Oxford Textbook of Interventional Cardiology (39). Abbreviations: HDL: high density lipoprotein; LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein; CRP: C-Reactive Protein; tPA: tissue plasminogen activator; PAI-1: Plasminogen activator inhibitor -1).

1.2.1 Dyslipidaemia

Diabetic patients have a characteristic but abnormal lipid profile probably due to a combination of insulin resistance and a general failure of insulin activity. This results in an increase in release from adipose tissue of free fatty acids, increased transport of free fatty acids to the liver and increased synthesis of VLDL (40;41). Other findings include elevated concentrations of small dense LDLs, triglyceride-rich VLDLs and low concentrations of HDL. This abnormal profile is associated with significantly increased cardiovascular risk (42;43). Triglyceride levels appear to be directly linked to the degree of glucose intolerance and glycaemic control but these have less of an effect on HDL and LDL levels (44).

1.2.2 Endothelial dysfunction

Endothelial dysfunction is a very early marker of atherosclerosis and is a key feature of the severe arteriopathy that can occur in the pre-diabetic state. The combination of hyperglycaemia, hypertension, dyslipidaemia and insulin resistance cause endothelial damage and the resulting endothelial dysfunction is a consequence of both microvascular and macrovascular complications developing (45-47). Further evidence supporting the hypothesis that macrovascular disease can arise in the pre-diabetic period is that these patients have an increased concentration of endothelial dysfunction markers including soluble vascular adhesion molecule (sVCAM), soluble intercellular adhesion molecule (sICAM), endothelin (ET)-1 and von Willebrand's Factor (vWF) (48).

Whilst it is well established that a clustering of traditional cardiovascular risk factors occurs in the pre-diabetic period, it seems that the state of insulin resistance additionally contributes to the increase in cardiovascular risk (49). Hyperglycaemia activates the protein kinase C (PKC) pathway and this is associated with both increased vascular contractility and endothelial dysfunction as well as a host of other abnormal cellular functions (50). It is thought that there is a link between endothelial dysfunction and inflammatory cytokines; both Interleukin-6 (IL-6) and C-reactive protein (CRP) have been linked to the development of central adiposity and diabetes mellitus (51-53). Further studies have demonstrated that use of pharmacological agents or reduction in visceral fat leads to a reduction in circulating concentrations of cytokines and soluble markers of endothelial activation resulting in an overall improvement in endothelial function (54).

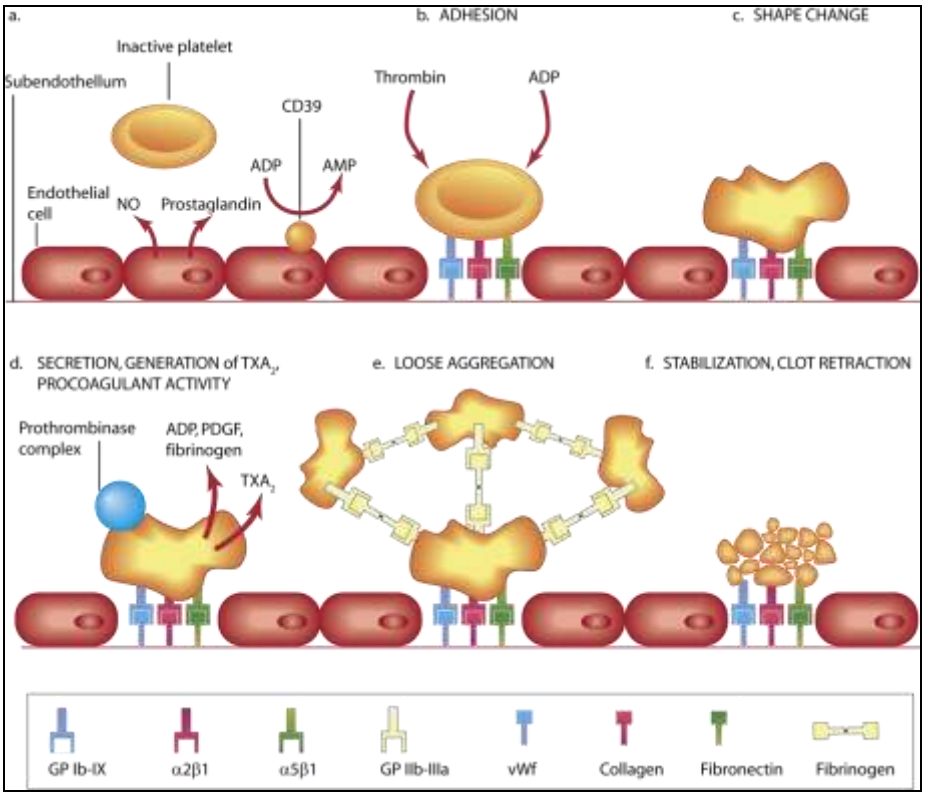
Other risk factors include oxidative stress (increased concentrations of markers including oxidized LDLs and F2-isoprostanes) and glycation of proteins resulting in formation of advanced glycation end-products (AGEs) in both LDL and within the collagen of the arterial wall which have a number of pro-atherogenic effects (55) - (56). Hyperglycaemia results in endothelial and platelet dysfunction causing impaired vasodilatation and accelerated atherogenesis (57).

1.2.3 Platelet hyper-reactivity

Patients with diabetes have increased risk of thrombogenic events due to increased platelet activity and this can be targeted by pharmacological interventions. Platelets in patients with diabetes are larger and have an increased number of glycoprotein (GP)

IIb/IIIa receptors (58) and also aggregate more easily to known agonists *in vitro* than platelets from non-diabetic patients (59). These platelets circulate in an activated state, possibly due to hyperglycaemia resulting in increased production of F2-isoprostane (57). Hyperglycaemia may also promote platelet mediated thrombosis – serum glucose levels have been shown to be an important multi-variate predictor of platelet dependent thrombosis (60). The role of platelets in thrombus formation is summarized in Figure 2.

Figure 2: The role of platelets in thrombus formation

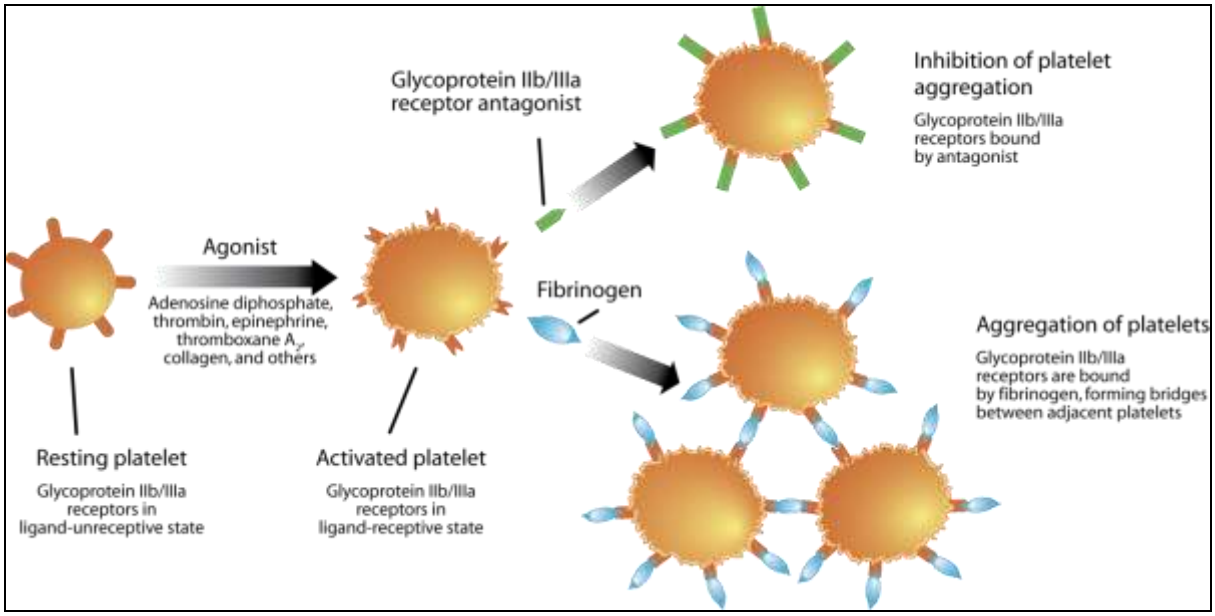


(adapted from Bhatt *et al* Nature Reviews Drug Discovery 2003 (61).) (a) Circulating platelets are usually maintained in an inactivated state by PC and NO released by endothelial cells lining the blood vessel walls. Endothelial cells also express CD39 on their surface which inhibits platelet activation by converting ADP, a potent inducer of platelet activation to AMP. (b, c) At sites of injury to the blood vessel wall, platelets adhere to the exposed sub-endothelium, through a complex series of interactions between collagen, vWF, fibronectin and their receptors on platelets. Both thrombin and ADP cause active conformation of platelets. (d) Activated platelets secrete ADP, PDGF, and fibrinogen from storage granules. TBX-A_2 is produced. (e) GPIIb/IIIa receptors on the surface of activated platelets bind fibrinogen, fibrinogen bridges form between the platelets and platelet aggregation occurs. This and the simultaneous formation of a fibrin mesh (not illustrated) lead to the formation of a platelet thrombus. (f) Clot retraction then occurs leading to formation of a stable thrombus. (Abbreviations: PC: Prostacyclin; NO: Nitric oxide; ADP: Adenosine diphosphate; AMP: Adenosine monophosphate; vWF: von Willebrand's Factor; GPIIB/IIIA: Glycoprotein IIB/IIIA; PDGF: Platelet Derived Growth Factor; TBX-A_2: Thromboxane-A2).

Endothelial dysfunction leads to a reduction in formation of both prostacyclin and NO; both are agents with potent anti-platelet aggregating properties and are released from intact endothelium. In diabetic patients and insulin resistance, the synthesis of agents which have pro-aggregating properties such as adenosine diphosphate (ADP) and thromboxane is upregulated, resulting in a further mechanism for platelet mediated athero-thrombosis (62). This forms the basis for the pronounced clinical benefit that patients with diabetes derive from antiplatelet therapies including aspirin (63-65), thienopyridine derivatives such as clopidogrel (66) and prasugrel (67) and GP IIb/IIIa inhibitors (68).

The GP IIb/IIIa receptor on the platelet surface binds fibrinogen. Once activated these receptors act as adhesion molecules resulting in platelet cross-linking, a key component of platelet aggregation (Figure 3). GP IIb/IIIa receptor inhibitors block platelet aggregation by binding to these receptors and disrupting the final common pathway (69). The use of GP IIb/IIIa inhibitors in patients with diabetes undergoing PCI has been shown to result in improved survival over both the short and long term (70;71).

Figure 3: Platelet Activation and Aggregation and Inhibition of Platelet Aggregation by Inhibitors of Glycoprotein IIB/IIIA Receptors



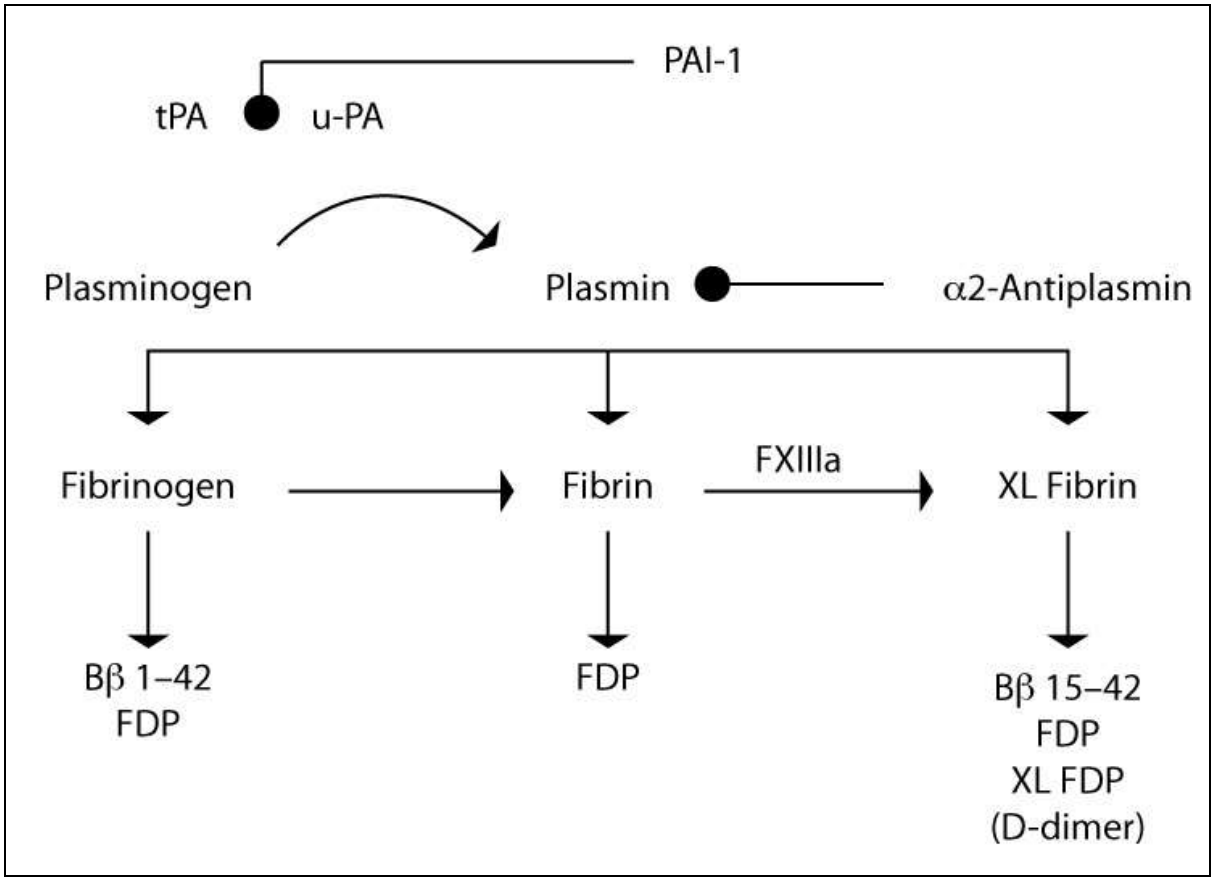
(Adapted from Yeghiazarians *et al* N Engl J Med 2000 (72)). Activation causes changes in the shape of platelets and conformational changes in glycoprotein IIb/IIIa receptors. Ligand-receptive glycoprotein IIb/IIIa receptors bind fibrinogen molecules. These form bridges between adjacent platelets and facilitate platelet aggregation. Inhibitors of glycoprotein IIB/IIIA receptors also bind to glycoprotein IIb/IIIa receptors preventing the fibrinogen from binding and preventing platelet aggregation.

1.2.4 Haemostatic abnormalities

A number of different haemostatic abnormalities are associated with diabetes. These include elevated levels of vWF, coagulation factors VII, VIII, X and fibrinogen (73-76). Reduced levels of protein C and antithrombin are also seen which are potent inhibitors of coagulation and therefore further contribute to the increased propensity for

coagulation (77).

Figure 4: The fibrinolytic pathway

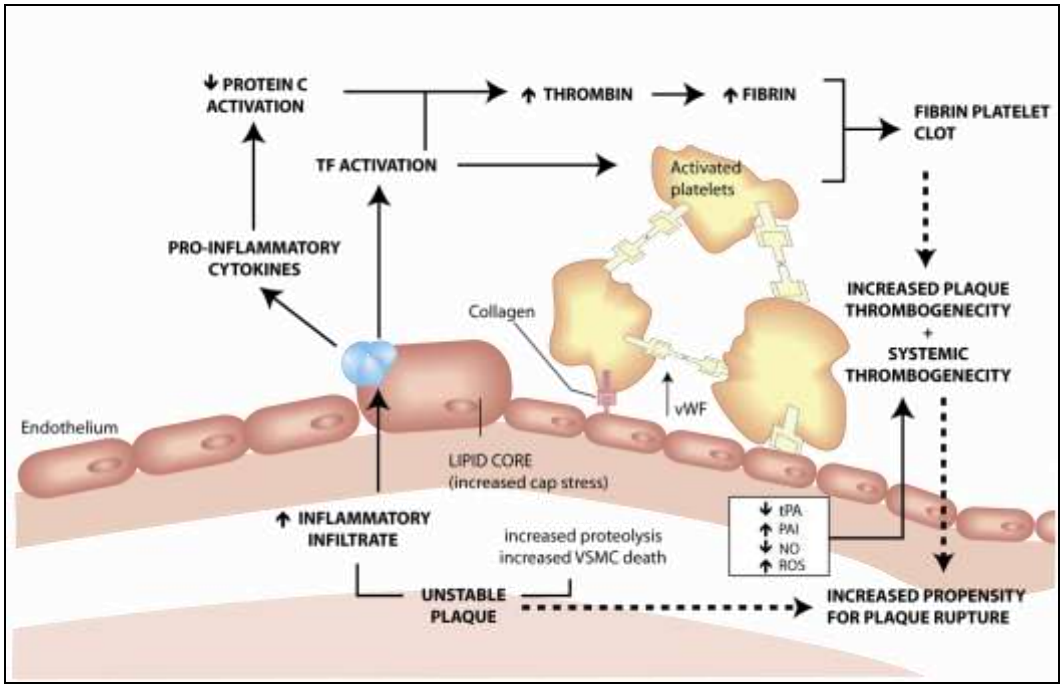


(Adapted from Deitcher *et al* clevelandclinicmed.com (78)). Physiological fibrinolysis is initiated by endothelial cell-derived tPA mediated conversion of plasminogen to plasmin. Plasmin can degrade fibrinogen and fibrin, thus limiting the size of a thrombus and helping to clear a thrombus once the vascular injury has been repaired. The fibrinolytic pathways are regulated by the inhibitory proteins α_2-antiplasmin and PA-I1. (Abbreviations: tPA: Tissue plasminogen activator; PAI-1: Plasminogen Activator Inhibitor-1).

In patients with diabetes and associated insulin resistance, suppression of the fibrinolytic pathway (Figure 4) occurs leading to increased concentrations of PAI-1 (79). There is a strong relationship between features of the metabolic syndrome and elevated PAI-1

levels with the correlation with triglyceride levels being particularly marked (79). Interestingly this is not seen in patients with T1DM in whom there is no consistent defect in fibrinolysis. Studies suggest that these patients have an increase in fibrinolytic activity and tPA concentrations and either normal or reduced levels of PAI-1 (80;81). Key processes involved in the formation of a pro-thrombotic state in diabetes are summarized in Figure 5:

Figure 5: Schematic diagram of the key processes involved in the development of a pro-thrombotic state in diabetes



(adapted from D'Souza *et al* Mol Cell Biochem 2009 (36)). Diabetes mellitus accelerates the athero-thrombotic process by decreasing fibribinolytic capacity and mediating a shift to a pro-coagulant state characterized by increased inflammatory mediators, PAI-1, plasma fibrinogen, vWF and thrombin. (a) Endothelial dysfunction and NO deficit in diabetes impair endogenous inhibition resulting in enhanced platelet susceptibility to aggregate in response to release of agonists from the inflammed endothelium. T-lymphocytes stimulate plaque macrophages resulting in increased expression of the potent procoagulant Tissue Factor. This highlights the relationship between arterial inflammation and athero-thrombosis. (b) Post-plaque rupture, adhesion molecules (e.g. vWF and collagen) activate circulating platelets resulting in aggregation. Tissue Factor is released from the lipid core and the subendothelial matrix activates the coagulation casade with thrombin generation and fibrin formation. The overall effect is the generation of an environment which facilitates the generation and persistence of coronary thrombi and may precipitate acute coronary syndromes. (Abbreviations: TF: Tissue Factor; vWF: von Willebrand's Factor; tPA: Tissue Plasminogen Activator; PAI-1: Plasminogen Activator Inhibitor-1; NO: Nitric oxide; ROS: Reactive Oxygen Species).

1.2.5 Adverse arterial remodelling

In patients with diabetes an accelerated process of both atherosclerosis and restenosis occurs (39;82). In the early stages of atherosclerotic plaque formation a process of negative arterial modelling occurs with lack of collateral vessel formation and an increased risk of late vessel occlusion following balloon angioplasty although this is not the case with intracoronary stenting (34;83-85). The lack of ability in patients with diabetes to collateralize occluded arteries is linked to the endothelial dysfunction described previously. Decreased expression of vascular endothelial growth factor (VEGF) in the myocardium and also low levels of the VEGF receptor have been implicated in this process (86).

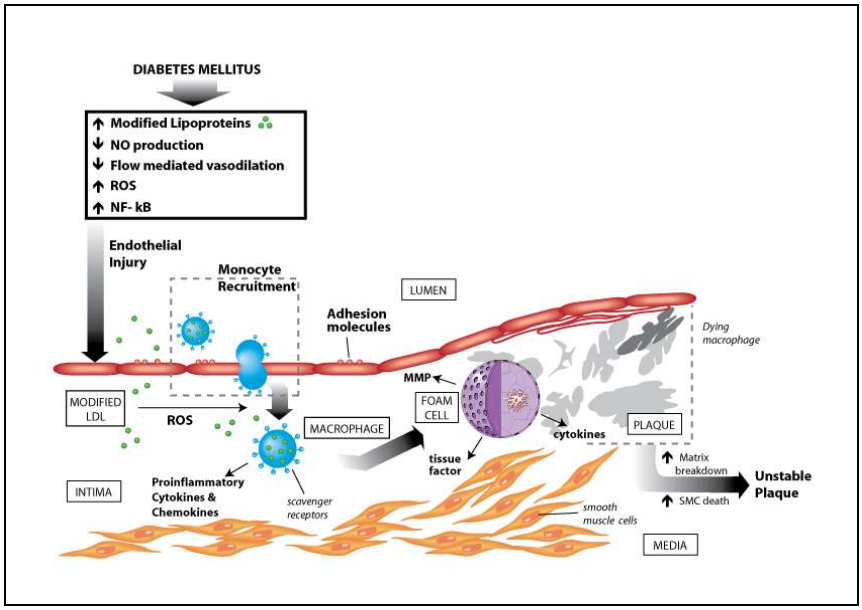
Patients with diabetes lack an adaptive arterial response and vessel contracture occurs to the extent that a lower plaque burden can cause a more significant stenosis in diabetic patients when compared to non-diabetic subjects (87). Patients with diabetes therefore display a tendency to have smaller arteries, with lesions of longer length and diffuse disease which provide a host of challenges to performing successful revascularization (39).

1.2.6 Accelerated atherosclerosis

Both endothelial dysfunction and the pro-thrombotic state which occur in patients with diabetes and have been previously described, contribute to the aggressive atherosclerotic process observed (summarized in Figure 6). Hyperglycaemia via a number of chemical pathways, leads to the formation of AGEs. Engagement of

RAGE, the cell surface receptor for AGEs activates an inflammatory process involving endothelial cells, smooth muscle cells and macrophages. These cells all play key roles in the process of atherogenesis and further multiply the inflammatory stimuli to the arterial wall. RAGE therefore plays a key role in the progression of atheroma and plaque vulnerability in diabetic patients (55;88;89). Administration of soluble RAGE has been shown to retard the progression of already established atheroma, reducing lesion size and altering plaque characteristics with reduced inflammation and increased stability (90).

Figure 6: Schematic diagram of the inflammatory mechanisms involved in plaque formation in diabetes accelerated atherosclerosis



Adapted from D'Souza *et al* Mol Cell Biochem 2009 (36). This figure illustrates the key interactions between the inflammatory infiltrate and the vascular wall in diabetes-accelerated atherosclerosis with a temporal sequence from left to right. Multiple atherosclerotic risk factors increase oxidative stress in vascular cells, activating signalling molecules such as NF-κB. This results in endothelial dysfunction characterised by decreased NO synthesis and increased entry of modified circulating lipoproteins into the vessel wall. In response to this inflammatory stimulus, the dysfunctional endothelium generates up-regulation of adhesion molecules (VCAM, ICAM), facilitating adherence and trans-endothelial migration of leucocytes into the tunica intima. In the intima, inflammatory mediators e.g. M-CSF augment expression of scavenger receptors which detect and internalise modified lipoprotein particles by macrophages. Ultimately this results in foam cell formation and initation of the atherosclerotic plaque. As the lesion evolves, lymphocytes and resident cells of the vascular wall secrete cytokines and growth factors. This encourages migration of vascular cells into the intima and penetration through the elastic lamina and collagenous matrix of the evolving plaque. MMP secreted by macrophages degrade the ECM which plays a key role in plaque stability. T-lymphocytes produce IFN-γ which limits collagen production. The overall result is a vulnerable plaque with extensive inflammatory infiltrate, a lipid core and a thin fibrous cap which is highly susceptible to rupture. (Abbreviations: ICAM: Inter-Cellular Adhesion Molecule, NO: Nitric oxide, VCAM: Vascular Cell Adhesion Molecule; NF-KB: Nuclear Factor Kappa B; M-CSF:Macrophage Colony Stimulating Factor; MMP: Matrix Metallo-Proteinases).

Haemoglobin A1c (HbA1c) is a protein (haemoglobin) that has undergone non-enzymatic glycation and provides a surrogate marker for glycaemic control over the previous 3 month period (the life-span of a red blood cell). Furthermore HbA1c correlates with AGE levels. The multi-factorial complexity of diabetic vascular disease suggests the need for tight glycaemic control and an overall strategy of risk factor modification (91;92). However uncertainties remain over very aggressive regimes with 2 recent trials showing that intensive therapy may be associated with increased mortality (93;94).

1.2.7 Cellular proliferation and restenosis

A number of studies have demonstrated that diabetes is an independent risk factor for restenosis following balloon angioplasty (83;84) with reported rates ranging from 35-71%. Restenosis occurs due to 3 factors: abrupt vessel closure, adverse arterial remodelling and neo-intimal proliferation.

Arterial remodeling and acute recoil of balloon injured vessels play a significant role in the restenosis process following balloon angioplasty with a lesser contribution from neo-intimal proliferation (95;96). In diabetic patients, collagen rich sclerotic content is increased in restenotic lesions undergoing remodeling and elastic recoil. This suggests that there is an aggressive fibrotic rather than proliferative response occurring in post angioplasty stenosis in diabetic vessels (97). Late vessel occlusion following balloon angioplasty is associated with a marked reduction in ejection fraction (98) and is a key determinant of long term mortality in patients with diabetes (99). Data from studies using intravascular ultrasound (IVUS) suggest that intimal hyperplasia is responsible for the

increased restenosis rates seen in diabetic patients (82). The advent of coronary stenting led to an overall reduction in restenosis rates in both diabetic and non-diabetic populations by eliminating abrupt vessel closure and vessel remodeling following PCI and also reduced the rate of occlusive stenosis in diabetic patients closer to the levels seen in non-diabetic patients (100;101).

Both insulin and glucose have been put forward as key players in the neo-intimal proliferation causing restenosis in patients with diabetes although no direct link has been identified associating glycaemic control with restenosis. Insulin is a weak growth factor and co-expression of insulin-like growth factor-1 (IGF-1) and platelet derived growth factors which both have powerful mitogenic effects may play a contributory role (102).

1.3 Cardiovascular disease in patients with CKD

Patients with CKD, when compared to age-matched controls have a much higher rate of cardiovascular mortality and this is multi-factorial in aetiology (103). It has been shown that the relative risk of all-cause death is increased by approximately three-fold in patients with a serum creatinine > 150 µmol/l (104). For patients on dialysis this risk is even higher – ten-fold to a hundred-fold higher than for age and sex-matched controls in the general population (105). Possibly due to this high mortality patients with severe CKD have predominantly been excluded from trials investigating interventions to ameliorate cardiovascular risk (106). A number of clinical trials currently underway are seeking to address this (107-109). Patients with CKD are in a persistent catabolic state resulting in a systemic syndrome of malnutrition, chronic inflammation and

atherosclerosis, with upregulation of many acute phase proteins and cytokines (110). This becomes particularly prevalent once patients reach CKD Stage 5 (estimated glomerular filtration rate (eGFR) < 15 ml/min) (111).

A computerised database (NEOERICA) from primary care in the United Kingdom has demonstrated the influence of CKD on the prevalence of cardiovascular comorbidities (112). The authors found that the prevalence of CHD is approximately 25% in a patient population with CKD Stage 3 (eGFR 30 – 59 ml/min) – Stage 5 (eGFR < 15 ml/min) and this is more than double that for patients with normal renal function. This increased prevalence is also seen in congestive cardiac failure (CCF), peripheral vascular disease (PVD) and cerebrovascular disease. Data from the Framingham Heart Study shows that when patients progress from normal renal function to a eGFR < 45 ml/min the burden of any cardiovascular disease is significantly higher (113) and this increased cardiovascular risk predicts a more rapid decline in renal function when adjusted for baseline eGFR (114). This may occur through an 'upregulated pro-inflammatory' state as has been described above (111). At the time of referral to a nephrologist approximately 1/3 of patients with CKD already have manifestations of CHD (103).

Data from the NEOERICA study (112) also demonstrated that > 50% of patients ≥ 85 years have CKD and questions remain as to whether this is simply part of a normal ageing process. If so, this might suggest that a proportion of CKD is not clinically relevant as many of these patients are not symptomatic and cardiovascular risk modification strategies in this age group do not have clear benefits in terms of improvement in morbidity and mortality (103). It is important to note however that conditions such as diabetes which contribute to the development of both

cardiovascular and renal disease may not present with typical symptoms of coronary ischaemia (115). The clinical challenge is therefore to establish on an individual patient basis the importance of impaired renal function and address cardiovascular risk modification appropriately.

1.3.1 Cardiovascular Risk Factors in Patients with CKD

Patients with CKD have higher rates of cardiovascular morbidity and mortality than are predicted by the Framingham model of cardiovascular risk (116). As has been described previously renal dysfunction leads to a catabolic state which leads to chronic inflammation resulting in accelerated atherosclerosis and the development of cardiovascular disease (117).

Hypertension, dyslipidaemia, diabetes and obesity are well established risk factors for cardiovascular disease and are highly prevalent in populations with CKD (107;118). Other cardiovascular risk factors have been identified to occur more frequently in patients with CKD than in a general population including anaemia, abnormal metabolism of calcium and phosphorous, carnitine deficiency, hyperhomocysteinemia, proteinuria, inflammation and oxidative stress and high lipoprotein a levels and small lipoprotein a (LpA) size (reviewed (103;107)). However the relative contribution of traditional and non-traditional risk factors to the risk of developing cardiovascular disease is not fully understood. In order to be of clinical relevance individual risk factors must be applicable to a general population. For example elevated levels of homocysteine are associated with increased risk of cardiovascular events in patients with CKD (119-122). Levels can be controlled with dietary supplementation of pyridoxine, Vitamin B12 and folic acid

(reviewed (123)). Measurement of homocysteine levels is not currently a part of routine clinical practice as such modification has not been able to demonstrate a consistent benefit in reducing cardiovascular risk in renal patients (124-127).

1. 3.1.1 Diabetes and CKD

Patients with diabetes and CKD are a particular challenge: they have a higher incidence of cardiovascular co-morbidities such as CHD and PVD than any other patient group with CKD (128;129). Mortality in diabetes patients becomes markedly elevated with the onset of proteinuria (130;131) and it is thought that this acts as a surrogate marker for endothelial dysfunction and therefore increased cardiovascular disease (132). In patients with diabetes, microalbuminuria is an independent predictor of increased cardiovascular mortality (129;133); the presence of microalbuminuria increases the risk of fatal CHD by a factor of 2 to 4. One possible explanation for this is that the early stages of diabetic nephropathy are associated with the co-existence of multiple cardiovascular risk factors including hypertension, dyslipidaemia and a hypercoagulable state (134).

Hypertension is a frequent co-morbidity in diabetic nephropathy even when creatinine levels remain within normal limits and can further exacerbate coronary disease in diabetic patients. Microalbuminuria is associated with a pro-atherogenic lipid profile including elevated concentrations of LDL and chylomicron remnants, decreased concentrations of HDL and increased concentrations of LpA (135-137). PAI-1 activity, factor VII and plasma fibrinogen are all up-regulated in patients with T1DM and microalbuminuria (138;139). Diabetic nephropathy also results in accelerated

accumulation of AGEs in both the circulation and tissue which correlates with the severity of renal impairment (88).

Strategies to modulate cardiovascular risk in diabetic patients include general measures such as weight loss and blood pressure control and glycaemic control (140). In an attempt to reduce the risk of cardiovascular disease, a number of different trials have looked at various different glucose lowering strategies. However, the effect of glucose lowering on cardiovascular outcomes remains an area of contentious debate as evidenced by the class IIb recommendation from the joint American Diabetes Association (ADA), American Heart Association and American College of Cardiology (141). One concern is that glucose lowering may be a weaker intervention than previously envisaged with individual trials being underpowered to detect changes in a chosen end-point. Another is that current therapies may negate the effects of glucose lowering e.g. weight gain which has 'knock on' effects on blood pressure and lipids (142). The largest study of intensive glucose lowering regimes compared to standard treatment for cardiovascular outcomes, combined data from 5 randomised controlled trials on 33040 patients (143). There were 1497 non-fatal MI, 2318 coronary events, 1127 strokes, 2892 deaths and 1391 cases of new or progressive heart failure. A 0.9% lowering in HbA1c (7.5% vs. 6.6%) was associated with a 17% reduction in non-fatal MI, a 15% reduction in coronary events and a trend towards decreased stroke risk. There was no association between intensive and standard treatment and mortality and heart failure. A recent meta-analysis adds data on 8 extra studies with 1493 additional participants (144). The cardiovascular data are broadly consistent with earlier reports, demonstrating a 15% reduction in non-fatal MI but no clear effect on other cardiovascular disease outcomes. There were 54 non-fatal MI, 209 deaths and 187

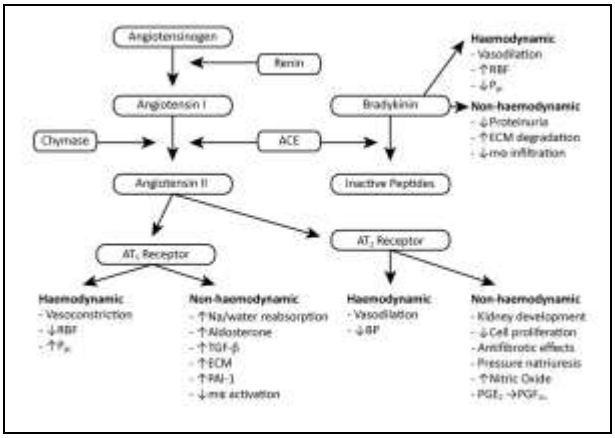
cases of new or worsening heart failure. It is important to note that the sensitivity analysis of intensive glucose lowering and heart failure events is dominated by one study, the PROspective pioglitazone Clinical Trial in macroVascular Events (PROactive) (145). The data are also consistent with earlier evidence that the cardiovascular benefit of intensive glucose lowering in isolation appears to be modest at best and that this is less efficacious and more difficult to achieve then interventions targeting lipid and blood pressure control; it would therefore suggest that a combined approach targeting all 3 risk factors is the optimal strategy (146). Further studies will be necessary to ascertain whether an absolute HbA1c target should be achieved or whether attempts should be targeted at a target percentage reduction from baseline.

Even more uncertainty exists as to the best index of glycaemic control in patients with advanced CKD (147). A large epidemiological study did not demonstrate a relationship between HbA1c and death in patients on dialysis (148). Conversely, a recent study from the United States in > 20 000 patients undergoing dialysis, showed that HbA1c levels were incrementally associated with a higher risk of cardiovascular death but only after adjustment for confounding variables (including age, length of time on dialysis and markers of nutrition and inflammation) (149). HbA1c may be unreliable in uraemia due to the unpredictable influences of carbamylated haemoglobin and/or variable red blood cell survival and may underestimate true glycaemic control. Glycated albumin is a possible alternative that requires further investigation (150).

Interventions in diabetic patients with renal impairment have concentrated on reducing conversion of normalbuminuria to microalbuminuria (151;152), delaying the onset of diabetic nephropathy in patients with microalbuminuria (153;154) and delaying further

deterioration in renal impairment once diabetic nephropathy has occurred (155;156). The main pathway targeted by these interventions is the renin angiotensin system (RAS) (Figure 7). Once diabetic nephropathy is established intervention with agents that block the RAS system have not been shown to impact on cardiovascular risk (155). This highlights the importance of instituting treatment at the early stages of kidney damage in order to improve cardiovascular outcome in patients with diabetes.

Figure 7: Schematic diagram depicting the Renin Angiotensin System



(adapted from Taal and Brenner Kidney International 2000 (157)). Abbreviations: ACE: Angiotensin Converting Enzyme; ECM: Extracellular matrix; mφ: Macrophage; PAI-1: Plasminogen Activator Inhibitor-1; PG: Prostaglandin; P_{gc}: glomerular capillary hydraulic pressure; RBF: Renal blood flow; TGF-β:Transforming Growth Factor-β; AT_1: Angiotensin II Type 1; AT_2: Angiotensin II Type 2).

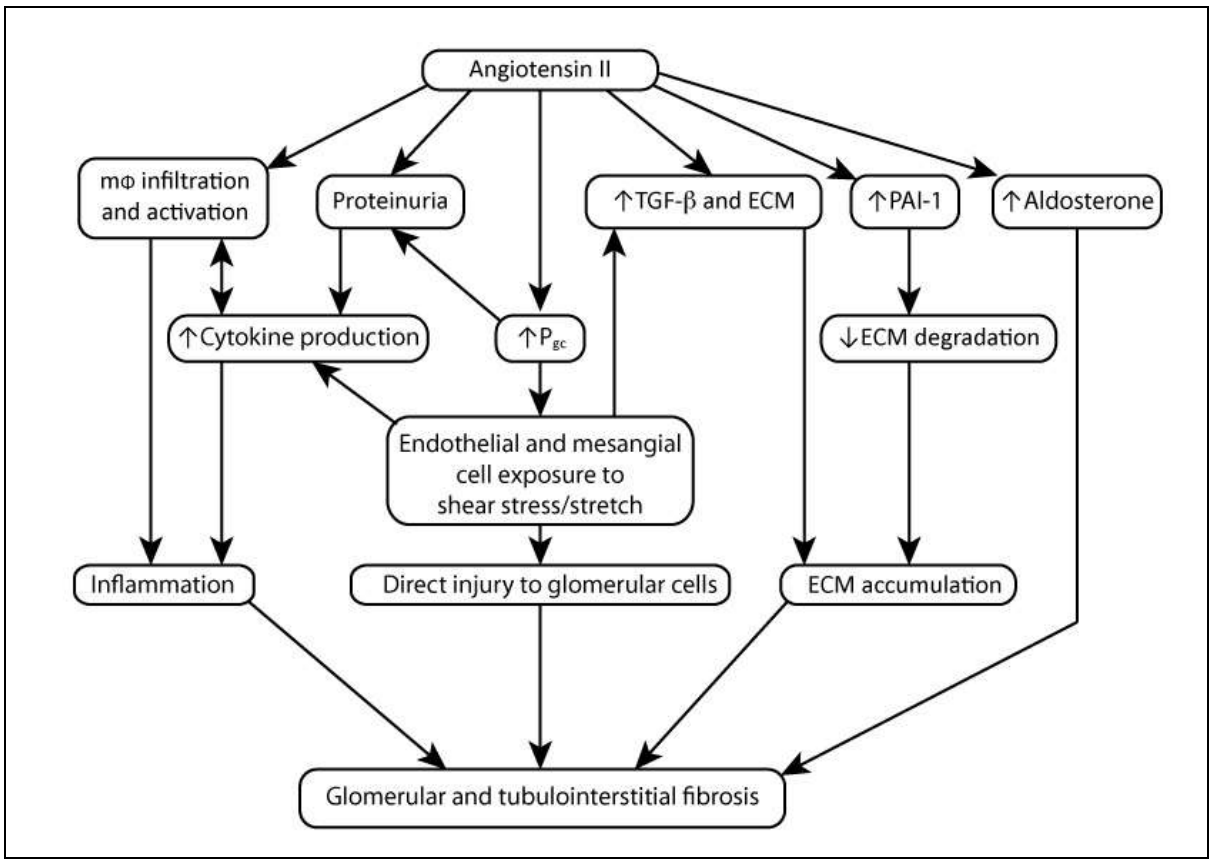
1.3.1.2 Hypertension and CKD

Renal impairment results in glomerular sclerosis and interstitial disease, activation of the RAS and leads to the development of fluid overload and increased arterial stiffness

resulting in hypertension. Essential hypertension itself, causes microvascular damage to the renal vascular bed and resulting renal impairment leads via RAS activation to further exacerbation of hypertension (103). Renal physiology is altered with increased filtration fraction of sodium and increased renovascular resistance (158). The majority of patients with CKD are hypertensive and prevalence rises with increasing severity of CKD: at eGFR <30 ml/min >90% of patients are hypertensive (111). As CKD progresses the diurnal variation in blood pressure starts to change, resulting in loss of the physiological nocturnal 'dip' and this is a marker for the development of left ventricular hypertrophy (LVH) (159). In dialysis patients the relationship between systolic blood pressure and death has a 'U'-shaped distribution, with both low and high levels of blood pressure associated with increased mortality

When hypertension is treated rates of progression of CKD significantly decrease (155;156;160-162). Again the key pathway targeted by therapeutic agents is the RAS showing the importance of blocking the formation of angiotensin II (Figure 8) which is both an endothelial growth factor in the renal vascular bed and also a powerful vasoconstrictor (163;164).

Figure 8: Diagrammatic representation of the central role of angiotensin II in the pathogenesis of progressive renal injury



(adapted from Taal *et al* Kidney International 2000 (157)). The role of angiotensin II in the pathogenesis of progressive renal injury and fibrosis following nephron loss. (Abbreviations: ECM: Extracellular matrix; mφ: Macrophage; PAI-1: Plasminogen Activator Inhibitor-1; P_{gc}: glomerular capillary hydraulic pressure; RBF: TGF-β:Transforming Growth Factor-β).

The ideal blood pressure level to aim for in patients with CKD remains an area of controversy. A meta-analysis looking at randomized controlled trials of ACE-inhibitors demonstrated that systolic blood pressure > 120 mmHg was associated with increased risk of progressive renal dysfunction. However this was also seen with systolic blood pressures < 110 mm Hg, probably due to relative hypoperfusion of the kidney with low

systolic blood pressure secondary to heart failure (165). The current British Renal Association Guidelines (166) reinforce the guidance from the National Institute of Clinical Excellence (NICE) (167) and suggest aiming for a target blood pressure of < 140/90 mmHg in patients without significant proteinuria and < 130/80 mmHg in patients with proteinuria or diabetes mellitus.

1.3.1.3 Dyslipidaemia

Dyslipidaemia is a common finding in patients with CKD Stage 3-4. The abnormal lipid profile is characterized by elevated serum triglyceride and VLDL cholesterol levels, decreased serum HDL cholesterol levels and normal or decreased serum LDL levels (168). Patients with impaired renal function also have increased levels of plasma LpA which is associated with an increased risk of cardiovascular disease (169;170).

A meta-analysis of randomized clinical trials of statins vs. placebo in over 6500 patients with CKD showed that statin use resulted in a significant reduction in lipid concentrations and the incidence of cardiovascular events. Moreover, statins were well tolerated without increased rates of adverse events such as liver dysfunction (171). A secondary analysis from the Treating to New Targets study demonstrated that 80 mg daily of atorvastatin significantly reduced cardiovascular events in patients with CKD as compared to a 10 mg dose and again was well-tolerated (172). On initial reflection these observations suggest that statins should be used in Stage 3-4 CKD in much the same way as they are used in the general population. It is important to note however that the vast majority of patients in these trials had GFR > 30 ml/min/1.73 m^2 (CKD Stages 1-3).

To date (2011), there have been only three randomized clinical trials of statin therapy in patients on dialysis completed and published in the international literature. The 4D Study (Die Deutsche Diabetes Dialyse Studie) found no significant benefit of statin therapy on cardiovascular outcomes despite significant reductions in LDL cholesterol. The AURORA (A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Haemodialysis) Trial was an international multicentre randomized double-blind trial of 2776 patients undergoing haemodialysis. Patients were randomly assigned to 10 mg rosuvastatin or placebo. The primary endpoint was the time to a major cardiovascular event (defined as non-fatal myocardial infarction (MI), non-fatal stroke or death from cardiovascular causes. Mean length of follow-up was 3.2 years. Rosuvastatin had no significant effect on the composite primary end-point. Furthermore whilst rosuvastatin lowered lipid levels there was no relationship between the primary cardiovascular end-point and either baseline LDL cholesterol or levels at 3 months. The SHARP (Study of Heart And Renal Protection) trial recently reported on the safety and efficacy of the combination of simvastatin with ezetimibe, an agent which decreases absorption of cholesterol from the intestine, in patients with CKD (173). 9270 patients with CKD (3023 on dialysis) with no known history of previous MI or coronary revascularisation procedures were randomly assigned to either the combination of simvastatin 20 mg and ezetimibe 10 mg daily versus matching placebo. The main pre-specified outcome was first major atherosclerotic event. Simvastatin-ezetimibe significantly reduced the incidence of atherosclerotic events but had no effect on survival. Further trials will be necessary to fully determine the role of statins and other lipid lowering agents in patients with CKD (108).

1.3.1.4 Vascular Calcification

Atherosclerotic disease in the general population is manifest by intimal fibro-fatty plaque formation which may then become calcified. The medial layer of elastic arteries may also become involved in atherosclerosis with thickening and development of calcification, a phenomenon known as 'medial calcinosis' (174;175). This results in increased pulse wave velocity, elevated pulse pressure and development of systolic hypertension (176;177). Medial calcinosis is more common in diabetes patients, the elderly and patients with CKD and is known to be associated with increased cardiovascular mortality (178;179).

There is currently an incomplete understanding of the pathophysiology of vascular calcification in CKD. Arterial calcification involves the transformation of vascular smooth muscle cells into bone forming osteoblast-like cells expressing bone matrix proteins which are involved in arterial calcification (180). Several other factors (including hyperphosphataemia, hyperparathyroidism, hyperhomocysteineaemia and elevated levels of AGEs) are elevated in patients with CKD and can accelerate vascular calcification (103). Abnormal bone mineralization also contributes with both high and low bone turnover states occurring in patients with renal dysfunction (181). In high bone turnover states, both osteoblast and osteoclast activity are increased, preventing adequate mineralization of newly formed bone. In low ('adynamic') bone turnover excessive hypercalcaemia is a frequent occurrence as the skeleton cannot act as an efficient buffer for calcium or phosphate. This situation can be further exacerbated by oral phosphate binders, taken by patients to attempt to prevent gastrointestinal phosphate absorption which may contain calcium.

In dialysis patients links exist between decreased bone mineral density and increased coronary artery calcification (182). Both high and low bone turnover states can lead to development of vascular calcification and whilst controlling phosphate levels is of critical importance, phosphate binders with high calcium content are arguably not desirable.

Adynamic bone disease often occurs in patients with relatively low levels of parathyroid hormone (PTH), < 150 pg.ml and may result in part from 1,25-dihydroxyvitamin D_3 deficiency (183). The most frequent bone abnormality in patients with CKD is high turnover bone disease. In pre-dialysis CKD patients, > 90% have this form of bone histology compared to < 5% who exhibit the adynamic modality (184). Recent data suggest that adynamic bone disease may also constitute a risk factor for CVD if there is con-comitant aluminium or calcium overload, due to the inability of the bone to buffer calcium (178;185). Whether induction of adynamic bone disease by low PTH levels, without evidence of aluminium or calcium is related to increased cardiovascular morbidity and mortality remains an area of debate. Recent studies of dialysis patients undergoing parathyroidectomy showed improvement in long-term survival following parathyroid gland removal (186). Further studies will be necessary to confirm the survival benefits associated with lower PTH levels (187).

Fibroblast growth factor 23 (FGF-23) has recently been discovered to have a role in the regulation of phosphate and mineral metabolism. Its main physiological role is in modulating phosphate excretion (188) . Levels are associated with both progression of CKD and mortality in diabetes patients (189). Loss of FGF-23 is implicated in vascular calcification and therefore genetic variation may influence calcification in CKD (103).

There is relatively little data in this area regarding dialysis and low clearance patients although there is some evidence that in new-onset haemodialysis patients progression of coronary calcification is less aggressive in those taking non-calcium containing phosphate binders e.g. sevelamer (190). A mortality benefit has not yet been shown in the overall dialysis population. There is evidence for a reduction in mortality in patients aged > 65 years after 2 years of sevelamer therapy compared to patients on a calcium based phosphate binder (191).

1.3.1.5 Left ventricular hypertrophy in CKD

Even in the very early stages of renal impairment there is a higher prevalence of LVH than occurs in the general population. A prospective echocardiographic study of patients attending a low clearance clinic demonstrated LVH in 27% of patients with a creatinine clearance (CrCl) > 50 ml/min. In patients with CrCl 25 – 50 ml/min and < 25 ml/min the prevalence was 31% and 45% respectively (192).

The cardiomyopathy of renal failure results from a combination of pressure overload and volume overload. In the early stages these are physiological ‘adaptive’ compensations to attempt to maintain adequate stroke volume. Arterial stiffness and calcification lead to the development of hypertension and development of concentric LVH. Activation of the RAS leads to salt and water retention, resulting in volume overload and left ventricular dilatation and this process is exacerbated by anaemia. The resulting cardiomyopathy leads to the development of both systolic and diastolic dysfunction (193).

1.3.1.6 Other risk factors

Proteinuria

Proteinuria is associated with both traditional and non-traditional risk factors for development of cardiovascular disease (107). Patients with proteinuria suffer particularly rapid deterioration in renal function and therefore might be more susceptible to the putative accelerated atherosclerosis that is attributable to low GFR. Albuminuria may additionally indicate diffuse endothelial dysfunction or be a surrogate marker for atherosclerotic plaque burden (134). Proteinuria is also associated with abnormalities of fibrinolysis, predisposing affected individuals to abnormal coagulation and thrombosis (194). Proteinuria is thought to directly increase cardiovascular risk rather than being a marker for increased pre-existing vascular burden as interventions that produce significant reductions in albuminuria are associated with substantial cardiovascular benefit (195).

Coagulation

Patients with CKD are more likely to express markers of a procoagulant state than a general population, despite having a higher risk of bleeding (107). Patients with Stage 3-4 CKD have higher levels of fibrinogen (a marker of coagulability associated with an increased risk of myocardial infarction) than is seen in patients with normal renal function (196). A meta-analysis of trials in which the primary outcome was failure of vascular access showed that anti-platelet therapy significantly reduced the risk of serious vascular events (197).

Anaemia

Anaemia is associated with a number of unfavourable conditions including cardiomyopathy and LVH (198). Several recent studies have compared ‘partial’ and ‘complete’ correction of anaemia with erythropoietin in patients with CKD and have failed to demonstrate a consistent benefit in terms of improved quality of life (107). At least 2 trials have been terminated prematurely because of concerns regarding futility or adverse events (199) (200). No trial to date has shown that erythropoiesis-stimulating therapy results in improved cardiovascular outcomes when compared to placebo in patients with CKD (201) (202).

1.3.2 Strategies for management of cardiovascular risk factors in CKD

Strategies for the management of cardiovascular risk in patients with CKD are summarized in Table 2.

Table 2: Strategies for Cardiovascular Risk Modification in patients with CKD

Risk Factor	**Stage of CKD**	
	3 – 4	**5D (Dialysis)**
Smoking	Recommend cessation	Recommend cessation
Diet	Ensure sodium intake < 2.4 g /day	Ensure sodium intake < 2.4 g /day
Exercise	Aim for 30 – 60 minutes of moderate intensity dynamic exercise 4 – 7 days/week	Aim for 30 – 60 minutes of moderate intensity dynamic exercise 4 – 7 days/week
Hypertension	**Treatment goal:** BP <130/80 mmHg **Pharmacotherapy:** Proteinuric CKD: (urine albumin:creatinine ratio ≥ 30 mg/mmol) should include and ACE-I or ARB Non-proteinuric CKD: (urine albumin:creatinine ratio < 30 mg/mmol) treat with either an ACE-I or ARB , a thiazide diuretic, a β-blocker (in patients < 60 years) or a long acting CCB	**Treatment goal:** Predialysis and postdialysis BP should be < 140/90 mmHg and <130/80 mm Hg respectively. Consider nocturnal dialysis if BP or ECF volume are difficult to control **Pharmacotherapy:** ACE-I and ARB are preferred. Diuretics are not recommended. Sotalol is contraindicated. Spironolactone with caution to avoid ↑K^+
Diabetes mellitus	**Treatment goal:** HbA1c < 7%, FPG 4-7 mmol/l **Pharmacotherapy:** Metformin is acceptable in stable Stage 1-3 CKD, Repaglinide is acceptable and needs no dose adjustment, short-acting sulphonylureas e.g. gliclazide are preferred over longer acting agents. Sulphonylureas and insulin require dose adjustment	**Treatment goal:** Optimal HbA1c is unknown **Pharmacotherapy:** Metformin should be discontinued, sulphonylureas and insulin require dose adjustment
Dyslipidaemia	**Treatment goal:** LDL cholesterol targets should follow guidelines for the general population **Pharmacotherapy:** Statin Dose adjustment required for fibrates only	**Treatment goal:** unknown **Pharmacotherapy:** Statin if benefits outweigh risks Dose adjustment required for fibrates only
Anaemia	**Treatment goal:** 10-12 g/dl Balance CV risks against QoL benefits on an individual basis **Pharmacotherapy:** Iron supplementation, Erythropoiesis stimulating agents	**Treatment goal:** 10-12 g/dl Balance CV risks against QoL benefits on an individual basis **Pharmacotherapy**: Iron supplementation, Erythropoiesis stimulating agents
Other	Aspirin daily if high CV risk or established CV disease and no contraindication	Aspirin daily if high CV risk or established CV disease and no contraindication

(adapted from Rucker and Tonelli Nature Review Nephrology 2009 (107) and incorporating current guidelines(166;203-206). Abbreviations: CKD: Chronic kidney disease; BP: Blood pressure; ACE-I: Angiotensin Converting Enzyme Inhibitor; ARB: Angiotensin II Receptor Blocker; CCB: Calcium channel blocker; FPG: Fasting Plasma Glucose; CV: Cardio-Vascular; QoL: Quality of Life).

1.4 Classification of Kidney Disease

1.4.1 Chronic Kidney Disease

CKD is an area of intensive clinical and epidemiological research (207). A number of different definitions of CKD are used in the literature which can cause difficulties with data comparison and also may call into question the validity and reliability of research findings (208-210). In 2002 the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) formulated guidelines providing clear definitions and classification of CKD (211). The classification defines CKD as the presence of kidney damage or glomerular filtration rate (GFR) of < 60 ml/min/1.73 m^2 for at least 3 months. CKD is further classified into 5 stages according to the estimated eGFR (Table 3).

Table 3: The National Kidney Foundation Kidney Disease Outcomes Quality Initiative

Stage	Clinical Features	GFR (ml/min/1.73m^2)
I	Kidney Damage* with normal or increased GFR	≥ 90
II	Kidney Damage* with a mild decrease in GFR	60 – 89
III	Moderate decrease in GFR	30 – 59
IV	Severe decrease in GFR	15 – 29
V	Kidney Failure	< 15 or dialysis

*Kidney Damage = pathological abnormalities or markers of disease present in the urine, blood or on imaging modalities

(Table adapted from Anderson and Glynn 2011 (207))

It was thought that these guidelines would establish a benchmark for both research and clinical practice (212). However there has been extensive criticism and calls for

amendments (213-215). These have included increasing the 'chronic' aspect of the definition by increasing the 3 month time frame to 6, 9 or 12 months (216). It has also been suggested that these guidelines 'over-label' elderly patients with mild impairment of renal function as having a disease (217). It is important to note that despite the calls for lengthening the chronicity aspect of the CKD definition, many large CKD prevalence and mortality studies including the National Health Nutrition and Examination Survey have chosen to use only one serum creatinine reading to define CKD, therefore not accounting for the inherent chronicity of this disease (218;219).

There has also been great debate with regard to the optimal method of measuring kidney function. The 'gold standard' to assess kidney function is direct measurement of GFR from 24 h urine collection by isotopic techniques but this is not practical in many settings (207); therefore equations are used to calculate an eGFR, rather than direct measurement.

The 2 equations most commonly used to estimate kidney function are the Cockcroft Gault equation (for determining creatinine clearance (CrCl)) (220) and the Modified Diet in Renal Disease (MDRD) equation (for estimating eGFR) (221). The majority of epidemiological studies use the MDRD equation and its use is also widespread in clinical laboratories (222-226). Historically the Cockcroft-Gault equation has been recommended for use when calculating medication dosage although recently the MDRD formula has been used for this purpose as well (227). However both equations have been criticised for biases and inaccuracy (213;228). A number of new equations have been proposed to attempt to overcome some of these limitations but need to be further

developed, validated and adopted through a process of international consensus (217;229-231).

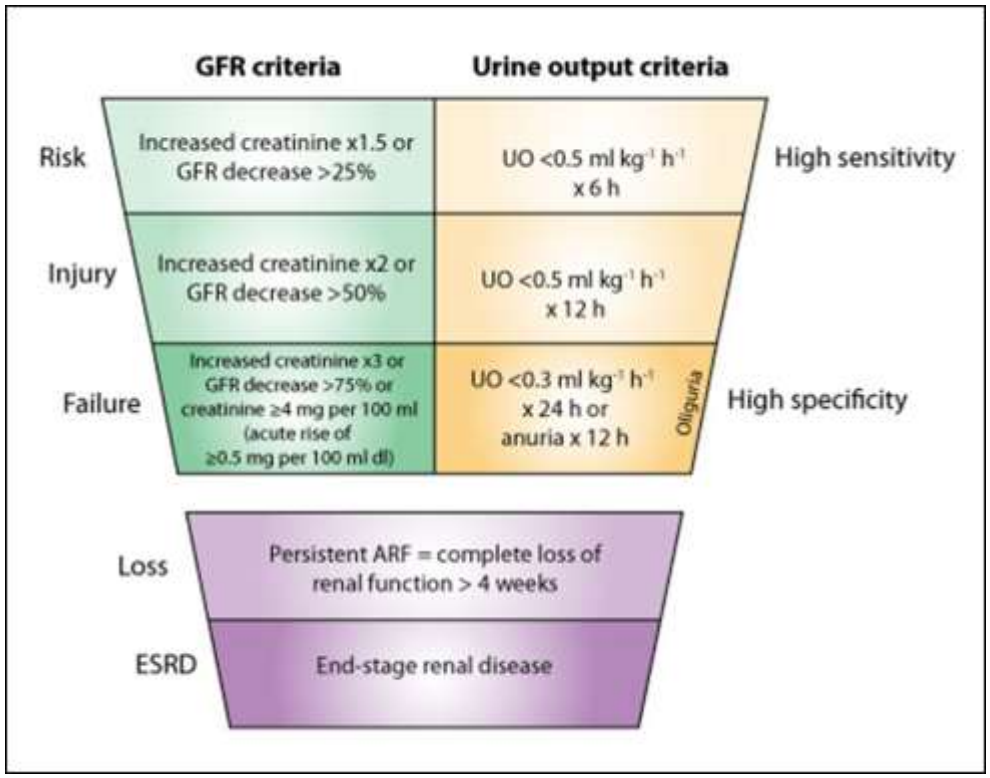
1.4.2 Acute Kidney Injury

The results of the Cardiac Angiography in REnally impaired patients (CARE) and Visipaque Angiography/interventions with Laboratory Outcomes in Renal insufficiency (VALOR) (232;233) studies discussed in more detail in section 1.6.3, highlight how the definition of AKI used can affect trial outcomes and there is also a need for consistent terminology to ensure that research is interpretable and valid comparisons between trials can be made. Studies to date, have defined the development of contrast induced AKI as an absolute increase in serum creatinine of 44 - 88 μmol/l (234) or as a percentage increase from baseline (25 - 50%) (235-237). Serum creatinine levels can vary by 10-20% in patients with normal renal function depending on hydration status and hence serum creatinine is an insensitive marker of GFR in these patients (238). In a small subset of diabetes hyperfiltration occurs which is dependent on the degree of hyperglycaemia (239). Moreover changes in serum creatinine level take at least 24 h to become apparent after a change in GFR has occurred. Whilst creatinine clearance is a more sensitive marker of GFR than serum creatinine, its use is limited by practical considerations as discussed in the section 1.4.1. Other markers that are currently being assessed include iothalamate and cystatin C (240;241).

1.4.2.1 RIFLE and AKIN

The RIFLE (Risk, Injury, Failure, Loss and ESRD) criteria were developed in an attempt to standardize and improve the care of patients with AKI (242;243). They combine the use of both GFR and urine output criteria and hence avoid the difficulties that may arise from using serum creatinine alone to stratify severity of renal impairment. Patients are grouped according to the RIFLE acronym: R – at Risk of AKI, I – with renal Injury, F – with renal Failure, L – with sustained Loss of renal function, E – End –stage renal disease (ESRD). This is summarised on the next page in Figure 9.

Figure 9: The RIFLE Classification for AKI



(Adapted from: Ricci *et al* Kidney International 2008 (244)). The shape of the figure denotes the fact that more patients (high sensitivity) will be included in the mild category, including some without actually having renal failure (less specificity). In contrast, at the bottom, the criteria are strict and therefore specific, but some patients will be missed. Abbreviations: GFR, glomerular filtration rate; UO, Urine Output; ARF, acute renal failure.

A number of studies have shown that the RIFLE criteria correlate with outcomes in many different populations (244) including in-hospital (245), critical care units (246), cardiac surgery (247) and patients on haemodialysis (248). Whether different aetiologies of AKI (e.g. contrast medium) affect the clinical outcome of each degree of renal dysfunction in

the RIFLE criteria is not currently known but would be valuable to be addressed in future studies of contrast induced AKI.

The Acute Kidney Injury Network (AKIN) was formed in 2004 and proposed a new definition for AKI (243). Compared with the RIFLE classification, this definition of contrast induced AKI requires a serum creatinine elevation of either ≥ 0.3 mg/dl or ≥ 50% above baseline (within a 48 h period), identical grades of oliguria and a similar severity staging system. Retrospective studies should be conducted to initiate the process of validating this definition of AKI with subsequent prospective studies incorporating sampling of putative AKI biomarkers (249).

1.5 The Diabetic Kidney

Diabetes is the leading cause of end-stage kidney disease and has reached epidemic proportions worldwide (132). Patients with diabetes have up to a 40% life-time risk of developing diabetic nephropathy which is one of the major micro-vascular complications (250).

1.5.1 Cellular and structural abnormalities in diabetic nephropathy

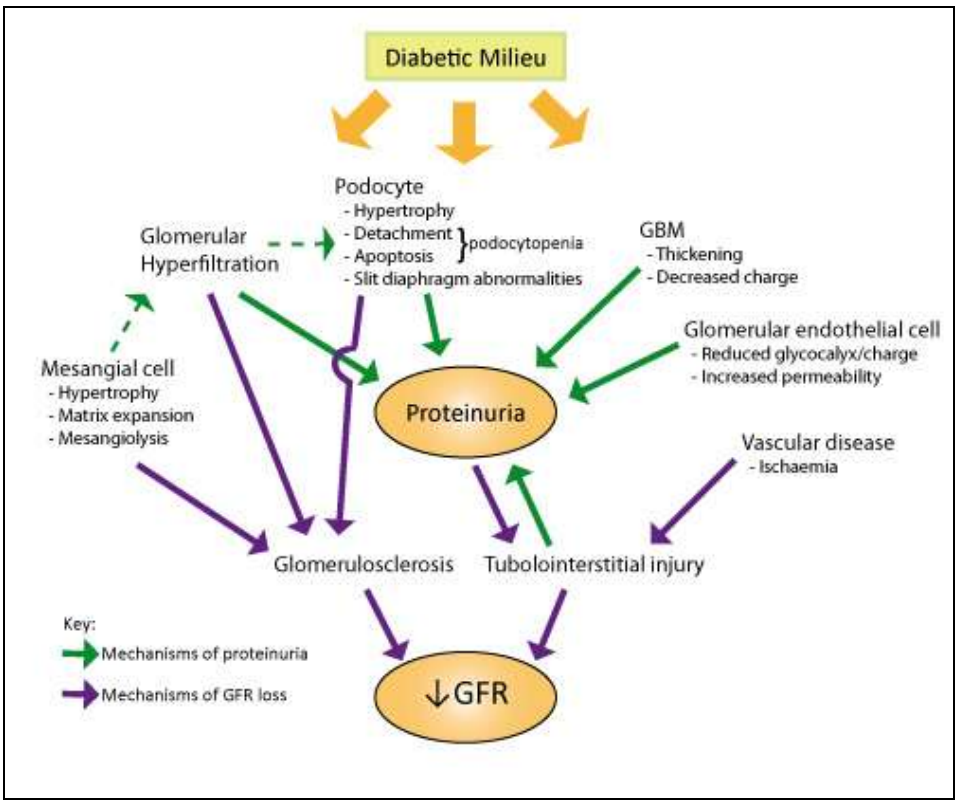
The kidney lesions in diabetic nephropathy are similar whether related to T1DM or T2DM (251). Cells involved include glomerular podocytes, mesangial and endothelial

cells, tubular epithelia and interstitial fibroblasts and vascular endothelia. Cellular and extra-cellular abnormalities occur in both the glomerular and tubule-interstitial compartments (252). These include hyperplasia/hypertrophy of various cells associated with thickening of the glomerular and tubular basement membranes and expansion of the tubule-interstitial and mesangial compartments (253). Other changes which occur include hyalinization of arterioles and thickening of the intrarenal arterial tree and its branches, resulting in impairment in 'autoregulation' of the microcirculation.

1.5.2 Pathogenesis of diabetic nephropathy

In one-third of patients, diabetic nephropathy is a progressive condition characterised by decline in GFR (Table 3) with increasing proteinuria (predominantly albuminuria) which acts as both a marker of disease severity, is used to guide treatment and is an independent risk factor for cardiovascular disease (130;131). Proteinuria also plays a central role in the pathogenesis of progressive renal dysfunction (summarised in Figure 10).

Figure 10: Schema unifying the mechanisms of proteinuria and decrease in GFR in DKD



(Adapted from Jefferson *et al* Kidney International 2008 (132)). Green arrows summarise events leading to albuminuria and proteinuria. Purple arrows summarise events leading to reduced GFR. The diabetic milieu acts on all cell types within the kidney (thick orange arrows) and these contribute either primarily or secondarily to the development of albuminuria/proteinuria and reduced GFR. At the level of the glomerulus, both haemodynamic effects and injury to the individual components of the glomerular filtration barrier primarily lead to proteinuria (green arrows). Tubulointerstitial injury may decrease tubular protein reuptake. Mesangial cell injury plays a secondary role with loss of glomerular filtration surface leading to glomerular hyperfiltration (dashed green arrows) and mesangiolysis leading to structural changes in the capillary loops.

Results from several recent studies show that a number of different phenotypes of diabetic nephropathy exist and in fact the majority of patients do not develop this 'classic' phenotype of progressive diabetic nephropathy. In some patients the

deterioration in renal function is characterised by a decrease in GFR and little or no albuminuria (254;255).

Table 3: Classification of diabetic nephropathy

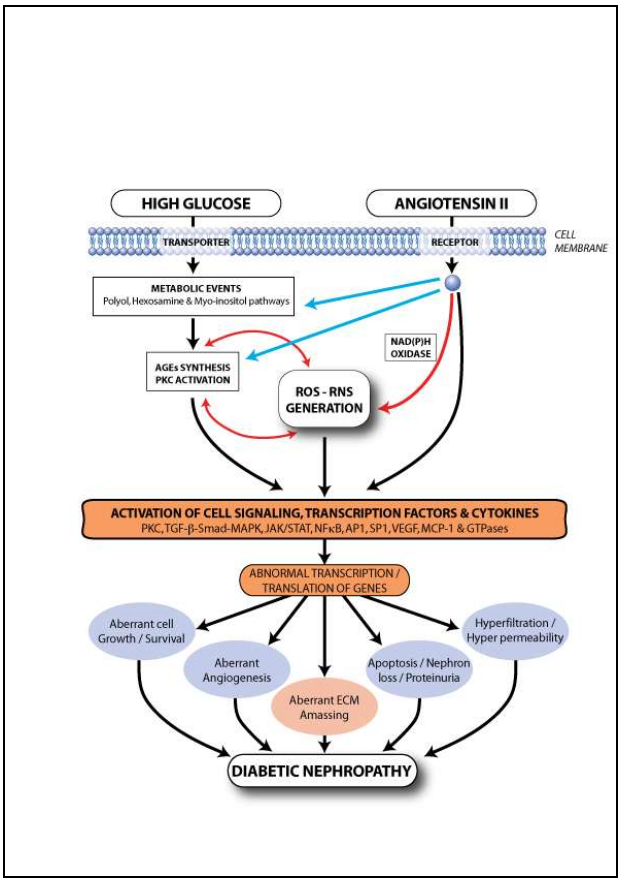
Stage of diabetic nephropathy	GFR (ml/min/1.73m^2)	Albuminuria (mg/g)
1	≥ 90	Absent or < 30
2	≥ 60	30 - 300
3	≥ 60	> 300
4	< 60	> 3000
5	< 15	Absent or present

(Adapted from Calvin and Pflueger Nature Reviews Nephrology (238)).

Diabetic nephropathy is characterised by renal vascular dysfunction with increased sensitivity to renal vasoconstrictors and renal ischaemia and a decrease in nitric oxide dependent vasodilatation (256;257). Ishimura *et al* showed that patients with Stage 1 diabetic nephropathy have elevated renal resistive indices in their renal vasculature indicating a loss of renal vasodilatory blood flow reserve (258). Patients with diabetes also have a lower renal blood flow (vasodilatory) response following administration of intravenous (i.v.) nitrate (259). Renal blood oxygenation is lower in patients with early stage diabetic nephropathy than in healthy controls following a water-load suggesting that there is impairment of oxygen delivery to the kidney (260). The abnormal vascular and endothelial function that occurs in diabetes (discussed in section 1.2) is likely to contribute to this impairment in renal oxygenation. Other factors which may play a role (summarised in Figure 11) include defective nitric oxide production, increased concentrations of AGEs, increased generation of cytokines and increased production of reactive oxygen species (ROS) (252;261). Data from animal studies have shown that

nitric oxide dependent renal vasodilatation plays a key role in counteracting the vasoconstrictive effects of contrast media (262). The responsiveness of this mechanism is decreased in patients with diabetes (258;261;263).

Figure 11: Summary of mechanisms leading to development of diabetic nephropathy and chronic renal failure



(adapted from Kanwar *et al* Exp Biol Med 2008(252)). Various mechanisms relevant to hyperglycaemia induced and angiotensin II induced activation of various signalling pathways. This causes upregulation of various genes and cellular dysfunction, resulting in the development of diabetic nephropathy and chronic renal failure. Abbreviations: AGEs: Advanced Glycation End-products; PKC: Protein Kinase C; TGF-β: Transforming Growth Factor-β; ROS: Reactive Oxygen Species, NFκB: Nuclear Factor kappa B; RNS: Reactive Nitrogen Species; ECM: Extracellular matrix; AP1: Activator protein1; SP1: Specificity protein 1, VEGF: Vascular Endothelial Growth Factor, MCP-1: Monocyte Chemoattractant Protein-1, GTP: Guanosine Triphosphate).

1.6 Contrast Induced Acute Kidney Injury

Contrast induced acute kidney injury (AKI); previously known as contrast induced nephropathy (CIN) is an important complication following procedures in the catheterisation laboratory (264-266). It is the third commonest cause of acute renal failure in hospitalised patients (266) and is associated with higher short and long term morbidity and mortality rates than are seen in patients without contrast induced AKI (267-271).

'Contrast induced AKI' is now the preferred terminology over the more familiar term 'CIN', since it reflects that deterioration in renal function following procedures using iodinated contrast media may be multi-factorial (267). CIN is a form of contrast induced AKI but is not the only mechanism for its occurrence (272).

The most common definition used in clinical trials to define contrast induced AKI is a rise in serum creatinine of 44.5 µmol/l (0.5 mg/dl), or a relative increase of serum creatinine > 25% from baseline (273). AKI usually manifests clinically within 3 days of contrast administration and peaks within 3-5 days, with a return to baseline creatinine seen within 10-21 days (273;274) although in some cases prolonged and even permanent decline in GFR may persist (272). Renal impairment is a frequent feature in patients with coronary artery disease and several studies have documented the adverse prognostic impact of CKD following percutaneous intervention (PCI) (275;276). As discussed in section 1.3.1 patients with CKD are at extremely high risk for developing CAD, with subsequent poor prognosis. The combination of CKD and diabetes is associated with particularly poor outcomes, since when the renal function is already impaired, the ability of the nephrons

to recover from additional insults is extremely limited. This is discussed in detail in section 1.6.1.2.

1.6.1 Incidence of AKI following Coronary Angiography or PCI

1.6.1.1 General Population

The incidence of contrast induced AKI is of the order of 3-15% in the general population undergoing procedures in the cardiac catheterisation laboratory (268;277). However, this rate may be much higher in some at–risk population groups (278-280).

1.6.1.2 Chronic Kidney Disease and Diabetes Mellitus

If renal function is already impaired by pathological processes then the kidney is far less able to tolerate the additional stress of excreting a contrast load (281). Pre-existing CKD (GFR <60 ml/min/1.73 m^2 of BSA) is one of the strongest risk factors for the development of AKI (218). In these patients there is a considerable loss of nephron units and residual renal function is at risk of further deterioration from renal insults (e.g. iodinated contrast, athero-embolism, renal-toxic medications, and cardiopulmonary bypass). Other co-morbidities that have been demonstrated to be important include congestive heart failure, left ventricular dysfunction, hypotension, hypertension, pre-procedure shock, acute myocardial infarction and possibly female gender (reviewed (281)). Diabetes mellitus has emerged as being a particularly important risk factor for

the development of contrast induced AKI as highlighted by data from the Mayo Clinic (268). In this retrospective analysis of 7586 patients, both elective and acute, undergoing coronary angiography and PCI at the Mayo Clinic, the authors report the higher risk to diabetic patients although this study was not specifically looking at contrast induced AKI. In patients with a baseline serum Cr <2.0 mg/dl (177 µmol/l), the diabetic patients had a significantly higher rate of AKI than the non-diabetic patients. Among patients with a serum Cr >2.0 mg/dl (177 µmol/l) all patients (both diabetic and non-diabetic) had a significantly increased risk of AKI. If the serum Cr was <1.1 mg/dl (97.4 µmol/l), the risk of AKI was 3.7% vs. 2.0%, p=0.05 in the diabetic vs. non diabetic patients and if the serum Cr was between 1.2 mg/dl (106.1 µmol/l) and 1.9 (170 µmol/l) the risk of AKI was 4.5% vs. 1.9%, p < 0.001. When serum Cr was >2.0 mg/dl both diabetic and non-diabetic patients experienced AKI at a high rate (22.4% for Cr 2.0-2.9 mg/dl (177-256.4 µmol/l and 30.6% for Cr >3.0 mg/dl (265.2 µmol/l)). From these data the overall incidence of AKI in patients with diabetes is of the order of 6% - an additional 2 fold increase over non diabetic patients in this study (268). These results are summarised in Table 4.

Table 4: Observed incidence of AKI stratified by baseline serum creatinine and diabetic status

Cr (mg/dl)	**Risk, all patients, %**	**Risk, diabetic patients, %**	**Risk, non-diabetic patients,%**	**Diabetic vs. non-diabetic patients, OR (95% CI) and *p value***
0 – 1.1	2.4 (n = 3965)	3.7 (n = 809)	2.0 (n = 3156)	1.86 (1.20, 2.89) *0.005*
1.2 – 1.9	2.5 (n = 3318)	4.5 (n = 710)	1.9 (n = 2608)	2.42 (1.54, 3.79) *< 0.001*

2.0 – 2.9	22.4 (n = 179)	22.4 (n = 67)	22.3 (n = 112)	1.00 (0.48, 2.08) *0.99*
≥ 3.0	30.6 (n = 124)	33.9 (n = 62)	27.4 (n = 62)	1.36 (0.63, 2.92) *0.44*

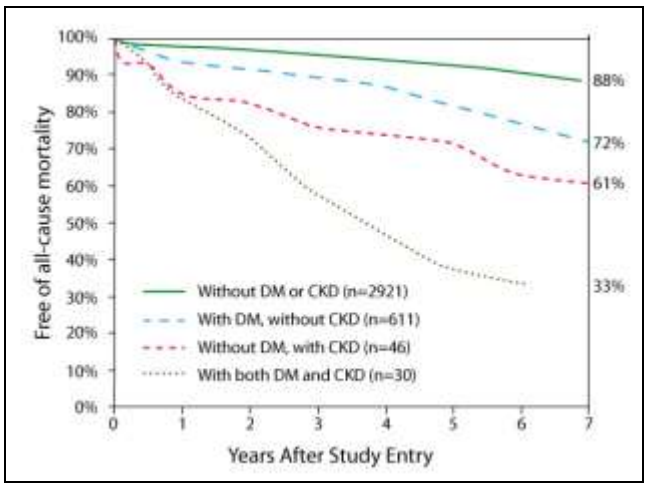
(Adapted from Rihal *et al* Circulation 2002 (268). Abbreviations: OR: Odds Ratio).

This study also demonstrated that AKI is associated with significantly increased rates of mortality. 22% of patients who developed AKI died during the index hospitalisation compared to 1.4% of patients without AKI ($p < 0.0001$). Among hospital survivors with AKI, 1 year and 5 year estimated mortality rates were 12.1% and 44.6%, respectively, much greater than the 3.7% and 14.5% mortality rates in patients without AKI ($p < 0.0001$).

The findings of another, albeit smaller study, emphasise the adverse prognosis of AKI after PCI in patients with baseline Cr elevation (278). 46% of the 161 patients whose Cr increased by >25% had died by 1 year compared with 19.4% of those whose Cr did not rise by this amount. The BARI Trial and Registry (recruited 1988-1991) of patients with multivessel coronary disease (n=1829) (353 patients with diabetes, 76 patients with CKD - defined as serum Cr >1.5mg/dl) provides further evidence (Figure 12). Patients with CKD were more likely to have a history of diabetes and hypertension, and CKD was the strongest predictor of all-cause mortality (128) and was associated with an increased risk of recurrent hospitalisation and subsequent bypass surgery.

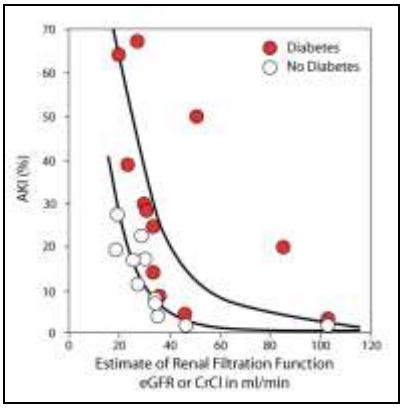
In earlier studies the risk of AKI post contrast has been reported to be as high as 33% in patients with diabetes (280). It has been suggested that diabetes acts as a 'risk amplifier' (Figure 13), that is to say that at any level of decreased eGFR, patients with diabetes have a higher risk of AKI (282).

Figure 12: Freedom from all cause death for patients with CKD and DM: Data from the BARI Trial and Registry



(Adapted from Szczech *et al* Circulation 2002 (128).) 7 year all-cause mortality was markedly different for patients on the basis of both the presence and absence of CKD and of DM. Mortality for patients with DM was 28% in those without CKD and 67% for patients with CKD.

Figure 13: Risk of contrast induced AKI according to baseline renal function and diabetes status



(Adapted from McCullough PA *et al* Am J Cardiol 2006 and McCullough PA J Am Coll Cardiol 2008 (267;277)).

1.6.1.3 Risk of contrast induced AKI requiring dialysis

The risk of contrast induced AKI requiring treatment with dialysis is approximately 1% in the general population undergoing coronary angiography or PCI (283-285), 4% in patients with underlying renal impairment (286) and 3% in patients undergoing primary PCI for ACS (287) (reviewed (267)). Although relatively rare patients who require haemodialysis post PCI have high hospital and 1 year mortality rates (278;283); post PCI patients requiring dialysis have a five-fold increase in in-hospital death compared with those who developed AKI but did not require dialysis (36% vs. 7%, $p<0.0000001$); mortality rate at 1 year in patients requiring dialysis were 54.5% vs. 6.4% respectively, $p<0.0001$ (281).

1.6.1.4 Outcomes following development of AKI

Contrast induced AKI has been associated with worsening of both short and long term outcomes, including death from cardiovascular causes (268;288). Kimura *et al* (289) studied 2349 patients with CKD who underwent coronary angiography or PCI. Relationships between CKD and AKI and mortality or cardiovascular disease were assessed using Cox regression analysis and identified both CKD and AKI as independent predictors of long term mortality and cardiovascular events. The results are summarised in Table 5.

Table 5: Cox regression analysis for long term endpoints in patients with CKD undergoing coronary angiography

		All patients	
End-point	Parameter	Unadjusted model	Multi-variable model
Death from any cause	CKD	2.23 (1.62 – 3.01)	1.51 (1.07 – 2.13)
	AKI	2.61 (1.44 – 4.71)	1.53 (0.84 – 2.91)
Composite end-point	CKD	2.55 (2.10 – 3.10)	1.72 (1.40 – 2.11)
	AKI	2.59 (1.78 – 3.78)	1.64 (1.09 – 2.46)

(Adapted from Kimura *et al* Nephrol Dial Transplant 2010 Values are described as HR (95% CI))

There has been a tendency to assume that contrast induced AKI identifies patients with a higher burden of comorbidity and therefore these longer term adverse outcomes reflect this (reviewed (290)). Recent prospective randomised trials in which a therapeutic intervention reduces both the incidence of contrast induced AKI and long term adverse events, raises the possibility that contrast induced AKI directly contributes to an increased risk of cardiovascular and renal sequelae (291;292). Episodes of AKI predispose patients to develop long term loss of renal function (293). Long term follow up of patients who have developed AKI shows that they experience a more precipitous decline in GFR than individuals who do not develop kidney injury (294). As described in Section 1.3, CKD is a strong risk factor for developing cardiovascular events (103;107;295).

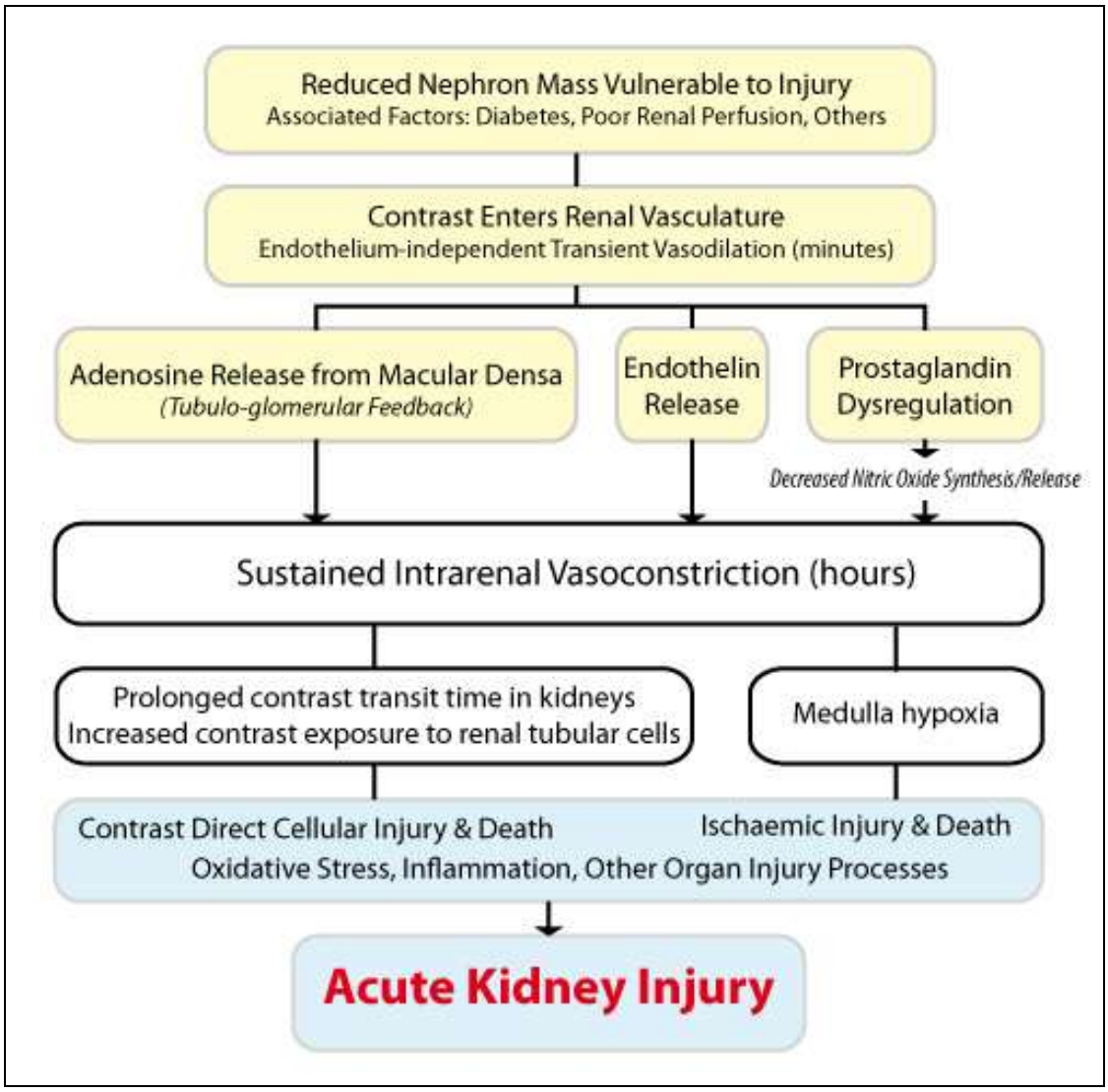
1.6.2 Pathophysiology

The pathophysiology of contrast induced AKI is multi-factorial in aetiology and is summarised in Figure 14. Athero-emboli and haemodynamic alterations leading to reduced renal perfusion may play a role in some cases, as may other co-morbidities that predispose patients receiving iodinated contrast to renal injury (267;272;296). Impaired renal function at baseline (eGFR < 60 ml/min) appears to be a pre-requisite for the development of contrast induced AKI (296). Two major hypotheses have been proposed to explain this finding: renal vasoconstriction and tubular injury (267;272).

A baseline reduction in nephron number is assumed, with superimposition of acute vasoconstriction caused by release of renal vasoconstrictors such as adenosine and endothelin triggered by iodinated contrast administration. Blockade of endogenous vasodilators including prostaglandins and nitric oxide occurs. This results in a transient increase in renal blood flow followed by a sustained reduction, lasting for several hours. This, in association with volume depletion or reduced renal blood flow from heart failure, results in increased blood viscosity, predisposing to medullary hypoxia and ischaemia (267;272). Iodinated contrast concentrates in the renal tubules and collecting ducts leading to direct cellular injury and death of renal tubule cells. The degree of renal cytotoxicity is directly related to the length of exposure those cells have to iodinated contrast. The sustained reduction in renal blood flow to the outer medulla leads to medullary hypoxia, ischaemic injury and renal tubular cell death. Other processes including oxidative stress and inflammation play an additive role resulting in further damage. Any superimposed insult such as sustained hypotension, bleeding, athero-

embolic shower from catheter exchange/manipulation or use of intra-aortic balloon pump (IABP) may further amplify this injury cascade (267).

Figure 14: Pathophysiology of contrast induced AKI



(Adapted from McCullough PA J Am Coll Cardiol 2008 (267)).

1.6.3 Role of Contrast Media on Risk of AKI

Iodinated contrast agents contain radio-opaque iodine atoms attached to water soluble carbon based molecules. Contrast media are classified according to osmolality, reflecting the total particle concentration of the solution – high-osmolal (HOCM) ~ 2000 mOsm/kg, low-osmolal (LOCM) 600-800 mOsm/kg and iso-osmolal (IOCM) 290 mOsm/kg, with decreasing renal toxicity according to these classifications. IOCM were originally developed to reduce the risk of contrast induced AKI but have a higher viscosity than LOCM (297;298). Increased viscosity has been shown in animal studies to reduce renal tubular flow and decrease GFR (299). There have therefore been some concerns regarding whether IOCM are beneficial (297;298;300;301).

The agents may also be described as ionic or non-ionic depending on whether the iodine moiety is bound to an organic (non-ionic) or ionic compound. An ionic compound dissociates or dissolves into charged particles when it enters a solution such as blood. Ionic agents were developed first and use is still relatively widespread but may cause additional complications. Non-ionic agents covalently bind the iodine and have fewer side-effects as they do not dissociate into the individual component molecules (302). Table 6 lists commonly used contrast agents and classifies them by both osmolality and ionicity.

Table 6: Classification of commonly used contrast agents

Compound	Generic Name	Brand Name	Type	Iodine content (mg/ml)	Osmolality (mOsm/kg)	Level
Ionic	Diatrizoate	Hypaque 50	Monomer	300	1550	High osmolar
Ionic	Metrizoate	Isopaque 370	Monomer	370	2100	High osmolar
Ionic	Ioxaglate	Hexabrix	Dimer	320	580	High osmolar
Non-ionic	Iopamidol	Isovue 370	Monomer	370	796	Low osmolar
Non-ionic	Iohexol	Omnipaque 350	Monomer	350	884	Low osmolar
Non-ionic	Ioxilan	Oxilan 350	Monomer	350	695	Low osmolar
Non-ionic	Iopromide	Ultravist 370	Monomer	370	774	Low osmolar
Non-ionic	Iodixanol	Visipaque 320	Dimer	320	290	Iso osmolar

In the last 40 years agents have become progressively more physiological, approaching the point of iso-osmolality. Red blood cell deformation, systemic vasodilatation, intra-renal vasoconstriction and direct renal tubular toxicity may all result with hyperosmolar contrast agents (267).

1.6.3.1 What is the contrast agent of choice in patients with CKD?

Early studies demonstrated a lower incidence of contrast induced AKI in patients with diabetic nephropathy who received an IOCM as compared to LOCM (303;304). However, subsequent larger scale studies did not confirm this finding (232;233). It can sometimes be difficult to compare studies due to the different definitions used to define contrast induced AKI. The double-blind, multi-centre CARE study used a definition of a rise in creatinine of > 50% from baseline and showed no difference in rates of AKI

between patients with diabetic nephropathy who received LOCM iopidamol and IOCM iodixanol (232). The VALOR trial looked at the incidence of AKI in patients with CKD and further stratified by the presence of diabetes (233). Both IOCM iodixanol and LOCM ioversol were assessed. AKI was defined as an absolute rise in serum creatinine ≥ 44.5 µmol/l (0.5 mg/dl). In patients with CKD but without diabetes (n = 145), no difference was observed in the incidence of contrast induced AKI between the 2 treatment groups. However in patients with CKD and diabetes (n = 154), the incidence of AKI was lower in the iodixanol group than the ioversol group (12.9% vs. 2.4%, p = 0.01).

The joint guidelines from the American College of Cardiology (ACC) and American Heart Association (AHA) for ACS patients with CKD, describe the usage of IOCM as a class I, level of evidence A recommendation (305). Use of IOCM is also recommended for renal dialysis patients to minimise the risk of volume overload and other complications between dialysis sessions. Use of LOCM is allowed for lower risk patients and i.v. administration.

Many studies have demonstrated that the volume of contrast agent is an important risk factor for contrast induced acute kidney injury (e.g. (237;306). Even small volumes of contrast (~30 ml) can have adverse effects on renal function in patients at particularly high risk (237). In this study of 59 azotemic diabetic patients undergoing coronary angiography as part of pre-transplant evaluation univariate analysis demonstrated a significant association between dye quantity (p=0.002) and development of AKI and the independence of this relationship was confirmed by stepwise logistic regression. This is especially important in the current era of PCI where increasingly larger volumes of contrast are being used reflecting the increasing complexity of the interventions being

undertaken. This is particularly true of PCI performed in patients with diabetes who generally have more extensive and more severe disease (34). It has been suggested that as a rule, the volume of contrast in ml should not exceed twice the baseline level of eGFR (307). Therefore for patients with significant CKD the operator should aim to use <30 ml for diagnostic coronary angiography and <100 ml for PCI, computed tomography (CT) and other intravascular studies.

It has been demonstrated in a number of studies that the risk of contrast induced AKI is higher after intra-arterial than i.v. contrast administration (308;309). However it should be remembered that in CT studies comparatively large volumes of contrast agent can be given in a compact i.v. bolus and then the risk of AKI may be increased (282).

Serial exposure to contrast medium and subsequent administration in the AKI setting is believed to further worsen renal function and may result in an increased chance of persistent renal dysfunction developing. Current clinical practice would in this setting favour a limited diagnostic catheterisation and PCI in the same setting over a diagnostic catheterisation and then a scheduled PCI within 10 days. It is important to note that there are no published studies of comparative data on this strategy and furthermore the optimal waiting period between a first and subsequent contrast medium exposure is also unknown.

1.6.4 Risk Factors

A number of risk factors have been identified for the development of contrast induced AKI. These can be divided into modifiable and non-modifiable factors and are summarized in Table 7.

Table 7: Risk factors for the development of contrast induced AKI

Fixed (non-modifiable) risk factors	Modifiable risk factors
Older age	Volume of contrast media
Diabetes mellitus	Hypotension
Pre-existing renal failure	Anaemia and blood loss
Advanced CCF	Dehydration
Low LVEF	Low serum albumin level (<35g/l)
Acute myocardial infarction	ACE inhibitors
Cardiogenic shock	Diuretics
Renal transplant	Non-steroidal anti-inflammatory drugs
	Nephrotoxic antibiotics
	IABP

(adapted from Mehran *et al* Kidney International Suppl 2006 (310). Abbreviations: ACE Angiotensin Converting Enzyme, CCF Congestive Cardiac Failure, IABP Intra-Aortic Balloon Pump, LVEF Left Ventricular Ejection Fraction).

1.6.4.1 Pre-existing renal disease

Pre-existing renal disease, as measured by an elevated serum creatinine level, is a key factor in the development of contrast induced AKI. The incidence of contrast induced AKI in patients with underlying CKD is reported in the literature at between 14.8-55% (268;271;283). In one contemporary study, despite pre-hydration and the use of non-ionic contrast media, contrast induced AKI occurred in one-third of 439 consecutive

patients who underwent PCI and had a baseline creatinine ≥ 1.8 mg/dl (~ 160 µmol/l) (271).

The higher the baseline serum creatinine concentration, the greater the risk of contrast induced AKI. This was very clearly shown by Hall *et al* (311) (summarized in Table 8).

Table 8: Relationship between baseline serum creatinine and risk of developing contrast induced AKI

Creatinine (mg/dl)	Risk of contrast induced AKI (%)
≤ 1.2	2
1.4-1.9	10.4
≥ 2.0	62

For patients with a baseline creatinine ≤ 1.2 mg/dl (≤ 107 µmol/l), the risk of contrast induced AKI was 2%. This increased five-fold to 10.4% for a creatinine in the range 1.4 - 1.9 mg/dl (125 – 169 µmol/l). For patients with creatinine ≥ 2.0 mg/dl (≥ 178 µmol/l) this rose even further to 62%.

There are limitations with baseline creatinine as a reliable marker for identifying patients at risk for developing contrast-induced AKI as there is marked variation with age, muscle mass and gender (310). In order to evaluate renal function accurately assessment of creatinine clearance should be performed. It may not be practical to measure creatinine clearance directly but it can easily be estimated using the Cockcroft-Gault Formula (220) or the Modification of Diet in Renal Disease (MDRD) equation (221). A number of studies have demonstrated that an estimated glomerular filtration rate (eGFR) of ≤ 60 ml/min/1.73m^2 is a reliable threshold for identifying patients at high risk for development of AKI (310). It is therefore recommended that eGFR is calculated prior to exposure to contrast agent as part of the risk assessment process (220;221).

1.6.4.2 Diabetes mellitus

A high proportion of patients undergoing coronary angiography or PCI have diabetes. This is due to the combination of the high prevalence of the disease and the wide spectrum of cardiovascular disease that can manifest as a consequence. The incidence of contrast induced AKI in diabetic patients in the literature varies from 5.7 – 29.4% (286;312). In diabetic patients with preserved renal function and absence of other risk factors, rates of contrast induced AKI are comparable to those of a non-diabetic population (312). In one study contrast induced AKI occurred in 27% of diabetic patients with a baseline serum creatinine 2.0 - 4.0 mg/dl and in 81% with serum creatinine > 4.0 mg/dl (313). In another study of diabetic patients, contrast induced AKI occurred in 15.1% of patients without CKD vs. 27.4% in those with CKD; *de novo* dialysis was instituted in 0.1 vs. 31% respectively (both $p < 0.0001$) (286).

1.6.4.3 Older Age

There is evidence from a number of studies that older age is an independent risk factor for the development of contrast induced AKI (280;314;315). The reasons for higher risk in the elderly have not been specifically studied. However these are likely to be multi-factorial in origin including age related changes in renal function such as decreased glomerular filtration rate, tubular secretion and concentrating ability. Furthermore the presence of multi – vessel coronary artery disease, requiring more complex intervention procedures with higher volumes of contrast media and more difficult vascular access due to tortuosity and calcification of vessels may further amplify risk (310).

1.6.4.4 Volume and Type of contrast media

The volume of contrast media used during coronary angiography or PCI is probably one of the most easily modifiable risk factors. There is a clear correlation between volume of contrast media used and risk of contrast induced AKI e.g. (237;283;315;316). Even relatively low amounts of contrast (< 100 ml) can result in permanent renal failure and/or the need for dialysis in patients with underlying CKD (237;317). One study in diabetic patients with CKD showed that for every 100 ml increment in contrast volume used, the result was a 30% increase in the odds of contrast induced AKI developing (odds ratio 1.30, 95% CI 1.16-1.46) (286).

Whether use of different types of contrast agent is of benefit in reducing rates of contrast induced AKI is an area of great debate. Katholi *et al* (318) and Harris *et al* (319) both showed that decrease in creatinine clearance was greater in patients receiving HOCM compared to LOCM. However Schwab *et al* (320) did not show any difference in nephrotoxic effects between several different studied contrast agents. In a meta-analysis of 45 trials, a greater increase in serum creatinine following administration of HOCM compared to LOCM was only seen in patients with pre-existing renal failure (321).

There are currently a number of different LOCM available. There is some evidence that differences in nephrotoxic effect appear to more evident with ionic than non-ionic agents. The ICON study (Ionic versus non-ionic Contrast to Obviate worsening Nephropathy after angioplasty in chronic renal failure patients) showed that contrast induced AKI is less likely to develop in high risk patients (baseline serum creatinine 1.5 – 3.5 mg/dl) when iso-osmolality, non-ionic iodixanol is used rather than low-osmolality,

non-ionic iohexol (322).

In current practice, non-ionic low osmolar contrast media remain the preferred choice in patients with renal impairment. Further studies will be necessary to clarify the issue of minimizing renal damage whilst using different contrast media (310).

1.6.4.5 Anaemia and blood loss

It is thought that anaemia contributes to renal ischaemia and hence development of contrast induced AKI. Data from the interventional cardiology database at Columbia University in New York (323), showed that rates of AKI increased as pre-procedure haematocrit quintile decreased (from 10.3% in the highest quintile with haematocrit ≥ 44.8% to 23.3% in the lowest quintile with haematocrit < 36.8%). Stratification by baseline eGFR and baseline haematocrit demonstrated that rates of AKI were the highest (28.8%) in patients with both the lowest level for both baseline eGFR and pre-procedure haematocrit. On multivariate analysis, lower baseline haematocrit was identified as an independent predictor for AKI development regardless of the presence or absence of CKD: each 3% decrease in baseline haematocrit resulted in a significant increase in the odds of AKI in patients with and without CKD (11% and 23%, respectively).

1.6.4.6 Other risk factors

Congestive cardiac failure, impaired left ventricular function, dehydration, hypotension and the use of IABP and some drugs (non-steroidal anti-inflammatory (NSAIDs), diuretics and ACE inhibitors) have all been identified as risk factors for the

development of AKI (310;324;325). The damaging effect of hypotension on renal function is well known (323). IABP insertion may be linked with the development of AKI through a number of different mechanisms: a) athero-emboli to the renal circulation during insertion, b) partial occlusion of renal blood flow due to malposition, c) as a marker of increased vascular complications and post PCI hypotension (reviewed (310)).

The role of ACE inhibitors is controversial. In one study patients receiving ACE inhibitors had a significant increase in serum creatinine post procedure compared with patients not receiving this medication (315). However use of ACE inhibitors only predicted the occurrence of contrast induced AKI on univariate but not multi-variate analysis.

1.6.4.7 A Risk Scoring System: The Mehran Risk Score

The development of various risk scoring systems now allows clinicians to evaluate an individual patient's risk of developing AKI following PCI. In the scoring system below (Table 9) (326), diabetes significantly adds to the risk of developing AKI, especially given that many patients with DM undergoing PCI have eGFR < 60 ml/min.

Table 9: The Mehran Risk Score for development of contrast induced AKI

		Score
Risk Factor	Hypotension	5
	IABP	5
	CCF	5
	Age >75 years	4
	Anaemia	3
	DM	3
	Contrast media volume	1 for every 100 ml
	Serum Cr >1.5mg/dl*	2
*Or eGFR <60 ml/min	40-60	2
	20-40	4
	<20	6
Risk Score	**Risk of AKI**	**Risk of dialysis**
<5	7.5%	0.04%
6-10	14.0%	0.12%
11-16	26.1%	1.09%
>16	57.3%	12.6%

(adapted from Mehran *et al* J Am Coll Cardiol 2004 (326)). 8315 patients undergoing PCI at Columbia University Medical Centre, New York over a 6 year period were randomly assigned to a development and validation dataset. 5571 patients in the development dataset had their baseline clinical and procedural characteristics considered as candidate univariate predictors for the development of contrast induced AKI (defined as either a rise of >25% from baseline or an absolute rise >0.5mg /dl). Multivariate logistic regression analysis was used to identify predictors of contrast induced AKI with a p value < 0.0001. From the odds ratio (OR) 8 variables (hypotension, intra-aortic balloon pump (IABP), congestive cardiac failure (CCF), CKD, diabetes, age >75 years, anaemia and contrast volume) were identified and assigned a weighted integer. The sum of the integers was a total risk score for each patient. Definitions: anaemia: baseline haematocrit value < 39% for men and < 36% for women; CCF: New York Heart Association (NYHA) Class III/IV ± history of pulmonary oedema; hypotension: systolic BP < 80 mmHg for at least 1 hour requiring inotropic support or IABP.

1.7 Strategies for renal protection

(Summarised in Figure 15)

1.7.1 Intravenous hydration

Due to the considerable morbidity and mortality associated with the development of renal injury post PCI, it is important to identify strategies that may confer a degree of renal protection. Volume expansion and treatment of dehydration has a key role in prevention of contrast induced AKI. Volume expansion results in increased renal peri-tubular blood flow and reduces tubular stasis of contrast medium. It is thought that this limits the contact between contrast and the tubular cells therefore decreasing the direct cytotoxic insult. Whilst the importance of good hydration with i.v. fluids has been identified, there are no robust data that favour a particular regimen (327;328). Isotonic crystalloid (normal saline or bicarbonate solution) is probably more efficacious than half-normal saline as it will remain in the intravascular space for a longer duration (327). It has been hypothesised that alkalinisation of tubular fluid might be beneficial by reducing the levels of pH dependent free radicals. Merten *et al* (328) showed that creatinine levels were less likely to rise >25% within 2 days following the administration of contrast in patients given isotonic sodium bicarbonate infusion rather than normal saline. However this remains a controversial intervention due to methodological concerns about this study (reviewed (329)). A recent meta-analysis demonstrated no benefit of hydration with sodium bicarbonate versus normal saline (330) and called for more good quality large scale trials to evaluate this further.

There is limited evidence to date to guide the choice of optimal rate and duration of the fluid regime. Maintaining urine output >150 ml/h (324) has been shown in one study to be associated with decreased rates of AKI. This was a prospective randomised controlled single-blind trial of 98 patients. Those in the control arm (n=55) were randomised to intravenous crystalloid (0.45% saline) at a rate of 150 ml/hour, commenced on arrival in the cardiac catheter lab and continued during the procedure. Those in the experimental arm (n=43) received the same regime of intravenous crystalloid, frusemide intravenously as a single dose (1 mg/kg up to a maximum of 100 mg) and intravenous dopamine (3 µg/kg/min) which was continued during the procedure. The forced diuresis resulted in higher urine flow rate (163.26 +/- 54.47 vs. 122.57 +/- 54.27 ml/hour) over the 24 h following contrast exposure (p=0.001). 2 patients in the experimental arms versus 5 in the control arm required dialysis with all 7 cases having urine flow rates <145 ml/h. The rates of renal failure in those with urine flow rates >150 ml/h in the post-procedure period were significantly lower 8/37 (21.6%) vs. 28/61 (45.9%), p=0.03.

At the London Chest a pragmatic approach is taken with 2 h of intravenous normal saline prior to the procedure with 6 h of hydration (oral or intravenous) post-procedure in all patients with eGFR < 50 ml/min. (331)

1.7.2 N-acetylcysteine (NAC)

NAC has the potential to decrease nephrotoxicity due to its anti-oxidant and vasodilatory effects. Animal studies have demonstrated that acetylcysteine decreases ischaemic renal failure, blocks expression of vascular cell adhesion molecule-1 (VCAM-1) and prevents activation of NF-κB in glomerular mesangial cells (332). A clinical trial of 83 patients with chronic renal insufficiency (defined as a serum creatinine >1.2 mg/dl or 106 μmol/l or a creatinine clearance <50 ml/min) undergoing CT with a low-osmolality contrast agent, assigned patients to receive either oral 600 mg acetylcysteine bd for 2 days (starting the day prior to the planned procedure) or placebo. All patients received intravenous hydration with 0.45% saline for 12 h pre and post contrast administration. A standard dose of 75 ml of low-osmolality contrast agent was used. The study demonstrated that serum creatinine levels rose by >0.5 mg/dl in 2% of patients who received NAC compared with 21% of patients in the control group ($p<0.01$, (333)). It has been noted that the event rate in the control group was unexpectedly high for patients receiving a low osmolality contrast agent. Until recently other trials have been limited by low power and lack of blinding and by variation of NAC dose level and regime (reviewed (282)). A number of meta-analyses have been published on this subject (285;334-343) and seven of these found a net benefit for NAC in the prevention of AKI. A review (339) found marked heterogeneity in 10 of 11 meta-analyses and also that it was only in studies where following NAC administration serum creatinine decreased below baseline values that AKI rates appear to be reduced. NAC reduces skeletal muscle production of creatinine into the blood stream and it has been hypothesised that it may falsely lower post-contrast creatinine and not actually protect against AKI (282).

The ACT (Acetylcysteine for the prevention of Contrast induced nephropathy Trial) recently reported at the AHA in November 2010 (344). This randomised multi-centre clinical trial of 2308 patients from Brazil compared high dose NAC (1200 mg bd orally, 2 days pre and 2 days post procedure) against placebo in patients undergoing coronary and vascular angiography. There was no increase in the primary endpoint of contrast induced AKI (12.7% in the NAC arm vs. 12.7% in the placebo arm, p = 0.97). Clinical endpoints at 30 days including mortality, cardiovascular mortality and dialysis were equivalent between the 2 groups. The study used a number of different contrast agents including 22% HOCM and fluid regimes were not standardised. Furthermore although the authors refer to the population as 'high risk' they only had to have one of the following to qualify: age >70 years, chronic renal failure (serum creatinine > 1.5 mg/dl), diabetes mellitus, heart failure and shock.

Routine use of NAC is currently not recommended in the ACC/AHA guidelines but it is a Class IIB recommendation in chronic renal failure patients in the European Society of Cardiology (ESC) guidelines (345). At the London Chest oral NAC 600 mg bd is given to all patients with eGFR <50 ml/min undergoing coronary angiography and PCI (331) for 48 h pre and post procedure.

1.7.3 Statins

Statins (3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors) have been shown to reduce myocardial infarction and death (346-348). It is thought that reduction of LDL is responsible for the majority of the observed benefit. Statins have been shown to demonstrate pleiotropic effects including improved endothelial function (e.g. at the

level of the glomerulus) and reduction in systemic inflammatory response and may have protective effects on the kidney. It has been shown that patients continued on statins during cardiovascular procedures including PCI and CABG have lower rates of AKI (349). Small randomised trials provide further supportive evidence although conclusive trials have not been reported to date (reviewed (350)).

1.7.4 Other Approaches

Forced diuresis with frusemide, mannitol or a combination of these agents has not been demonstrated to reduce the incidence of contrast induced AKI when compared to intravenous hydration alone (351;352). There have been small randomised trials of various vasodilators including atrial natriuretic peptide (353;354) prostaglandins (355) and endothelin receptor antagonists (356) but similarly none of these agents have demonstrated a reduction in the risk of AKI when compared to intravenous hydration. Aminophylline, an adenosine receptor inhibitor, has been proposed as an agent that may reduce the risk of contrast induced AKI but there is no conclusive supporting evidence for this so far (329;357;358), reviewed (329). The choice and volume of contrast agent utilised is also important as outlined previously (section 1.6.3).

A novel therapeutic approach using a device allowing delivery of direct renal arterial infusion has recently been described (359). This has the advantage of allowing local drug delivery, with a high local dose whilst avoiding systemic adverse effects due to renal 1st pass metabolism. This study was a post-market registry which enrolled 501

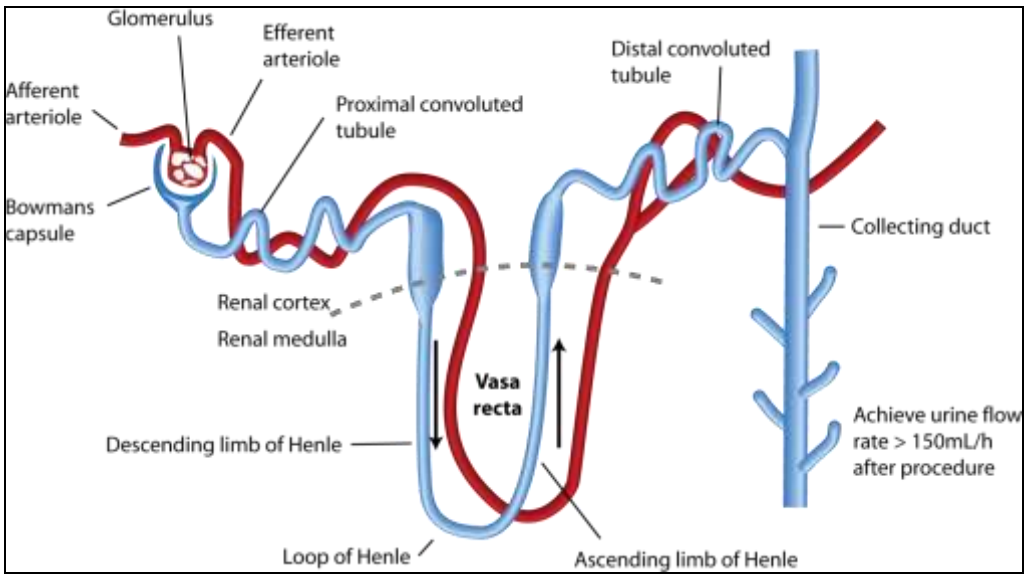
patients at high risk for AKI. Bilateral renal artery cannulation was successful in 94.2% with a mean cannulation time of 2 min. Patients received an infusion of either fenoldopam mesylate, sodium bicarbonate, alprostadil or B type natriuretic peptide. Mean creatinine levels did not change significantly (baseline, 24 and 48 h post procedure: 1.95. 1.99 and 1.98 mg/dl, p = NS). In the 285 patients who received fenoldopam and were followed for 48 h, the incidence of AKI was 71% lower than predicted (8.1% actual vs. 28% predicted by the Mehran risk score, p < 0.0001). Only 4 (1.4%) of patients required dialysis vs. the 2.6% predicted (p = NS). Further large randomised studies will be required to further validate these initial promising results.

1.7.5 Role of Haemodialysis/Haemofiltration

The role of haemodialysis in patients at high risk for the development of AKI remains unclear. Iodinated contrast is water soluble and therefore can be removed by dialysis. Patients already receiving renal replacement therapy (RRT) should have a dialysis session shortly following contrast administration to remove the contrast from the body and avoid possible complications of volume overload or late contrast reactions. There is currently no evidence that prophylactic dialysis in patients with advanced CKD (not on RRT) reduces the risk of AKI even when this intervention is carried out within 1 h of contrast administration. There may however be a role for haemofiltration in very high risk patients as some small studies have shown this to be beneficial (360;361). In 114 patients with advanced kidney disease (defined as a serum creatinine > 2mg/dl (176.8 µmol/l)) an increase in serum creatinine levels was significantly lower in patients randomly assigned to receive haemofiltration (n=58) pre and post contrast exposure as opposed to those assigned to receive fluid alone (5% vs. 50%, p<0.001). In-hospital

death was also significantly less frequent in those who received haemofiltration (2% vs. 14% in the control arm p=0.02) (361). Serum creatinine is directly affected by the intervention, and the relationship between the intervention and reduction in mortality is not clear. The resources required to deliver this therapeutic intervention are considerable and therefore the authors concluded that this would only be a realistic option for the most severe cases. In order to target patients who would benefit from this intervention after a contrast load, earlier identification of those at risk is necessary.

Figure 15: Strategies for renal protection



(adapted from McCullough PA: Chapter 27 Contrast Induced Acute Kidney Injury, Oxford Textbook of Interventional Cardiology 2010 (282)).

1.7.6 Remote Ischaemic Pre-Conditioning

Murry *et al* first proposed the hypothesis of 'conditioning' the myocardium to protect it against ischaemia-reperfusion injury (362). They observed that intermittent occlusion of the coronary artery to induce short bouts of myocardial ischaemia and reperfusion led to the development of myocardial resistance to a subsequent more prolonged episode of ischaemia and resulted in a significant reduction in infarction size. They termed this phenomenon 'ischaemic pre-conditioning' (IPC). The IPC stimulus results in a 'biphasic response with 2 distinct 'windows' of myocardial protection. The first, or 'classical IPC' lasts 3-4 hours and then disappears (363). The 'second window of protection, SWOP' or delayed/late preconditioning window, reappears 24 hours later and may confer cardioprotection for up to 72 hours (364).

The cardioprotective potential of IPC was first demonstrated in patients undergoing coronary artery bypass grafting (365;366). The IPC protocol involved cross clamping of the aorta and was shown to decrease myocardial injury. However the IPC technique used was both impractical and highly invasive. A more convenient, less invasive means of achieving cardioprotection may be conferred through 'remote ischaemic preconditioning' (RIPC) where brief ischaemia in a tissue/organ confers protection on a distant tissue or organ from a prolonged episode of ischaemia.

RIPC was first described by Przyklenk *et al* in 1993 (367). The authors showed that in anaesthetised dogs, episodes of circumflex coronary artery occlusion interspersed with periods of reperfusion before a sustained occlusion of the left anterior descending (LAD) artery with subsequent reperfusion, resulted in a significant reduction in the size of

myocardial infarction in the LAD vascular bed (6 +/- 2% of the risk zone versus 16 +/-5% in non-pre-conditioned controls, $p < 0.05$). Furthermore segment shortening of the LAD region was improved by preconditioning the circumflex vascular bed, again reflecting smaller infarction size. Preconditioning the circumflex vascular bed did not affect collateral flow within the LAD coronary bed. The authors also demonstrated that the magnitude of infarction reduction (35%) was equivalent to that of standard preconditioning within the same vascular bed (reviewed (368)).

Recent studies have suggested that there may be benefit from RIPC in decreasing rates of renal injury (369-371;371). The first of these studies, by Ali *et al* showed that RIPC reduced both myocardial and renal injury after elective abdominal aortic aneurysm repair (369). This was a randomised study performed in 82 patients undergoing elective abdominal aortic aneurysm (AAA) surgery which is a major operation associated with significant myocardial and renal injury. The study utilised invasive lower limb ischaemia with 2 cycles of intermittent cross clamping of the iliac arteries for 10 minutes followed by 10 minutes of reperfusion. Myocardial injury was defined as a cardiac troponin I > 0.40 ng/ml, myocardial infarction by the ACC/AHA definitions (305) and renal injury by a serum creatinine >177 μmol/l. RIPC reduced the incidence of myocardial injury by 27% (39% versus 12%, p=0.05), myocardial infarction by 22% (27% vs 5%, p=0.006) and renal impairment by 23% (30% vs 7%, p=0.009). Hoole *et al* extended the concept of RIPC using 3 cycles of upper limb ischaemia to a population undergoing elective percutaneous coronary intervention (370). Subjects were randomised either to 3 cycles of upper limb RIPC (n=104) or a control arm (n=98). RIPC significantly decreased median troponin I concentrations when compared to controls at 24 hours (0.06 ng/ml vs 0.16 ng/ml, p=0.04). RIPC was associated with less chest pain and ECG changes

during the PCI procedure and at 6 months there were fewer major adverse cardiac events when compared to controls (4 versus 13, hazard ratio 0.28, p=0.018).

In order to become adopted as an effective therapeutic strategy, it will be important to determine whether the myocardium is amenable to IPC in the presence of pathological conditions such as the metabolic syndrome and diabetes mellitus – this is currently not known (371;372). The data from animal models has been conflicting (reviewed (373)) and studies in humans have not included sufficient numbers of patients with diabetes (370;371;374).

One possibility is that there is an abnormality in protein kinase signalling in the PI3k-Akt kinase pathway of the diabetic heart. This pathway is thought to be involved in relaying the cardioprotective signal in the index ischaemic phase (363) and also inhibits the opening of the mitochondrial transition pore (375) in the inner mitochondrial membrane. Opening of this pore in the first few minutes of reperfusion mediates cell death by uncoupling oxidative phosphorylation and inducing mitochondrial swelling (376). Research by Tsang *et al* suggests that the effect of IPC may be restored by augmenting the IPC stimulus (377). Administration of hypoglycaemic agents such as glimepiride or rosiglitazone (reviewed (378)) may also restore the effects of IPC, since they act to augment levels of Akt. It is however important to note that some medications e.g. nicorandil and anti-diabetic agents have the potential to interfere with the endogenous cardioprotection conferred by IPC. This includes the sulphonylurea gliblencamide which increases basal and postprandial insulin secretion by closing the ATP dependent potassium channel in pancreatic beta cells. It is also thought to antagonise IPC by closing similar channels in the myocardium (379). However other sulphonylureas

including glipizide, gliclazide and glimepiride do not antagonise IPC (reviewed (378)). Further studies will be necessary to evaluate the role of RIPC in ameliorating contrast induced AKI, particularly in patients with diabetes.

1.8 Use of Biomarkers

In both AKI and CKD, prompt recognition and early intervention can significantly improve the otherwise very poor prognosis. Currently, in standard clinical practice, AKI is diagnosed by measuring serial serum creatinine levels but this may be problematic for a number of reasons. Creatinine is not a reliable indicator during acute fluctuations in kidney function (380-382). Serum creatinine levels can vary widely even in healthy individuals depending upon age, sex, muscle mass and metabolism, medications, nutrition and hydration status reviewed (383). A number of both acute and chronic kidney conditions can exist with no increase in serum creatinine evident due to the concept of 'renal reserve': this estimates that at least 50% of kidney function must be lost before it becomes clinically evident with a rise in serum creatinine. During acute changes in glomerular filtration, creatinine does not provide an accurate depiction of kidney function until a steady-state equilibrium (between production and decreased excretion of creatinine) has been achieved (380), a process that takes at least several hours. Finally an increase in serum creatinine is a late marker of functional change in GFR and lags behind key structural changes occurring in the kidney during the early stages of AKI (249).

Animal studies (reviewed (383)) have demonstrated that whilst AKI can be prevented and/or treated by various strategies, these must be instituted very early, after the initial insult and before the serum creatinine starts to rise. Typically creatinine begins to rise between 48-72 h following this initial insult. Conventional urine markers such as casts or fractional excretion of sodium are insensitive and non-specific for early recognition of AKI (381).

1.8.1 The need for a 'troponin-like' biomarker of AKI

A biomarker for AKI that is easily measured and unaffected by other biological variables, with the potential for both early identification and risk stratification would be a key therapeutic advance in the care of hospitalised patients where the overall incidence of AKI is estimated at 5-7% (380-382). As already discussed this rate may be even higher in some at risk groups undergoing exposure to contrast agents such as those found in the cardiac catheter laboratory (218;268;281). The deleterious influence of AKI to clinical outcomes in critically ill patients is well known (384-386). AKI has also been shown to be a key risk factor for the development of non-renal complications and to independently contribute to mortality (387). Technological developments in functional genomics and proteomics have led to the identification of a number of novel serum and urine biomarkers in both AKI and CKD. Our aim is to identify a highly sensitive and specific biomarker or if necessary develop a 'kidney injury risk panel' to perform the same role. This would be similar to that used in diagnosing an acute coronary syndrome where one combination used comprises of creatinine kinase, troponin and myoglobin.

1.8.2 The Ideal Acute Kidney Injury Biomarker

(adapted from Devarajan Nephrology 2010 (383)).

There are a number of important requirements for the ideal biomarker. These are:

1. Non-invasive using easily accessible samples such as urine or blood with the ability to perform either bedside testing or evaluate in a standard clinical laboratory

2. Rapid and reliable measurement using established standardised clinical assay platforms across a range of co-morbidities e.g. diabetes, coronary heart disease

3. Sensitive – facilitating early identification of AKI, with a wide dynamic range and cut off values allowing relevant risk stratification

4. Robust biomarker performance on statistical analysis including accuracy testing by receiver operating characteristic curves (388-390).

5. Highly specific for AKI, allowing the identification of AKI sub-types and different aetiologies

6. Cheap and cost effective

Biomarkers may serve a number of other purposes in AKI (388-390). They may have a role in identifying:

1. Primary anatomical location of the injury (e.g. proximal tubule, distal tubule, interstitium or vasculature)

2. Duration of AKI, CKD or ‘acute on chronic’ injury

3. Aetiology of AKI (e.g. ischaemia, sepsis, toxins, multi-factorial)

4. Risk stratification and prognosis (e.g. duration/severity of AKI, need for dialysis/filtration, length of hospital stay or mortality)

5. Response to AKI interventions

Finally AKI biomarkers may have an important role in the development of drugs and therapeutic interventions to treat AKI allowing nephrologic care to be individually tailored to the patient.

1.9 Biomarkers to be measured

These markers have been chosen to allow the identification of a possible ‘panel’ to predict the risk of contrast induced AKI – similar to that used in ACS non-ST elevation myocardial infarction (NSTEMI) (CK, Myoglobin, Troponin). The markers include both serum and urine neutrophil gelatinase associated lipocalin (NGAL), interleukin-18 (IL-18) and cystatin C. All have been implicated in the development of AKI but not all have been studied in post contrast AKI or in patients with CKD.

1.9.1 Microalbuminuria

Microalbuminuria, the pathological excretion of urinary albumin at levels below the threshold of detection by conventional urinary dipstick (30-300 mg/l), is thought to mark the onset of altered glomerular structure and function. There are some recent data from animal studies which suggest that the normal glomerular filter may leak albumin at higher levels than thought previously and albuminuria may reflect alterations in the proximal tubule cell retrieval pathway (391). Hence microalbuminuria may provide a useful marker of AKI and proximal tubule damage. There are concerns that lack of specificity may compromise utility as microalbuminuria may also occur in the setting of strenuous exercise, urinary tract infection, dehydration, fever and poor glycaemic control (392).

1.9.2 Neutrophil gelatinase associated lipocalin

NGAL is a small 25 kDa protein that was first identified bound to matrix metalloproteinase-9 (MMP-9) from neutrophils with a role as an innate immunity antibacterial factor (393). NGAL forms a barrel shaped tertiary structure with a hydrophobic calyx that binds small lipophilic molecules (394). The major ligands for NGAL are siderophores, small iron-binding molecules. Siderophores are synthesised by bacteria to acquire iron from their surroundings and NGAL exerts a bacteriostatic effect by depleting siderophores. However siderophores produced by eukaryotes play a key role in NGAL mediated iron shuttling that is critical in various cellular processes such as proliferation and differentiation (395). NGAL is expressed at very low levels in

several human tissues but acutely rises following injury to epithelial cells including the renal tubule, colon, liver and lung (383). NGAL is thought to play a role in enhancing the epithelial phenotype both during kidney development and following AKI (393).

In kidney animal models, NGAL has been identified as one of the earliest genes or proteins to be elevated following ischaemic or nephrotoxic injury (393;396-399). From this animal work it was discovered that NGAL was easily detectable soon after AKI and this lead to a number of translational studies evaluating NGAL as a non-invasive biomarker of human AKI. In adults with established AKI of various aetiologies (defined as a doubling of serum creatinine from baseline), a marked increase in both urine and serum NGAL was documented by Western blotting compared with controls (398). Urine and serum NGAL levels correlated with serum creatinine. Furthermore kidney biopsies in patients with AKI demonstrated intense accumulation of immunoreactive NGAL in the cortical tubules. This established NGAL as a sensitive biomarker of AKI (tubular damage) in humans. A number of subsequent studies (reviewed (383) have identified NGAL as an early diagnostic urine and plasma biomarker for AKI in various clinical settings in both adults and children, as summarised in the tables below (Tables 10 and 11)

Table 10: Urine NGAL for the early prediction of AKI

Reference	Setting	Subjects (n)	Sensitivity	Specificity	AUC-ROC (CI)
Mishra, Lancet 2005	Cardiac surgery	71	1.0	0.98	0.99 (NR)
Wagener, Anesthesiology 2006	Cardiac surgery	81	0.73	0.78	0.80 (0.57-1.03)
Koyner, Kidney Int 2008	Cardiac surgery	72	0.49	0.79	0.69 (0.57-0.82)
Wagener, Am J Kidney Dis. 2008	Cardiac surgery	426	NR	NR	0.61 (0.54-0.68)
Xin, Ren Fail 2008	Cardiac surgery	33	0.71	0.73	0.88 (NR)
Bennett, J Am Soc Nephrol 2008	Cardiac surgery	196	0.82	0.9	0.93 (NR)
Portilla, Kidney Int 2008	Cardiac surgery	40	1.0	1.0	1.00 (NR)
Tuladhar, J Cardiovasc Pharmacol 2009	Cardiac surgery	50	0.93	0.78	0.96 (0.9-1.0)
Bachorzewska-Gajewska, Am J Nephrol 2006	Contrast	100	NR	NR	NR
Ling, Nephron Clin Practice 2008	Contrast	40	0.77	0.71	0.73 (0.54-0.93)
Hirsch, Pediatr Nephrol 2007	Contrast	91	0.73	1.0	0.92 (NR)
Nickolas, Ann Intern Med 2008	Emergency room	635	0.9	0.99	0.95 (0.88-1.0)
Zappitelli, Crit Care 2007	Critical care	150	0.77	0.72	0.78 (0.62-0.95)
Makris, Clin Chem Lab Med 2009	Critical care	31	0.91	0.95	0.98 (0.82-0.98)
Siew, J Am Soc Nephrol 2009	Critical care	451	NR	NR	0.71 (0.63-0.78)
Parikh, Am J Transplant 2006	Kidney transplant	63	0.9	0.83	0.90 (0.71-1.0)
Hall, J Am Soc Nephrol 2010	Kidney transplant	91	0.77	0.74	0.81 (0.70-0.92)

(adapted from Devarajan *et al* Nephrology 2010 (383). Abbreviations: AUC-ROC, area under the receiver-operating characteristic curve; CI, 95% confidence interval; NR, not-reported).

Table 11: Plasma NGAL for the early prediction of AKI

Reference	Setting	Subjects (n)	Sensitivity	Specificity	AUC-ROC (CI)
Mishra, Lancet 2005	Cardiac surgery	71	0.7	0.94	0.91 (NR)
Koyner, Kidney Int 2008	Cardiac surgery	72	NR	NR	0.54 (0.4-0.67)
Dent, Crit Care 2007	Cardiac surgery	120	0.84	0.94	0 96 (0.94-0.99)
Tuladhar, J Cardiovasc Pharmacol 2009	Cardiac surgery	50	0.8	0.67	0.85 (0.73-0.97)
Haase-Fielitz, Crit Care Med 2009	Cardiac surgery	100	0.79	0.78	0.8 (0.63-0.96)
Malyszko, Ren Fail 2009	Contrast	91	0.73	1.0	0.91 (NR)
Wheeler, Crit Care Med 2008	Critical care	143	0.86	0.39	0.68 (0.56-0.79)
Cruz, Int Care Med 2010	Critical care	301	0.73	0.81	0.78 (0.65-0.90)
Bolignano, Nephrol Dial Transplant 2008	Critical care	88	0.82	0.97	0.92 (0.85-0.97)
Niemann, Liver Transplant 2009	Liver transplant	59	0.68	0.8	0.79 (NR)

(adapted from Devarajan *et al* Nephrology 2010 (383). AUC-ROC, area under the receiver-operating characteristic curve; CI, 95% confidence interval; NR, not-reported).

Urine and plasma levels are elevated 2 h post cardio-pulmonary bypass and 4 h post contrast administration in patients who subsequently develop AKI (400;401). Measurements may be influenced by pre-existing renal disease and systemic or urinary tract infections. In CKD NGAL has been demonstrated to be a marker (plasma and urine) of kidney disease and its severity (402;403). Further studies will be necessary to determine the role of NGAL in models of CKD and to define its association with CKD progression (380). NGAL has not been assessed post contrast in a population including CKD patients.

1.9.3 Interleukin-18 (IL-18)

IL-18 is a pro-inflammatory cytokine that is induced in the renal tubule following AKI. It is produced as a 24 kDa inactive precursor which requires cleavage by caspase 1 to generate its biologically active form (404;405). A cleaved form is detectable in urine. IL-18 has immunomodulatory properties and appears to play a key role in host cell defence against a number of infections (reviewed (392)). It has low sensitivity but high specificity for AKI (reviewed (401)). It is more specific to ischaemic AKI and other forms of tubular necrosis (406;407). Urinary levels are elevated 48 h before clinically evident AKI (rise in serum creatinine) (407). IL-18 does not appear to be affected by pre-renal uraemia, CKD or urinary tract infections and appears to offer prognostic information regarding severity and mortality at the time of AKI diagnosis. It has not been formally assessed as a marker of post contrast AKI (380).

1.9.4 Other biomarkers of renal injury

Other potential candidates as biomarkers of contrast induced AKI are discussed below. This list is not exhaustive but from review of the literature were the most promising markers for identifying renal injury early post procedure.

1.9.4.1 Cystatin C

Cystatin C is a cysteine protease inhibitor that is synthesised and released at a relatively constant rate by all nucleated cells. It is freely filtered by the glomerulus, completely reabsorbed by the proximal tubule and not secreted thereby providing a surrogate

marker of glomerular filtration. Plasma levels are elevated 8 hours post administration of contrast and urinary excretion has been shown to predict the requirement for renal replacement therapy in patients with established AKI approximately 1 day earlier than creatinine (408). It is primarily a sensitive marker of reduction in glomerular filtration and not a marker of kidney injury *per se* (380). From reviewing the literature plasma levels of cystatin C out-perform creatinine as a marker of kidney function. A 50% rise in cystatin C 2 days prior to clinical AKI has a 55% sensitivity and a 95% specificity for predicting AKI (409). However levels may be affected by HIV and glucocorticoid use and the variability and bias in using cystatin C to predict GFR were no better than equations using serum creatinine. It has been widely reviewed as a promising GFR marker in both AKI (as outlined above) and CKD but further studies are necessary to determine whether it is truly a better biomarker than serum creatinine and to define the populations in which its use is appropriate (380).

1.9.4.2 N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme and a sensitive urine marker of proximal tubule injury with a role in mortality risk prediction following AKI (410). This study also showed NAG has to be elevated in AKI following a number of disease states including cirrhosis and sepsis (410) In a study of 90 adult patients undergoing bypass surgery at Columbia University in New York, 36 patients developed AKI. The AUC for NAG on ROC analysis immediately and 3 hours post procedure was 0.61 and 0.63. However when combined with NGAL and Kidney Injury Molecule-1 (KIM-1) the AUC improved to 0.75 and 0.78 respectively (411). NAG has not been evaluated as a marker of contrast induced AKI.

1.9.4.3 Kidney Injury Molecule 1

KIM-1 is a transmembrane protein which is not expressed in normal kidney tissue but is up-regulated in de-differentiated proximal tubule cells following ischaemic or nephrotoxic AKI and a proteolytic processed domain is easily detectable in urine (412). It readily distinguishes ischaemic AKI from pre-renal uraemia and chronic kidney disease (413). From the New York registry described in section 1.9.4.2, KIM-1 had an AUC on ROC analysis of 0.68 and 0.65 immediately and 3 hours post bypass surgery respectively (411). Elevated urine levels have been associated with adverse outcomes in hospitalised patients who develop AKI (410). In a small study of 70 diabetic patients with normal baseline renal function undergoing coronary angiography, no significant increase in KIM-1 was seen within the first 48 hours post procedure (414). There are some methodological questions that arise on reviewing this study. Data are also provided for a control cohort of non-diabetic patients and the rate of contrast induced AKI was higher in this group than in the patients with diabetes. This is surprising since as discussed in section 1.6.4.2 diabetes is an important risk factor for development of AKI. Furthermore no p values were provided to demonstrate how well matched the 2 cohorts were with regard to baseline characteristics, although the authors state that the groups were age matched. Finally for a diagnostic procedure the mean volume of contrast used was very high at a mean of 170.5 ml. To my knowledge there are no other studies assessing KIM-1 as a marker of contrast induced AKI.

1.9.4.4 Liver Type Fatty Acid Binding Protein (L-FABP)

This is a 14kDa protein expressed in the proximal tubule of normal kidney tissue. Its primary function is to facilitate intra-cellular long chain fatty acid transport. Both expression and urinary excretion are increased in the setting of non-diabetic CKD (415). Levels at 4h post bypass surgery in paediatric patients have been shown on logistic regression analysis to independently predict development of kidney injury with an AUC on ROC analysis of 0.81, sensitivity 0.714 and specificity 0.684 for a 24-fold increase in urinary L-FABP (416). However as the name may suggest expression is not specific to the kidney and this protein is expressed in a number of tissues with active fatty acid metabolism such as the heart and the liver (417;418). One small study (n=70) has evaluated L-FABP post coronary angiography in patients with normal baseline renal function. Urinary L-FABP levels were shown to be significantly raised at 24 hours post procedure ($p<0.05$) in patients who subsequently developed AKI (414). There are some methodological concerns that arise from this study as mentioned in section 1.9.4.3. To my knowledge there are no other studies assessing L-FABP as a marker of contrast induced AKI.

1.9.4.5. Homocysteine

Homocysteine is a known risk factor for cardiovascular disease as discussed previously in section 1.3.1. Recent data have shown that levels independently predict development of contrast induced AKI in patients undergoing PCI (419). Hyperhomocysteinemia induces oxidative stress and endothelial dysfunction, which are some of the proposed pathophysiologic mechanisms of contrast-induced AKI discussed in more detail in section 1.6.2. The incidence of contrast induced AKI was significantly greater in

patients in the third homocysteine tertile (from lowest to highest, 4.7%, 7.3%, and 24.2%, p <0.001). Furthermore, the homocysteine levels were significantly greater in patients with contrast induced AKI than in those without (16.9 ± 4.9 vs. 13.5 ± 4.2 µmol/L, p <0.001). In multiple logistic regression models, hyperhomocysteinemia was an independent risk factor for contrast induced AKI
(per the SD change in the plasma homocysteine level [4.44 µmol/L], odds ratio 1.70, 95% confidence interval 1.07 to 2.71, p = 0.025) after adjusting for major risk factors such as age, diabetes, and baseline cardiac and renal function. In subgroup analyses according to diabetes, acute coronary syndrome, or baseline estimated glomerular filtration rate, significant graded associations were found between the homocysteine level and the incidence of contrast induced AKI. This was an observational study and in order to identify if a true causal relationship is present, further trials with interventions to lower homocysteine levels will be necessary.

1.10 Outline of thesis

The aim of the work presented in this thesis was to identify early markers of kidney injury post i.v. contrast following coronary angiography or PCI in patients with diabetes and CKD. These were compared against the current 'gold standard' marker of renal injury, creatinine in order to attempt to establish a panel of 'renal injury markers' that could translate into clinical practice. Patients with diabetes and CKD are known to have worse cardiovascular outcomes and are at extremely high risk of developing AKI and yet have been excluded from many of the studies investigating biomarkers of renal injury. Our research group has had a longstanding interest in diabetic coronary disease and

this was an exciting opportunity for collaboration between the departments of cardiology and renal medicine at Barts and The London NHS Trust.

This was a prospective observational study and the design is discussed in Chapter 2. All patients were recruited at a single site, The London Chest Hospital which is a tertiary centre for interventional cardiology procedures. Chapter 3 details the baseline characteristics and clinical procedural data collected. The influence of structural renal disease on the development of AKI is discussed in Chapter 4, along with assessment of a number of risk scores such as The Mehran Score and Syntax Score in a high risk cohort of patients. Chapter 5 describes the various assays and all laboratory techniques as well as the statistical methodology underlying Receiver Operating Characteristic (ROC) curve analysis, which is used to assess the predictive power of the biomarker panel. The results of a biomarker panel including NGAL and IL-18 are discussed in Chapter 6, with an exploratory analysis including the various risk scores as predictive markers. In each chapter a brief discussion is included at the end, with a comprehensive final discussion in Chapter 7.

Chapter Two

Study Design

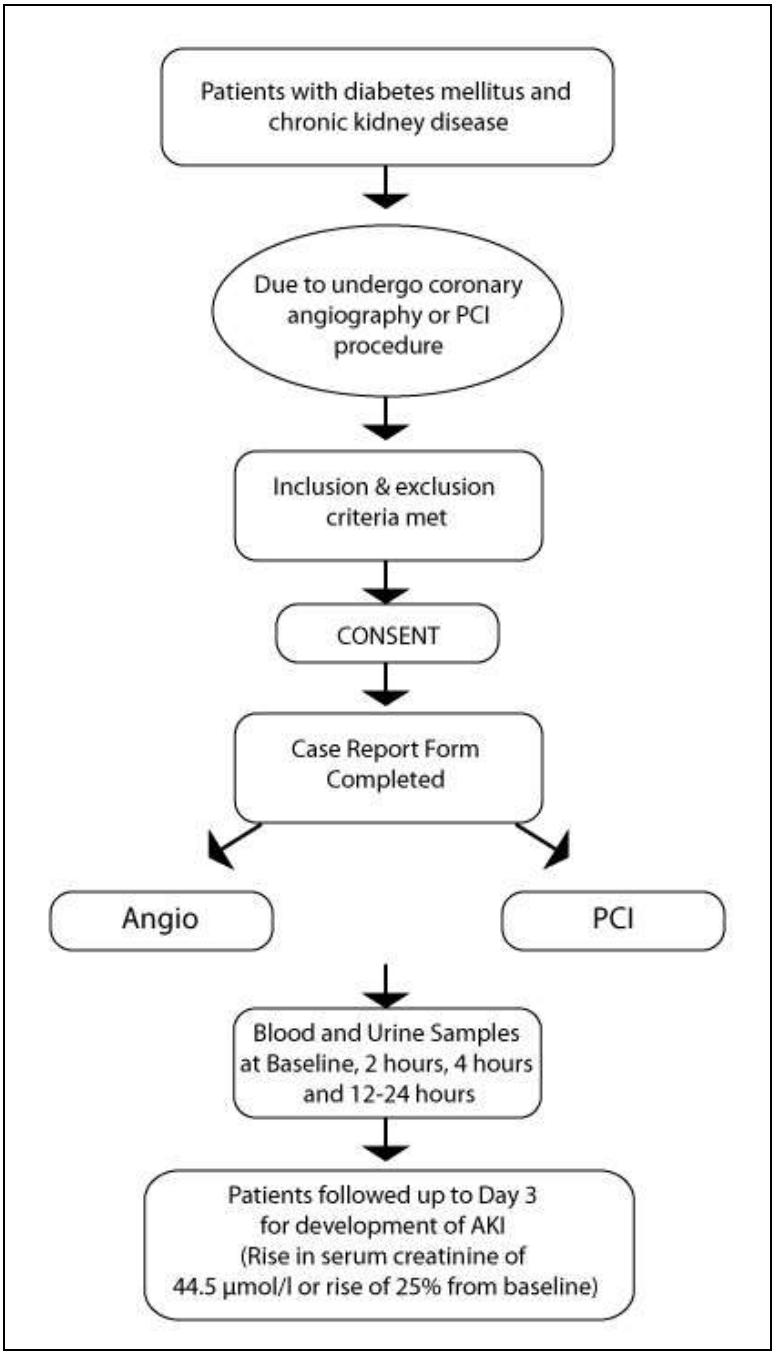
Chapter 2: Study Design

This was a single-centre prospective study of patients with diabetes with a baseline eGFR < 60 ml/min undergoing coronary angiography and / or PCI at The London Chest Hospital. AKI was defined as an increase in creatinine ≥ 44.5 µmol/l or a rise of > 25% from baseline. Both elective and unstable patients were included but due to other co-existent studies in the department patients with ST elevation myocardial infarction (STEMI) were excluded. In a subset of patients renal angiography was performed to assess for structural renal disease. Informed consent was obtained in all cases. The study was approved by the East London and City NHS Research Ethics Committee 1 (09/H0703/29) and registered on clinicaltrials.gov (NCT00948116).

2.1 Study Objective

The primary objective of this study was to identify in patients with diabetes techniques to predict who will develop AKI post coronary angiography PCI earlier than is currently possible. This is based on the hypothesis that a panel of biomarkers can predict development of contrast induced AKI earlier than a rise in serum creatinine. The study design is summarised in Figure 16 and was a single-centre observational study of biomarkers reflecting kidney injury in patients with diabetes mellitus undergoing coronary angiography and PCI.

Figure 16: Biomarkers Study Design



Consort diagram summarising study pathway. Samples were immediately centrifuged and stored at -80°C prior to assay for NGAL and Interleukin-18.

2.2 Sample Size Calculation

From a retrospective audit of the cardiac catheter laboratory database and a review of the literature we estimated that a sample size of approximately 250 patients with diabetes mellitus and CKD (eGFR < 60 ml/ml) would be needed. We envisaged that that we would encounter at least 50 cases of AKI from this cohort (based on an expected incidence of AKI between 15-30 % in this group). Looking for a study rate difference of at least 25%, for power of 95% and confidence intervals of 95% (with Fleiss correction) we would need at least 204 evaluable patients (to avoid Type 2 error). In view of a potential drop-out rate of 10-15% we therefore intended to recruit up to 250 patients (Power calculation performed using Arcus Medical Statistical Programme). The study was powered on the rate of anticipated AKI in the population rather than on the biomarkers diagnostic ability as the biomarkers had not been evaluated for this type of study population or following contrast administration at the time this study was conceived.

2.3 Study Duration

It was expected that the study would take between 12-18 months to recruit. 208 patients were recruited between 1st August 2009 and 21st July 2010. At this time recruitment stopped as we had reached a sufficient number of events for the study to be adequately powered.

2.4 Statistical Methods

Baseline characteristics were assessed with paired student t tests (parametric) and Mann-Whitney U test (non-parametric) for continuous variables. Chi squared and where appropriate Fisher's Exact test were used for categorical variables. Results are presented as mean (SD) for parametric variables and median (IQR) for non-parametric variables. Categorical variables are presented as n (%). Logistic regression analysis was used to establish the predictive relationship between biomarker elevation and development of contrast induced AKI. Where the biomarker was also a continuous variable linear regression analysis was used to assess the relationship between the biomarkers and other continuous measurements. Receiver Operating Characteristic (ROC) Curves were obtained by plotting sensitivity and specificity of individual markers at different cut-offs as has previously been described (407). All analyses were performed using Stata 10.1 (StataCorp, College Station, Texas). A p value <0.05 was considered to be statistically significant.

2.5 The London Chest Hospital Heart Attack Centre Guidelines for the Prevention of Contrast Nephropathy

The London Chest Hospital Heart Attack Centre Guidelines for the Prevention of Contrast Nephropathy are available on the Barts and The London NHS Trust Intranet (331). The eGFR for all patients undergoing coronary angiography or PCI must be

known (except in emergency cases such as primary PCI for acute STEMI) and is usually provided directly from the biochemistry laboratory. If it is not available then this can be easily calculated using the Cockcroft Gault Formula. An online calculator is available (http://nephron.org/cgi-bin/CGSI.cgi). Other formulas are available which do not include body weight (e.g. MDRD, Modification of Diet in Renal Disease, http://nephron.org/cgi-bin/MDRD_GFR/cgi). These are described in more detail in the Appendix along with a copy of The London Chest Hospital Guidelines for Prevention of Contrast Nephropathy.

2.5.1 The Cockcroft-Gault Formula

This estimates creatinine clearance (eC_{Cr}) in ml/min as follows:

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times Constant}{\text{Serum Creatinine (in } \mu\text{mol/L)}}$$

Where *Constant* is *1.23* for men and *1.04* for women

and was first reported in 1976 (220). This calculation is very dependent on age. For example a 20 year old person will have twice the creatinine clearance as an 80 year old for the same level of serum creatinine. It also shows that a woman will have a 15% lower creatinine clearance for the same level of creatinine than a man.

2.5.2 N-acetylcysteine

It is currently established practice that all patients with an eGFR < 50ml/min undergoing coronary angiography or PCI at the London Chest Hospital receive treatment with N-Acetylcysteine (NAC) 600 mg po bd prior to the procedure and continued for 24 hours following (total 4 doses). There is no evidence in the literature, nor from consensus expert opinion that there is any interaction between NAC and the biomarkers under investigation (420).

2.5.3 Intravenous Fluids

Patients with eGFR >50 ml/min do not require any special measures (e.g. NAC, hydration) and can take free fluids orally until the time of the procedure. For patients with eGFR ≤50 ml/min a litre of 0.9% sodium chloride should be commenced intravenously at least 2 h prior to the procedure and continued for a total of 8 hours. Free access to clear oral fluids should be available before and after the procedure.

2.5.4 Contrast agent

All coronary angiogram and PCI procedures were performed at The London Chest Hospital and used iodixanol (VISIPAQUE™, GE Healthcare, Norway) as the contrast agent. Iodixanol is a non-ionic, iso-osmolar agent (see section 1.6).

2.5.5 Medication discontinuation

It is advised that all diabetic patients discontinue metformin for 24 hours prior to the procedure and for 48 h afterwards due to the risk of lactic acidosis. In patients with GFR <50 ml/min it is advised that consideration should be given to stopping nephrotoxic agents such as ACE inhibitors, diuretics or NSAIDs on an individual patient basis.

2.6 Blood Sampling

Blood (serum) samples were taken at the following time intervals:

Baseline – FBC, U+E, CRP, Bone, LFT, Clotting, Glucose, HbA1c, lipids, CK, Troponin T (20 ml).

Baseline, 2 hours, 4 hours and 12-24 hours post procedure biomarkers (4 x 20 ml – serum samples).

Pre-discharge biomarkers CK, CK-MB, Troponin and U+E (2 x 20 ml).

U+E 3 days post procedure (5 ml).

Total Blood Volume: 145 ml.

All samples except for the biomarkers were analysed as according to routine clinical practice in the haematology and chemical pathology departments at Barts and The

London NHS Trust. The process for biomarkers samples is described in section 2.8 and 2.9.

(FBC = Full Blood Count, U+E = Urea and Electrolytes, LFT = Liver Function Tests, CK = Creatinine Kinase, HbA1c = glycated haemoglobin).

2.7 Urine Sampling

Urine samples were taken at the following time intervals:

Baseline – Urine albumin:creatinine ratio, biomarkers (100 ml)

2 hours, 4 hours and 12-24 hours post procedure biomarkers (3 x 100 ml).

Pre-discharge biomarkers (100 ml).

Total Urine Volume: 500 ml.

We selected these time points for blood and urine samples to assess our panel of markers on the day of the patient's procedure. This is important as the majority of coronary angiography and an increasing number of PCI procedures are performed on a day case basis (22). The current 'gold standard' marker of AKI – serum creatinine does not rise until 48-72 h post procedure. We therefore wanted to ascertain whether any of our biomarkers rose in the period that all patients remain in hospital – i.e. up to 4 h

following procedure. In those patients who stay overnight for procedures it is routine for them to have blood tests the next day – at least to include an FBC and U + E and sometimes also a CK and Troponin. We therefore also took serum and urine samples for our biomarker panel at this time-point in order to assess if there was an additional value from this to our biomarker panel for predicting AKI. This was typically 12-24 h post procedure. An initial plan to also take a sample at 8-12 h had to be abandoned for logistical reasons.

2.8 Sample Storage

Biomarker samples were immediately centrifuged at 3000 rpm (1600 *g*) for 10 minutes and the supernatant fluid was aliquoted into 2 ml cryotubes. All samples were stored at -80°C in allocated freezer space at the London Chest Hospital. Batches were transferred on ice to Professor Yaqoob's laboratory at The William Harvey Research Institute for analysis.

2.9 Analytical Assay

Blood (serum) samples were used for determination of biomarkers and comparison to serum creatinine and urine for biomarker presence and comparison to albumin:creatinine ratio. Commercially available ELISA (Enzyme Linked Immunosorbent Assay) kits (R and D Systems, Oxford, United Kingdom) were used to measure marker concentration.

ELISA, is a biochemical technique which is based on the 'antibody sandwich principle'. A capture antibody, specific to the analyte of interest is bound to a microtitre plate to create the solid phase. Unbound antibody is removed by washing the plate and a blocking reagent is added. Following a wash, samples, standards and controls are then incubated with the solid phase antibody which captures the analyte. After washing away unbound analyte, a conjugated detection antibody (e.g. biotin conjugated) is added. This detection antibody binds to a different epitope of the molecule being measured, completing the sandwich. A further wash removes unbound detection antibody and a detection reagent e.g. streptavidin-HRP (horse-radish peroxidase) is added. The plate is washed and a substrate solution e.g. tetramethylbenzidine (TMB)/hydrogen peroxide is added; colour develops in proportion to the quantity of bound analyte (TMB/peroxide turns blue when modified by HRP). Colour development is stopped with the addition of an acidic 'stop solution' which turns the solution yellow. The optical density of the yellow colour is read on a microtitre plate reader.

The values of the unknown samples are assigned in relation to a standard curve. Software allows various methods of 'curve fitting' to be tried e.g. linear, log-log to determine which curve best fits the data. Once an acceptable standard curve is obtained the assay is optimised to meet performance requirements. Further details of this process for the individual assays are given in Chapter 5.

2.10 Inclusion Criteria

In order to be eligible to enter the study, the patients had to meet the following criteria:

1. Age > 18 years, known diagnosis of diabetes mellitus or BM on arrival consistent with probable diagnosis of diabetes, eGFR <60 ml/min

2. Undergoing a coronary angiography or PCI procedure

3. Agreed to the additional collection of blood and urine samples as outlined above

4. Agreed to access of their clinical records for the collection of relevant medical data

5. No history or signs of drug abuse

6. Able to understand and sign the written Informed Consent Form

7. Able and willing to follow the Protocol requirements.

2.11 Exclusion Criteria

Patients were not admitted to the study if they met any of the following exclusion criteria:

1. Cardiogenic shock

2. Pregnancy

3. Patient on renal replacement therapy (haemodialysis/CAPD/renal transplant)

4. Known clinically significant infection such as HIV, Hepatitis or TB.

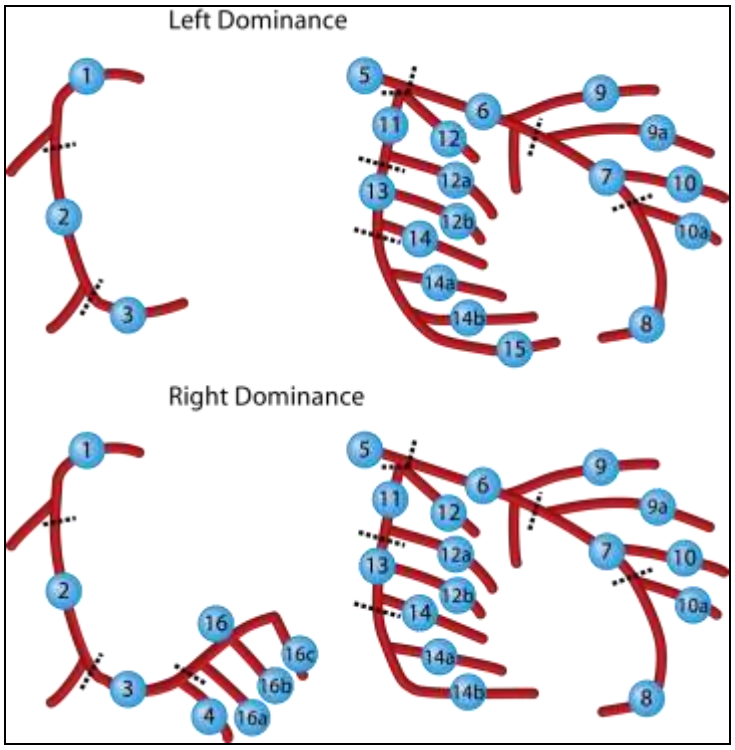
5. Any patient determined not able to make a reasoned, informed consent prior to the planned interventional procedure.

2.12 SYNTAX Score

The SYNTAX score allows quantification of the complexity of coronary artery disease by taking into consideration not only the number of significant lesions but also the complexity of each lesion independently. Higher SYNTAX scores, indicative of more complex disease are hypothesised to represent a bigger therapeutic challenge and potentially to have a poorer prognosis (421). It is important for the non-clinician and indeed the non-cardiologist to understand this complex scoring system and how this translates into interpreting the severity of CAD. Whilst on first inspection, this may appear to have little relevance to the main subject matter discussed in this thesis, that is the development of AKI post contrast, understanding is essential in order to interpret the data presented in Chapter 4.

The definition of the coronary tree segments (Figure 17) is based on the classification proposed by the AHA (422) and modified for the ARTS I and II Trials (423).

Figure 17: Definition of the coronary tree segments



1. RCA proximal: From the ostium to one half the distance to the acute margin of the heart.
2. RCA mid: From the end of first segment to acute margin of heart.
3. RCA distal: From the acute margin of the heart to the origin of the posterior descending artery.
4. Posterior descending artery: Running in the posterior interventricular groove.
16. Posterolateral branch from RCA: Posterolateral branch originating from the distal coronary artery distal to the crux.
16a. Posterolateral branch from RCA: First posterolateral branch from segment 16.
16b. Posterolateral branch from RCA: Second posterolateral branch from segment 16.
16c. Posterolateral branch from RCA: Third posterolateral branch from segment 16.
5. Left main: From the ostium of the LCA through bifurcation into left anterior descending and left circumflex branches.
6. LAD proximal: Proximal to and including first major septal branch.
7. LAD mid: LAD immediately distal to origin of first septal branch and extending to the point where LAD forms an angle (RAO view). If this angle is not identifiable this segment ends at one half the distance from the first septal to the apex of the heart.
8. LAD apical: Terminal portion of LAD, beginning at the end of previous segment and extending to or beyond the apex.
9. First diagonal: The first diagonal originating from segment 6 or 7.
9a. First diagonal a: Additional first diagonal originating from segment 6 or 7, before segment 8.
10. Second diagonal: Originating from segment 8 or the transition between segment 7 and 8.
10a. Second diagonal a: Additional second diagonal originating from segment 8.
11. Proximal circumflex artery: Main stem of circumflex from its origin of left main and including origin of first obtuse marginal branch.
12. Intermediate/anterolateral artery: Branch from bifurcating left main other than proximal LAD or LCX. It belongs to the circumflex territory.
12a. Obtuse marginal a: First side branch of circumflex running in general to the area of obtuse margin of the heart.
12b. Obtuse marginal b: Second additional branch of circumflex running in the same direction as 12.
13. Distal circumflex artery: The stem of the circumflex distal to the origin of the most distal obtuse marginal branch, and running along the posterior left atrioventricular groove. Caliber may be small or artery absent.
14. Left posterolateral: Running to the posterolateral surface of the left ventricle. May be absent or a division of obtuse marginal branch.
14a. Left posterolateral a: Distal from 14 and running in the same direction.
14b. Left posterolateral b: Distal from 14 and 14 a and running in the same direction.
15. Posterior descending: Most distal part of dominant left circumflex when present. It gives origin to septal branches. When this artery is present, segment 4 is usually absent.

(Adapted from Sianos *et al* EuroIntervention 2005 (421)). Abbreviations: RCA: right coronary artery, LAD: left anterior descending.

A lesion is defined as significant when it causes a ≥ 50% reduction in luminal diameter on visual assessment in vessels > 1.5 mm diameter. The algorithm includes occlusive (100% stenosis) and non-occlusive (50-99% stenosis) but does not further stratify percent diameter of stenoses. Various weighting factors are given to account for the difficulty in percutaneous treatment (summarised in Table 12).

Table 12: Coronary artery segment weighting factors

Segment Number	Segment Name	Right dominance	Left dominance
1	RCA proximal	1	0
2	RCA mid	1	0
3	RCA distal	1	0
4	Posterior descending artery	1	n.a.
16	Posterolateral branch from RCA	0.5	n.a.
16a	Posterolateral branch from RCA	0.5	n.a.
16b	Posterolateral branch from RCA	0.5	n.a.
16c	Posterolateral branch from RCA	0.5	n.a.
5	Left main	5	6
6	LAD proximal	3.5	3.5
7	LAD mid	2.5	2.5
8	LAD apical	1	1
9	First diagonal	1	1
9a	First diagonal a	1	1
10	Second diagonal	0.5	0.5
10a	Second diagonal a	0.5	0.5
11	Proximal circumflex artery	1.5	2.5
12	Intermediate/anterolateral artery	1	1
12a	Obtuse marginal a	1	1
12b	Obtuse marginal b	1	1
13	Distal circumflex artery	0.5	1.5
14	Left posterolateral	0.5	1
14a	Left posterolateral a	0.5	1
14b	Left posterolateral b	0.5	1
15	Posterior descending	n.a.	1

(Adapted from Sianos *et al* EuroIntervention 2005 (421)). Abbreviations: RCA: right coronary artery, LAD: left anterior descending.

All other adverse lesions characteristics assessed using the SYNTAX score have an additive value (Table 13).

Table 13: Adverse characteristic lesion scoring

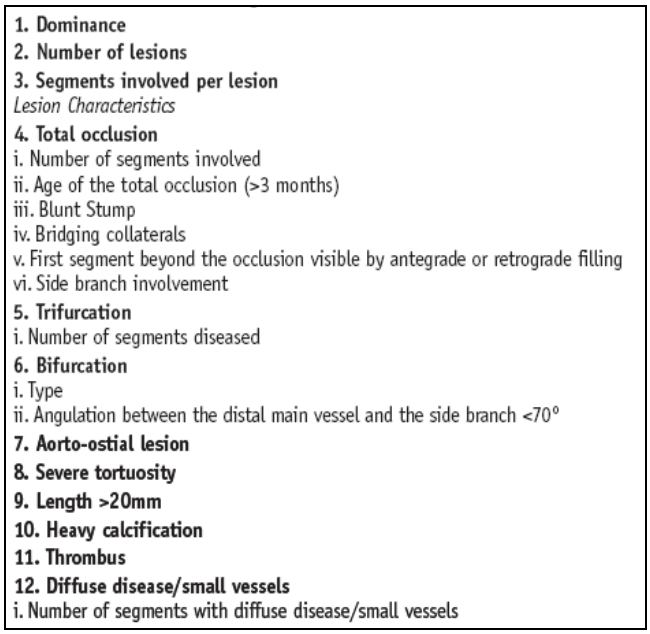
Lesion Type	Lesion Score
Diameter reduction*	
- Total occlusion	X 5
- Significant lesion (50-99%)	X 2
Total Occlusion (TO)	
- Age >3 months or unknown	+1
- Blunt stump	+1
- Bridging	+1
- First segment visible beyond TO	+1/ non-visible segment
- Side branch (SB) - – Yes SB < 1.5 mm** - Yes both SB < & ≥ 1.5 mm	 +1 +1
Trifurcations	
- 1 diseased segment	+3
- 2 diseased segments	+4
- 3 diseased segments	+5
- 4 diseased segments	+6
Bifurcations	
- Type A, B, C	+1
- Type D, E, F, G	+2
- Angulation <70°	+1
Aorto-ostial stenosis	+1
Severe tortuosity	+2
Length > 20 mm	+1
Heavy calcification	+2
Thrombus	+1
Diffuse disease/small vessels	+1/per segment number

(Adapted from Sianos *et al* EuroIntervention 2005 (421)). Key: x: multiplication, +: addition. * In the SYNTAX algorithm there is no question for % luminal diameter reduction. The lesions are considered as significant (50-99% luminal diameter reduction) or occlusive. **If all the side-branches are 1.5 mm diameter, no points are added since the lesion is considered as a bifurcation and it will be scored accordingly.

2.12.1 The SYNTAX Score Algorithm

The Syntax Score is calculated by a computer program (available as an online download: http://www.syntaxscore.com/) made up of sequential and interactive self-guided questions. These are summarised in Table 14. All patients in this study were scored by myself and Dr Akhil Kapur, Consultant Interventional Cardiologist. On completion of the algorithm a detailed report is automatically generated summarising all the adverse characteristics, the individual score for each lesion and the total SYNTAX score. For the purposes of this study we used Version 2.02 of the online calculator. The score can help to guide revascularisation strategy. For patients with a low SYNTAX score (0-22) the risk-benefit ratio favours PCI, for patients with an intermediate score (23-32) either PCI or CABG may be appropriate and for a high score (≥33) CABG is the preferred option (26).

Table 14: The SYNTAX Score Algorithm



(Adapted from Sianos *et al* EuroIntervention 2005 (421)). Algorithm definitions are given in the Appendix.

Patients with previous CABG cannot receive a SYNTAX score as such patients were excluded from the trials from which the score was developed. Our study included 41 such patients.

2.12.2 The Clinical SYNTAX Score

One of the main limitations of the SYNTAX score is that anatomical (lesion based) scoring systems have been shown to have a lower accuracy in predicting mortality than systems which include clinical characteristics (424). Whilst clinical risk scores such as EuroSCORE have been well-validated in cardiac surgery populations (425;426), there are currently only very limited data of such systems for patients undergoing PCI

(427) (428). A simple ACEF (Age, serum Creatinine and Ejection Fraction) score has recently been described and validated (429) in patients undergoing elective CABG. It is thought that more complex scores which use multiple individual variables may reduce the overall accuracy of the model (430).

In order to attempt to overcome the perceived limitations of the SYNTAX score, the authors of the SYNTAX trial (26), combined the SYNTAX score with a modified ACEF score (incorporating creatinine clearance rather than serum creatinine) creating a clinical SYNTAX score (CSS) (431). This was then assessed on the Arterial Revascularisation Therapies Study (ARTS) II dataset (n=512) (25) for improvement in ability to predict mortality and MACCE (Major Adverse Cardiovascular and Cerebrovascular Events) (431). Clinical outcomes were assessed for MACCE and mortality at 1 and 5 years follow up and further stratified by CSS tertiles: $CSS_{low} \leq 15.6$ (n=170), $15.6 < CSS_{med} \leq 27.5$ (n=171) and $CSS_{high} > 27.5$ (n=171). At 1 year follow-up rates of repeat revascularisation and MACCE were significantly higher in the highest tertile group (Table 15). At 5 year follow-up, CSS_{high} had a similar rate of myocardial infarction, demonstrated a trend towards a significantly higher rate of death, and significantly higher rates of repeat revascularisation and overall MACCE as compared to patients in the lower 2 tertiles. Univariate and multivariate predictors of MACCE are summarised in Table 16. The C statistics for the CSS, SYNTAX Score and ACEF score were respectively for 5 year mortality were 0.69, 0.62 and 0.65 and for 5 year MACCE were 0.62, 0.59 and 0.57 ($p<0.05$ for all).

Table 15: Clinical Outcomes at 1-Year Follow-Up stratified by CSS Tertile

Variable, n (%) unless stated	CSS ≤15.6 (n=170)	15.6 <CSS≤27.5 (n=171)	CSS >27.5 (n=171)	p value
Hierarchical				
Death	1 (0.6)	0 (0.0)	4 (2.3)	0.09
Cerebrovascular Accident	0 (0.0)	1 (0.6)	3 (1.8)	
MI	2 (1.2)	1 (0.6)	4 (2.3)	
Q wave	1 (0.6)	1 (0.6)	2 (1.2)	
Non-Q wave	1 (0.6)	1 (0.60	2 (1.2)	
Repeat revascularisation	8 (4.7)	0 (0.0)	21 (12.3)	
CABG	2 (1.2)	11 (6.4)	5 (2.9)	
PCI	6 (3.5)	2 (1.2)	16 (9.4)	
MACCE	11 (6.5)	13 (7.6)	32 (18.7)	0.001
Non- Hierarchical				
Cerebrovascular Accident	0 (0.0)	1 (0.6)	3 (1.8)	0.33
MI	2 (1.2)	1 (0.6)	6 (3.5)	0.14
Q wave	1 (0.6)	1 (0.6)	3 (1.8)	0.63
Non-Q wave	1 (0.6)	0 (0.0	3 (1.8)	0.23
Repeat revascularisation	9 (5.3)	11 (6.4)	27 (15.8)	0.002
CABG	7 (4.1)	9 (5.3)	21 (12.3)	0.009
PCI	2 (1.2)	3 (1.8)	6 (3.5)	0.41

(Adapted from Garg *et al* Circ Cardiovasc Interv (431)). Abbreviations: CSS: Clinical Syntax Score, MACCE: Major Adverse Cardiac and Cerebrovascular Event, MI: Myocardial Infarction, CABG: Coronary Artery Bypass Grafts, PCI: Percutaneous Coronary Intervention.

Table 16: Univariate and Multivariable Predictors of MACCE at 5-Year Follow-up

	Univariate Predictors of MACCE at 5 years		Multivariable Predictors of MACCE at 5 years	
Variable	**[95% CI]**	**p value**	**[95% CI]**	**p value**
Age	1.02 [1.00-1.04]	0.03		
Diabetes	1.80 [1.28-2.54]	0.001	1.55 [1.09-2.19]	0.01
Peripheral vascular disease	2.01 [1.18-3.44]	0.01	1.97 [1.14-3.41]	0.02
Log SYNTAX Score	5.62 [2.32-13.62]	<0.0001		
Log ACEF	7.11 [1.56-32.45]	0.01		
Log Clinical SYNTAX score	1.81 [1.42-2.29]	<0.0001	1.77 [1.02-3.07]	0.04
No. of diseased lesions	1.43 [1.17-1.73]	<0.0001		
Incomplete revascularisation	1.56 [1.12-2.17]	0.009	1.43 [1.01-2.02]	0.045

(Adapted from Garg *et al* Circ Cardiovasc Interv (431)). Abbreviations: MACCE: Major Adverse Cardiac and Cerebrovascular Event, CI: Confidence Interval).

The British Society of Echocardiography Guidelines were followed when assessing left ventricular (LV) function: normal ≥ 55%, mild impairment 45 – 54%, moderate impairment 36 – 44% and severe impairment ≤ 35% (432). Where echocardiogram data was not available, LV gram data (from time of angiography, if available) was used as follows: a score of 60% for normal/good, 50% for mild impairment, 40% for moderate impairment and 30% for severe impairment.

2.13 Ethics and Clinical Trial Registration

Following formal peer review at both The London Chest Hospital and The William Harvey Research Institute, the study received approval from Barts and The London NHS Trust Research and Development (R and D) Department (R and D 006580). Subsequently the study was assessed by The East London and City Research

Ethics Committee 1 and received formal ethical approval (NRES: 09/H0703/29). The study was then registered at clinicaltrials.gov (NCT 00948116).

2.14 Study Organisation

The study was sponsored by Barts and The London NHS Trust. Patients were recruited from The London Chest Hospital. The weekly cardiac catheter laboratory 'admissions list' was screened to identify suitable patients, in addition to a daily review of all transfers and admissions to the Coronary Care Unit (CCU) and cardiology wards at The London Chest Hospital. All baseline demographic and clinical data were recorded onto a specially designed paper Case Report Form (CRF). This allowed anonymised retrieval of data and these were then entered into a specifically designed electronic database. A copy of the CRF is included in the Appendix. The paper CRFs were stored in files in a locked office at The London Chest Hospital in accordance with good clinical practice.

Funding for the study was from an unrestricted research grant from Boston Scientific. Since this was an investigator initiated study the study design data management, coordination and data analysis were all undertaken independently from the source of funding. The study commenced on 14th August 2009 and the last patient was recruited on 26th July 2010. The design of the study is summarised in Figure 16.

2.15 Limitations of Study Design

The study described in this thesis was designed as a single centre observational study of biomarkers in a population with CKD and diabetes mellitus. This was part of a planned programme of work including 2 further studies for which I wrote 2 further complete protocols and successfully completed the ethics application. These further studies included possible therapeutic interventions to prevent / diminish AKI.

One of the main limitations of the design was that there was no control group for this study. The most logical control group would have been a non-diabetic population with CKD but in order to have adequate power the number of patients needed would have been outside the scope of a single recruiter and the limited time period available, due to the lower incidence of AKI in this population. Other populations that could have been considered include those with CKD undergoing CT procedures although this would have reflected i.v. rather than intra-arterial contrast administration and furthermore for CT scanning the contrast is given in a single bolus (rather than repeated small 5 – 10 ml injections as is the case in the cardiac catheter lab). Finally at the time of the study recruiting, the CT scanner at The London Chest was being refurbished and this meant that all CT scans were being done at other sites several miles away. Since sample processing and storage were on the London Chest site this was not felt to be a feasible control group to be studied. It was therefore planned that on completion of the study outlined in this thesis, having identified the most promising 2 – 3 biomarkers from the candidates discussed in section 1.9 that a larger scale study would be conducted by myself as part of a planned post – doctoral programme of research.

Chapter Three

Baseline Characteristics of the Study Population

and Clinical Procedural Data

Chapter 3: Baseline Characteristics of the Study Population and Clinical Procedural Data

3.1 Study Population

Our aim was to identify patients with diabetes mellitus and CKD (as described in Chapter 2) undergoing coronary angiography or PCI. Recruitment is summarised in Figure 18. Patients were recruited at The London Chest Hospital between 14th August 2009 and 26th July 2010. 234 patients were screened for the study, recorded in the Screening Log (as described in section 2.14) and of these 208 gave written consent and participated. Prior to consent patients were given a study Patient Information Sheet (see Appendix) and details of the study, the procedures and the risks were fully explained. Consent was indicated in the Research Section of the routine Patient Agreement to Investigation or Treatment Form (Barts and The London NHS Trust Consent Form 3). This was then signed and dated by the patient. Completed consent forms were filed in the patient's notes with a copy given to the patient.

All data were originally entered onto a paper Case Report Form (CRF) which was designed to allow anonymised retrieval of data (see Appendix). The first page of the CRF contained the patients contact details and allowed the assignation of a study ID number. This first page was then kept in a separate file ensuring that all data retrieval was anonymised to the study ID number. The relevant sections of the form were completed on the day of procedure and at follow-up. Subsequently all the data were

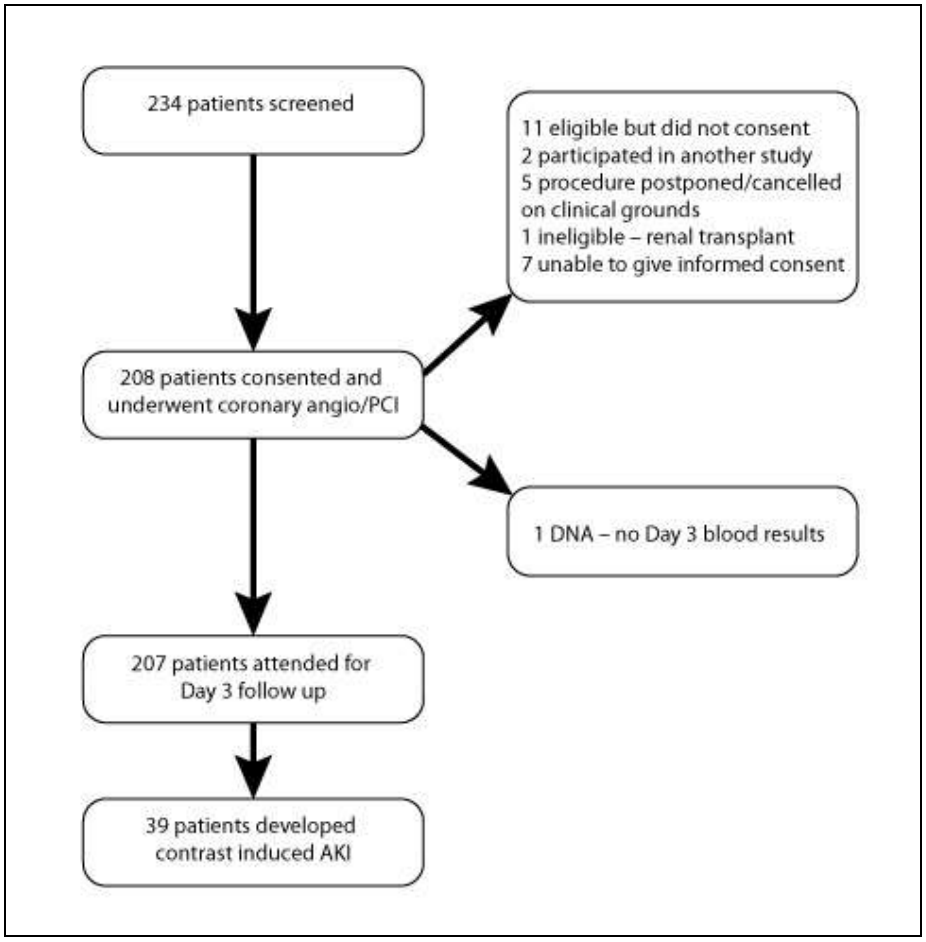
entered into a specifically designed database which coded from the CRF and again ensured anonymisation of data. From this database all analysis was performed.

116 patients had a coronary angiogram, 55 patients underwent PCI alone and 36 had a coronary angiogram with subsequent PCI on the same day. Following the study procedure 35 patients were referred for a PCI procedure and 27 were referred for CABG. 207 patients attended for Day 3 blood tests and of these 39 (18.8%) patients developed the main clinical end-point of contrast induced AKI.

3.2 Baseline Characteristics of the Study Population

Baseline characteristics of the study population are summarised in Table 17. The results are tabulated to compare those who did not develop contrast induced AKI versus those who did develop this event.

Figure 18: Consort diagram of Study Population



Abbreviations: Angio: Coronary angiogram, PCI: Percutaneous coronary intervention, DNA: did not attend, AKI: Acute Kidney Injury.

Table 17: Baseline characteristics of study population

	No AKI Outcome n = 168	**AKI Outcome n = 39**	**p value**
Age, mean (SD)	70.8 (8.5)	71.9 (9.4)	0.500
Male (%)	109 (64.9)	27 (69.2)	0.606
Ethnicity (%)			
Caucasian	66 (39.3)	14 (35.9)	0.838
South Asian	81 (48.2)	18 (46.2)	
East Asian	2 (1.2)	1 (2.6)	
Afro-Caribbean	8 (4.8)	3 (7.7)	
African	7 (4.2)	1 (2.6)	
Other	4 (2.4)	2 (5.1)	
BMI, mean, SD	28.6 (5.4)	29.1 (6.1)	0.651
Diabetes type (%)			
Type 1	3 (1.8)	0 (0)	0.401
Type 2	165 (98.2)	39 (100)	
Years with diabetes, mean (SD)	14.3 (10.0)	14.6 (10.4)	0.846
Diabetes Treatment (%)			
Diet	16 (11.8)	6 (17.1)	0.405
Oral	71 (52.2)	14 (40.0)	
Insulin	49 (36.0)	15 (42.9)	
Smoking Status (%)			
Current	10 (6.0)	2 (5.1)	0.634
Ex	86 (51.2)	17 (43.6)	
Never	72 (42.9)	20 (51.3)	
Family history of CHD (%)	84 (50.0)	20 (51.3)	0.885
Hypertension (%)	154 (91.7)	34 (87.2)	0.382
SBP, mean (SD)	134.4 (20.8)	134.4 (23.2)	0.988
DBP, mean (SD)	70.9 (12.1)	68.4 (13.6)	0.254

(Table 17 continued)

Hyperlipidaemia (%)	164 (97.6)	38 (97.4)	0.946
Previous CVA (%)	20 (12.0)	8 (20.5)	0.161
PVD (%)	16 (9.5)	4 (10.3)	0.889
Lung Disease (%)	14 (8.4)	7 (18.0)	0.075
Previous MI (%)	66 (39.3)	15 (38.5)	0.924
Known Valvular Heart Disease (%)	28 (16.7)	7 (18.0)	0.847
Previous PCI (%)	54 (32.1)	13 (33.3)	0.886
Previous CABG (%)	32 (19.1)	9 (23.1)	0.569
Heart Failure (%)	32 (19.2)	7 (18.0)	0.862
Angina (%)	143 (85.1)	35 (89.7)	0.453
Silent ischaemia (%)	7 (4.2)	3 (7.7)	0.355
CCS Class (%)			
0	7 (4.2)	0 (0)	0.136
I	14 (8.3)	4 (10.3)	
II	112 (66.7)	22 (56.4)	
III	34 (20.2)	12 (30.8)	
IV	1 (0.6)	0 (0)	
IVb	0 (0)	1 (2.6)	
NYHA (%)			
I	12 (7.1)	0 (0)	0.048
II	119 (70.8)	23 (59.0)	
III	35 (20.8)	15 (38.5)	
IV	2 (1.2)	1 (2.6)	

(Abbreviations used: NYHA: New York Heart Association classification of heart failure, CCS: Canadian Cardiovascular Society functional classification of angina – see Appendix)

Overall there were no significant differences between the 2 groups in terms of demographics, cardiac risk factors and co-morbidities. 204 patients had Type 2 diabetes with only 3 patients with Type 1 diabetes included in the study. The number of years patients had been diagnosed with diabetes was similar between the 2 groups at approximately 14.5 years. Furthermore diabetes treatment with respect to diet, oral medication and insulin therapy did not significantly differ between the 2 groups. It will be noted that the study included a high proportion of patients of South Asian origin. This reflects the demographics of the local population and the population of East London from which The London Chest Hospital receives referrals. Moreover patients of South Asian origin are known to be high risk for developing both diabetes and coronary artery disease (CHD) and this has been reported in a number of other studies (433-436).

There was a significant trend towards patients in the AKI group having a higher NYHA Class ($p = 0.048$). This is based on patient reported symptoms and is therefore subjective but this was further supported by objective data from the echo reports (104 patients) and LV gram performed at the time of procedure in 174 patients (Table 22).

3.3 Medication Use

Diabetes medication use at baseline is shown in Table 18 and other medication use in Table 19.

Table 18: Diabetes medication use at baseline

Medication	**No AKI Outcome n = 152 (%)**	**AKI Outcome n = 33 (%)**	**p value**
Metformin	70 (41.7)	10 (25.6)	0.640
Sulphonylureas	56 (33.3)	11 (28.2)	0.537
Thiazolinediones	9 (5.4)	2 (5.1)	0.954
Insulin	81 (48.2)	20 (52.3)	0.730

There were no significant differences in prescribing of diabetes medication between the 2 groups. Metformin was discontinued for 24 h pre-procedure and 48 h afterwards.

Table 19: Medication use at baseline

Medication	**No AKI Outcome n = 168 (%)**	**AKI Outcome n = 39 (%)**	**p value**
Aspirin	162 (96.4)	37 (94.9)	0.650
Clopidogrel	111 (66.1)	26 (66.7)	0.944
Warfarin	10 (6.0)	1 (2.6)	0.395
Heparin			
Low molecular weight	19 (11.3)	5 (12.8)	0.862
Unfractionated	1 (0.6)	0 (0)	
Other anticoagulant	6 (3.6)	3 (7.7)	0.259
Beta-blockers	117 (69.6)	24 (61.5)	0.328
Calcium channel antagonists			
Dihydropyridine	47 (28.0)	13 (33.3)	0.506
Non-dihydropyridine	19 (11.3)	6 (15.4)	0.482
Nitrates	92 (54.8)	25 (64.1)	0.289
Lipid lowering agents (LLA)			
Statin	151 (89.9)	36 (92.3)	0.792
Statin and other LLA	6 (3.6)	1 (2.6)	
Other LLA	2 (1.2)	1 (2.6)	
ACE - Inhibitors	105 (62.5)	22 (56.4)	0.482
Angiotensin II Receptor Blockers	42 (25.0)	8 (20.5)	0.555
Loop diuretic	62 (26.9)	23 (59.0)	0.012
Thiazide diuretic	37 (22.0)	4 (10.3)	0.097
Digoxin	10 (6.0)	0 (0)	0.118
Amiodarone	2 (1.2)	0 (0)	0.494
Thyroxine	14 (8.3)	2 (5.1)	0.500

There was a significantly higher rate of loop diuretic prescription in the group that developed AKI (59.0% vs. 26.9%, p = 0.012). This reflects the difference in NYHA Classification already reported (Table 17) and the more severe LV function from echo and LV gram data (Table 22). High numbers of patients were prescribed statin therapy or ACE inhibitors/Angiotensin II Receptor Blockers (AIIRBs) as is recommended in guidelines for this population (section 1.3). No patients in this study discontinued ACE inhibitors or AIIRBs prior to undergoing coronary angiography or PCI.

3.4 Baseline Blood and Urine Results

Patients were assessed for eligibility for the study based on 'screening' blood results (creatinine and eGFR) taken at the time of pre-admission appointment or from referral paperwork from referring centres. These results along with baseline results from the time of the procedure are summarised in Table 20.

Table 20: Screening and Baseline Blood Results

Blood result, Median (IQR)	No AKI outcome n = 168	AKI outcome n = 39	p value
Baseline results			
Glucose (mmol/l)	7.0 (5.7, 9.1)	7.5 (6.4, 10.9)	0.106
HbA1c (%)	7.4 (6.9, 8.5)	7.6 (6.7, 8.7)	0.785
HbA1c (mmol/mol)	57 (52, 69)	60 (50, 72)	0.794
Hb (g/dl)	11.4 (10.3, 12.7)	11.0 (9.8, 12.4)	0.125
HCt (units)	0.35 (0.32, 0.38)	0.33 (0.29, 0.38)	0.055
WCC (x 10^9/l)	7.4 (6.5, 8.8)	7.4 (6.2, 8.5)	0.722
Platelets (x 10^9/l)	212 (172, 262)	208 (166, 250)	0.423
INR	1 (1, 1.1)	1 (1, 1.1)	0.532
CRP (mg/l)	5 (5, 8)	6 (5, 10)	0.114
Sodium (mmol/l)	140 (139, 142)	140 (138, 142)	0.465
Potassium (mmol/l)	4.4 (4.1, 4.7)	4.5 (4.1, 5.1)	0.244
Urea (mmol/l)	9.2 (7.1, 11.5)	11.4 (8.3, 16.3)	0.004
Baseline Creatinine (µmol/l)	113 (94, 138)	143 (109, 156)	0.002
Baseline GFR (ml/min)	54 (40, 63)	42 (36, 54)	0.002
Corrected Calcium* (mmol/l)	2.2 (2.1, 2.3)	2.2 (2.2, 2.3)	0.297
Phosphate (mmol/l)	1.24 (1.09, 1.41)	1.25 (1.11, 1.36)	0.886
Albumin (g/l)	42 (39, 44)	40 (39, 44)	0.534
ALP (units)	69 (59, 89)	74 (65, 96)	0.127
ALT (units)	19 (15, 26)	19 (15, 27)	0.804
Creatinine kinase (IU/l)	105 (68, 149)	90 (58, 194)	0.597
Troponin T (µg/l)	0.01 (0.01, 0.03)	0.01 (0.01, 0.06)	0.067
Cholesterol (mmol/l)	3.4 (3.0, 4.1)	3.5 (3.0, 4.4)	0.708
LDL (mmol/l)	1.6 (1.3, 2.2)	1.8 (1.4, 2.3)	0.746
HDL mmol/l)	1.09 (0.88, 1.36)	1.06 (0.81, 1.55)	0.984
Triglyceride (mmol/l)	1.36 (1.03, 1.92)	1.44 (1.19, 1.76)	0.341
Screening results			
Screening Creatinine (µmol/l)	130 (110, 155)	155 (123, 176)	0.005
Screening GFR (ml/min)	46 (36, 54)	39 (34, 46)	0.011

*Calcium is reported as a corrected value for serum albumin levels – see Appendix. (Abbreviations: HbA1c: glycated haemoglobin; Hb: haemoglobin; HCt: Haematocrit; WCC: White Cell Count; INR: International Normalised Ratio; CRP: C-Reactive Protein; GFR: Glomerular Filtration Rate; ALP: Alkaline phosphatase; ALT: Alanine transaminase; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein).

The screening creatinine in both groups is higher than the baseline creatinine. This is to be expected as baseline results were taken at time of procedure and therefore would reflect the hydration that the majority of these patients would have received (Table 22). Renal function is significantly worse in the patients in the group who developed AKI versus the group who did not (reflected by elevation in both baseline and screening creatinine, elevated serum urea and decreased baseline and screening GFR). Again this is to be expected as pre-existing renal disease is one of the most important risk factors for the development of AKI (Section 1.6, Table 7 and 8).

HbA1c does not vary significantly between the 2 groups and a reasonable level of glycaemic control is achieved at 7.4 – 7.6%. We reported HbA1c using both the well-known DCCT (Diabetes Control and Complications Trial) % (437) and the new IFCC (International Federation of Clinical Chemistry) units of mmol/mol (438) since it has been recommended by the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD) and the International Diabetes Federation that these are adopted (439). For ease of reference we have given the conversion in the Appendix.

There is a trend towards a lower haematocrit in the patients who developed AKI ($p = 0.055$). As has already been discussed (section 1.6), anaemia is a significant risk factor for the development of contrast induced AKI, probably due to low effective intravascular volume. The study also included a high proportion of patients of South Asian origin and due to dietary and cultural reasons in addition to CKD these patients may be particularly prone to anaemia (435).

Prior to procedure patients were requested to provide a urine sample (section 2.5). An aliquot was centrifuged (3000 rpm, 1600 *g*) and frozen at -80°C with the remainder

sent to the clinical chemistry laboratory for assessment of urine albumin and protein levels. These are reported in Table 21.

Table 21: Urine results at baseline

Urine results, median (IQR)	No AKI outcome n = 168	AKI outcome n = 39	p value
Albumin : Creatinine ratio (mmol/l)	2.6 (1.6, 10.2)	4 (2.6, 19.2)	0.087
Protein : Creatinine ratio (mg/mmol)	45 (24, 100)	45 (22, 93)	0.521

Although there was no significant difference in urine protein levels as assessed by the protein : creatinine ratio, a trend was observed towards a higher level of urine albumin measured by the albumin : creatinine ratio in the patients who developed AKI.

3.5 Procedural Data

Key data from the patients' procedures are summarised in Table 22. The majority of the procedures included were elective. Due to other studies taking place in the department we were not able to include ACS patients with ST elevation myocardial infarction (STEMI) in this study which contributes to the vast majority of the emergency workload. We did however include both non-ST elevation myocardial infarction (NSTEMI) and unstable angina patients. The case mix with regard to stable and unstable cases was similar between the 2 groups and just over 25% of patients included had suffered a

recent infarct. Similar proportions of patients underwent angiography and PCI in the 2 groups.

A minority of patients (30 in total), underwent the study procedure as part of a planned staged revascularisation. There may be a number of reasons for a staged strategy including the presence of multi-vessel disease, complex lesion anatomy where contrast dose may be high or determining the culprit lesion for the patient's ischaemic symptoms.

Approximately 50% of procedures were performed by the radial route in both groups. Radial angiography as a technique was introduced to the London Chest Hospital in 2004 and this reflects the overall proportion of cases which are done radially at our centre (22). Radial access is increasingly popular as it allows the patient greater movement after the case and facilitates procedures being done on a day case basis. Furthermore complications such as bleeding from the access site are much more immediately apparent. There was no significant difference between radial and femoral access and incidence of AKI ($p = 0.332$).

Table 22: Procedural Details

	No AKI Outcome n = 168	**AKI Outcome n = 39**	**p value**
Type of procedure (%)			
Elective	118 (70.2)	25 (64.1)	0.444
Urgent	49 (29.2)	13 (33.3)	
Emergency	1 (0.6)	1 (2.6)	
Recent MI (%)	47 (28.0)	10 (25.6)	0.769
Point of Access (%)			
Radial	92 (54.8)	18 (46.2)	0.332
Femoral	76 (45.2)	21 (53.9)	
Type of procedure (%)			
Angio	98 (58.3)	18 (46.2)	0.298
PCI	41 (24.4)	14 (35.9)	
Both	29 (17.3)	7 (18.0)	
Staged procedure	25 (14.9)	5 (12.8)	0.742
Ejection Fraction, mean (SD)	48.9 (14.5)	44.4 (15.9)	0.181
LV function*			
Normal	79 (55.6)	15 (46.9)	0.027
Mild impairment	26 (18.3)	1 (3.1)	
Moderate impairment	19 (13.4)	8 (25.0)	
Severe impairment	18 (12.7)	8 (25.0)	
LV gram	67 (39.9)	8 (20.5)	0.023
Echo	83 (49.4)	21 (55.3)	0.514
IV fluids (%)	119 (70.8)	34 (87.2)	0.036
NAC (%)	122 (72.19)	34 (87.18)	0.051
Contrast dose, median (IQR)	140 (100,210)	180 (100, 240)	0.398
Procedure time, median (IQR)	55 (40, 85)	60 (45, 80)	0.574
Referred for further PCI	32 (19.3)	3 (7.7)	0.084
Referred for further CABG	20 (12.1)	7 (18.0)	0.327

(* The British Society of Echocardiography Guidelines (Cheesman *et al* Heart 1998 (432)) were followed when assessing LV function: normal: ≥ 55%, mild impairment: 45-54%, moderate impairment: 36-44%, severe impairment ≤ 35%).

LV function data were collated from a number of sources including pre-procedure echocardiograms, cardiac magnetic resonance imaging (CMR), stress echocardiogram and nuclear medicine scans as well as LV gram performed at the time of procedure. The patients who developed AKI had significantly worse LV function than those who did not develop this complication ($p = 0.027$). This supports the previous data from NYHA Class and diuretic prescribing mentioned earlier in this chapter. NYHA Class III-IV heart failure carries significant risk for developing AKI as discussed in section 1.6.

A significantly higher proportion of patients in the non-AKI arm underwent LV gram at the time of procedure ($p = 0.023$). There are no formal guidelines at The London Chest Hospital regarding performing an LV gram in patients with CKD. This decision is made on a case by case basis at the operator's discretion. The importance of volume of dye as a risk in the development of contrast induced AKI was discussed in section 1.6. Since the patients in the AKI group had significantly worse renal function prior to procedure, it may be that operators considered them to be higher risk cases and this is why a lower proportion of patients in this group had an LV gram during the procedure. Although the median contrast volume in the AKI group was numerically higher (180 ml vs. 140 ml in the non-AKI group), this was not a statistically significant difference ($p = 0.398$). In clinical practice every additional 10 ml of dye is felt to constitute a risk to the patient, with operators attempting to minimise every dose in high risk groups, however in the risk scoring systems such as the Mehran Risk Score (discussed in more detail in sections 1.6.4.7 and 4.2), heightened risk is only ascribed to every additional 100 ml contrast (326).

Use of both NAC and i.v. fluids was higher in the patients who developed AKI. This is as one might expect as we know this group had worse renal function at baseline and The London Chest Hospital Guidelines for prevent of contrast induced AKI (section 2.5) recommend NAC and fluids only for patients with GFR < 50 ml/min.

3.6 Post procedural Blood Results

In those patients who were in-patients following their study procedure it is routine clinical practice for a FBC and U and E to be checked prior to discharge. This time-point is between 12 and 24 hours following the procedure and the results are summarised in Table 23.

Table 23: Pre-discharge blood results

	No AKI outcome (n = 70)	**AKI outcome (n = 27)**	**p value**
Haemoglobin (g/dl)	10.8 (10.0, 12.4)	10.8 (9.5, 12.4)	0.675
Urea (mmol/l)	9.3 (6.7, 11.7)	11.4 (9.3. 17.0)	0.005
Creatinine (µmol/l)	120 (102, 149)	137 (129, 167)	0.005
eGFR (ml/min)	49 (39, 59)	42 (30, 46)	0.002

The main difference is that renal function is significantly worse in the group who developed AKI at day 3. This group also had worse renal function at baseline as seen in section 3.4.

3.7 Day 3 Blood Results

207 patients attended for Day 3 follow-up blood results. 1 patient did not attend and we were not able to obtain follow-up blood results from his GP. 39 (18.8%) developed AKI. The results are summarised in Table 24.

Table 24: Day 3 Blood Results

	No AKI outcome (n = 168)	**AKI outcome (n = 39)**	**p value**
Haemoglobin (g/dl)	11.4 (10.6, 12.6)	10.1 (9.3, 11.4)	0.001
Creatinine (µmol/l)	126 (104, 149)	183 (152, 213)	< 0.001
eGFR (ml/min)	49 (40, 55)	30 (25, 39)	< 0.001

1 patient required inotropic support but no patients required haemofiltration or haemodialysis. As would be expected, serum creatinine was significantly elevated in the AKI group at day 3 compared to the non-AKI group (188.3 µmol/l vs. 134.6 µmol/l, p <0.001). Haemoglobin was significantly lower in the AKI group at Day 3. Anaemia is known to be an important risk factor for the development of AKI (section 1.6.4).

3.8 Discussion

Diabetes mellitus is one of the main risk factors for the development of coronary artery disease (10). Once coronary artery disease has developed these patients

are then at high risk for the development of ACS (17-20;59). From the Framingham study (10) it is known that even after adjustment for other factors, diabetic patients have higher mortality rates and also a higher incidence of reinfarction and heart failure in both the acute and the post-infarct setting. Patients with diabetes and CKD are a particular challenge as they have a higher incidence of cardiovascular comorbidities than any other patient group and yet have been excluded from the majority of clinical trials to date (440-442). Due to their increased cardiovascular morbidity and mortality (7;13;443), there is frequently a need for patients with diabetes to undergo coronary angiography and PCI procedures. However when renal function is already impaired by pathological processes the kidney is far less able to tolerate the additional stress of excreting a contrast load (281). This risk is further amplified for patients with diabetes (268) as described in section 1.6. Therefore the patients who participated in our study were a high risk cohort for developing contrast induced AKI since in order to be eligible they had to have a confirmed diagnosis of diabetes and CKD (eGFR < 60 ml/min).

It would be extremely useful to be able to identify those patients most at risk early, to allow possible therapeutic interventions to be initiated promptly. Possible interventions include more intensive i.v. hydration regimes, use of vasodilators such as dopamine, fenoldopam, endothelin receptor antagonists or aminophylline (329). Such attempts have to date been limited as the current 'gold standard' biomarker serum creatinine does not start to rise until 48-72

hours post the injury event (265). Furthermore serum creatinine concentrations are influenced by a number of other factors including age, muscle mass, hydration and nutritional status (383). Additionally a rise in serum creatinine may not be clinically detectable until almost 50% of renal function has been lost and serum creatinine concentrations are not a reflection of acute GFR change in the acute setting since a period of hours-days must elapse before equilibrium between steady-state production and decreased creatinine excretion is achieved (380) (444).

The incidence of contrast induced AKI in our cohort was 18.8% (n = 39). This included both unstable and elective patients with diabetes and CKD but due to other concurrent studies in the department patients with STEMI were excluded. Due to problems with inconsistent definitions of contrast induced AKI being used, it can be difficult to compare results from different studies in the medical literature. We used the definition that has been most widely used of a rise in serum creatinine of > 25% from baseline or an absolute rise of 44.5 µmol/l. It will be important for future studies that definitions become standardised as discussed in section 1.4.

The majority of patients enrolled in our study had Type 2 diabetes, with only 3 patients with Type 1 diabetes included. The mean duration of diabetes was approximately 14.5 years. Diabetes treatment with respect to diet, oral medication and insulin therapy was similar between those patients who

developed AKI. Reasonable glycaemic control was observed in the studied patients with median HbA1c of 7.4 in the non-AKI group and 7.6 in the AKI group ($p = 0.785$). There was no significant difference in median blood glucose levels at time of procedure 7.0 mmol/l vs. 7.5 mmol/l, respectively, ($p = 0.106$).

A recent study by Stolker *et al* (445), showed that pre-procedural glucose levels are a risk marker for contrast induced AKI in patients without diabetes with acute MI (STEMI and NSTEMI) who underwent coronary angiography during their index admission. This was not observed in patients with diabetes possibly due to the higher risk for contrast induced AKI in these patients. The findings were valid even after adjustment for confounding variables such as impaired baseline renal function. In a previous study, fasting blood glucose was shown to be an independent predictor of contrast induced AKI in patients with the metabolic syndrome (446). Hyperglycaemia is commonly observed in critically ill patients and occurs in > 40% of patients without diabetes but with acute MI (447). The aetiology of hyperglycaemia in this setting is thought to be due to a number of factors including stress–related neuro-hormonal alterations, activation of the renin-angiotensin-aldosterone system, and expression of various cytokines (296). Hyperglycaemia may be acting as a ‘stress test’ for the failure of endogenous insulin reserves to adequately control blood glucose levels or be a consequence of insulin resistance (447). The results of the study by Stolker *et al* raises the possibility of interventions to treat blood glucose levels at the time of procedure such as intensive insulin regimes to reduce risk of contrast

induced AKI. Further studies will be necessary to ascertain if pre-procedural hyperglycaemia is a risk marker for contrast induced AKI in elective and general populations undergoing coronary imaging procedures using iodinated contrast media.

The study presented in this thesis included a high proportion of patients of South Asian origin, reflective of the demographics of the local population and also the population of East London from which The London Chest Hospital received referrals. Patients of South Asian origin are known to be at high risk for development of diabetes, CKD and CHD, with these conditions often coexisting in the same patient; and this has been reported in a number of other studies (433-436). These patients do not have an increase in the rates of the traditional and modifiable risk factors for CHD of hypertension, hypercholesterolaemia and cigarette smoking. South Asian patients are observed to have an increase of other cardiovascular risk factors including central adiposity and physical inactivity. Other factors which have been implicated include reduced HDL cholesterol, hypertriglyceridaemia, hyperinsulinaemia, reduced beta cell function and various prothrombotic factors (448-450). Ethnicity did not have any influence on the development of contrast induced AKI ($p = 0.838$).

Rates of use of ACE inhibitors ($n = 127$) and AIIRBs ($n = 50$) were high in this study (Table 19). There was no significant difference in use of ACE inhibitors ($p = 0.482$) or AIIRBs ($p = 0.555$) on patient outcome. In landmark clinical trials

pharmacological inhibition of the renin angiotensin system attenuated the decline in renal function associated with CKD (154-156;451-454). However these trials have several limitations and concerns arise regarding the general applicability of their findings to the 'real-world' particularly regarding older patients (> 65 years) with CKD (455-457).

The Renin Angiotensin Aldosterone System (RAAS) blockade clinical trials generally enrolled younger patients with well-preserved baseline renal function, the exception to this being the RENAAL and IDNT trials which recruited patients with quite advanced CKD with baseline serum creatinine of 1.9 and 1.7 mg/dl, respectively (155;451). Furthermore there is little evidence that the benefits of reno-protection extend beyond blood pressure control. A post-hoc substudy analysis from the HOPE trial showed that patients in the ramipril arm achieved significantly lower 24 hour BP levels when compared to the control arm (457). In other trials which reported BP independent effects such as RENAAL (losartan) and IDNT (captopril), the treatment arms showed mean arterial BP values lower than the placebo arm of ≥ 3 mmHg (155;451). In the ONTARGET trial lower BP levels were reported in the combination arm (telmisartan and ramipril) vs. the monotherapy arm (454). Marked emphasis has been given to the magnitude of reduction of levels of proteinuria in patients studied as a definitive renal end-point although this has not been validated in any studies (456;458). Finally in many of the RAAS blockade trials, the rates of discontinuation of medication have been extremely high. In the RENAAL trial 46.5% of patients discontinued losartan

and 53.5% discontinued the placebo treatment (155). The implications of study drug discontinuation rates on statistical analysis and validity of study outcomes is hypothetical and is an area of strong debate even amongst experts in the area (455).

Whether ACE inhibitors and AIIRBs should be discontinued prior to patients undergoing procedures involving iodinated contrast media remains an area of controversy. Some studies suggest that these drugs are of benefit and limit the effects of contrast induced AKI (269;459); others report that concurrent use of ACE inhibitors and AIIRBs exacerbates contrast induced AKI in patients with CKD (460-463). A recent post-hoc analysis from the Dialysis Versus Diuresis (DVD) Trial of 412 patients (29.1% diabetic) with serum creatinine ≥ 1.3 mg/dl and ≤ 3.5 mg/dl, demonstrated that patients treated with either ACE inhibitors or AIIRBs prior to exposure to contrast developed significantly higher rates of contrast induced AKI within 72 h (464). Following adjustment for confounding co-morbidities, treatment with either an ACE inhibitor or AIIRB was an independent risk predictor for development of contrast induced AKI (11.9% vs. 4.3%, p = 0.006). Multivariate analyses using logistic regression identified RAAS blockade to be an independent risk predictor for contrast induced AKI (OR 3.082, 95%CI 1.234 – 7.698, p = 0.016). Komenda *et al* (463) found that renal outcomes were improved when ACE inhibitors were withheld from patients for 2 days prior to coronary angiography. A trial by Gupta *et al* (459) which concluded that captopril was reno-protective administered the first dose of the drug just 1 h prior to

coronary angiography. It would appear unlikely that this would have allowed sufficient patients drug exposure prior to contrast being administered. Other limitations of this study include small size (n = 71) and that use of peri-procedural i.v. fluid regimes (a therapy that is known to be beneficial in preventing contrast induced AKI) was not standardised and was therefore at the operator's discretion. Rosenstock *et al* (465) also reported that the withholding ACE inhibitors or AIIRBs in patients with CKD prior to coronary angiography and contrast exposure was not beneficial. A major limitation of this study was that these drugs were only withheld for 24 h prior to contrast exposure. In patients with significant CKD, knowledge of pharmacokinetics would indicate that 24 h was an inadequate time period for the effect of RAAS blockade to disappear (466;467). This therefore could explain the failure of this study to demonstrate any benefit associated with withholding ACE inhibitors or AIIRBs prior to contrast exposure. This would also support the findings of Komenda *et al* (463) as previously discussed in this section, where withholding ACE inhibitors or AIIRBs for a longer time period was associated with improved renal outcomes.

On balance it would appear that the results in the literature are predominantly in favour of discontinuing RAAS blockade prior to exposure to iodinated contrast media for patients with CKD (315;460-464). However the limitations of small case series and post-hoc analyses should be recognised and this should drive the establishment of large scale multi-centre randomised trials to confirm these

findings.

There was a significant trend towards patients who developed AKI having a higher baseline NYHA Class ($p = 0.048$). This is based on patient reported symptoms and therefore is subjective but this was further supported by objective data on LV function from both imaging reports and LV gram data performed at the time of procedure (section 3.5). LV function was significantly worse in the patients who developed AKI ($p = 0.027$), with higher proportions of patients having moderate or severe LV dysfunction. Heart failure is a well-recognised risk factor for the development of contrast induced AKI (310). It is thought that this results in decreased renal blood flow, leading to increased viscosity, predisposing to medullary hypoxia and ischaemia (296). It may be difficult to adequately hydrate these patients prior to procedure and there may also be high rates of concomitant use of nephrotoxic medications such as ACE inhibitors / AIIRBs and diuretics.

This is further supported by the higher rate of loop diuretic prescription in the patients who developed AKI as reported in section 3.3. There is no evidence in the literature to date of any interaction between the biomarkers studied and loop diuretics and whilst these agents might ‘dilute’ the baseline concentration, a delta would still be observed following development of AKI.

Since the volume of contrast used is an important predictor for the development of AKI (237;326) and with the wide availability of non-invasive imaging

techniques for assessing LV function including nuclear imaging, echocardiography, cardiac MRI, it could be argued as to whether it is appropriate for patients with CKD to undergo an LV gram at the time of their angiography or PCI procedure. There are currently no formal guidelines regarding performance of LV gram at our institution and this is therefore at operator discretion. The minimum contrast volume for an LV gram using pressure injection systems (as are found in the majority of cardiac catheterisation laboratories in the United Kingdom) is 25 ml whilst a manual injection can be done with 10 ml. It has been suggested that for patients with CKD the volume of contrast (ml) should not exceed twice the baseline level of eGFR (307). In our cohort the median volumes of contrast used were 140 ml and 180 ml in the non-AKI and AKI groups respectively. This is compared to a median baseline eGFR of 54 ml/min and 42 ml/min. Since our institution is a tertiary referral centre and also a teaching hospital, these high contrast volumes may be reflective of the complexity of the cases referred and also of the training of junior operators.

Use of both NAC and i.v. fluids was significantly higher in the patients who developed AKI ($p = 0.036$ and 0.051). At first glance this might seem counter-intuitive but the AKI group had worse renal function at baseline (Table 20) and The London Chest Hospital Guidelines for Prevention of Contrast Induced AKI, recommend NAC and fluids only for patients with eGFR < 50 ml/min. Therefore patients in the study with eGFR 51-60 ml/min did not receive these agents. This appears to be a rather arbitrary cut-off as CKD Stage 3 or moderate CKD is

defined as eGFR 30 – 59 ml/min. Following the completion of this study, the hospital guidelines are currently under review and it has been proposed that use of NAC and i.v. hydration is extended to patients with eGFR 51-60 ml/min.

Limitations of the Study and Further Work

This was a single centre study performed in a tertiary referral cardiac unit. There may therefore have been an element of selection bias with a tendency to more complex cases being referred from peripheral hospitals. Our reported rate of contrast induced AKI and the relatively high volumes of contrast used in the study may be reflective of this.

A pragmatic approach with regard to i.v. hydration prior to the coronary angiogram or PCI procedure has had to be taken in order to ensure efficient use of both hospital beds and laboratory time and work flow. This generally precludes patients being admitted the night before their procedure for i.v. hydration if they have eGFR < 50 ml/min. The hospital guidelines recommend a minimum of 2 h of i.v. fluids in these patients prior to procedure and do not monitor urine output. As discussed in section 1.7, the majority of trials looking at i.v. hydration regimes have included a minimum of 6 hours of i.v. fluids prior to procedure.

The study included only elective and unstable patients with diabetes and CKD. STEMI patients were excluded due to other studies which were occurring in the department. To be truly reflective of a ‘real-world’ population with diabetes

and CKD undergoing coronary angiography or PCI it would be important for these patients to be included in further studies. The protocol was for patients to be followed up until Day 3 post procedure. Although data were collected on the outcome of all contrast induced AKI events it would be extremely interesting to follow up the patients over a longer term basis to ascertain the prognosis of developing contrast induced AKI on future morbidity and mortality.

Patients were referred from at least 5 peripheral hospitals all with their own clinical laboratories. There can be some variation in the assays used to report serum creatinine at different institutions. All patients who participated in the study were invited back to The London Chest Hospital for an appointment for a Day 3 blood test. However due to logistical reasons a number of patients were not able to do this and therefore results were obtained from their local hospital or general practitioner which may have led to us not correctly identifying all cases of contrast induced AKI.

The Hospital Guidelines are currently under review and some suggestions for further consideration are documented in Chapter 7.

Chapter Four

Renal Angiogram, Mehran Risk Score and SYNTAX Score Data

Chapter 4: Renal Angiogram Data, Mehran Risk Score and SYNTAX Score Data

4.1 Renal Angiogram data

There is a paucity of data in the medical literature with regard to structural renal disease and the development of contrast induced AKI. Structural renal artery disease is likely to result in diminished perfusion of the kidney and therefore might render it more vulnerable to contrast induced AKI. (Hypoperfusion of the kidney is a known risk factor for the development of AKI (section 1.6)). We therefore included renal angiography in the study protocol, to be performed at the operator's discretion.

56 patients underwent a renal angiogram at the time of the procedure. This was performed using a technique of selective intubation of the left and right renal arteries using a multi-purpose catheter. This was a minimal additional dye load as with selective intubation of the renal vessels an adequate image can be obtained with 2 ml of contrast agent. There are no established scoring systems for assessing severity of reno-vascular disease. We therefore scored the anatomy in a similar manner to that we use for coronary lesions as normal, mild stenosis, moderate stenosis and severe stenosis. The results are summarised in Table 25.

Table 25: Renal angiogram data

	No AKI Outcome	AKI Outcome	p value
Renal angiogram (%)	n = 44 (26.2)	n = 12 (30.8)	0.562
Normal	38 (86.4)	7 (58.3)	0.11
Mild stenosis	2 (4.6)	2 (16.7)	
Moderate stenosis	1 (2.3)	0 (0)	
Severe stenosis	3 (6.8)	3 (25.0)	

There was no significant difference in anatomical severity of renal artery disease between the 2 groups. 7 patients (12.5%) had moderate or severe renal artery stenosis. Of these 1 had a critical restenosis in a previous renal artery stent that due to language difficulties was not known about at the time of procedure. She underwent a balloon angioplasty to this lesion with excellent angiographic result. All patients with moderate to severe renal artery stenosis were referred to our renal colleagues for advice regarding further management.

We then looked at the usage of ACE inhibitors, AIIRBs and diuretics in these patients. The results are summarised in Table 26.

Table 26: Prescription of ACE inhibitors, Angiotensin II Receptor Blockers and Diuretics in patients who underwent renal angiography

Renal artery score	**Not on ACE-I (%)**	**On ACE-I (%)**	**p value**
Normal	15 (83.3)	30 (79.0)	
Mild stenosis	0 (0)	4 (10.5)	
Moderate stenosis	0 (0)	1 (2.6)	
Severe stenosis	3 (16.7)	3 (7.9)	0.464
Renal artery score	**Not on AIIRB (%)**	**On AIIRB (%)**	**p value**
Normal	36 (83.7)	9 (69.2)	
Mild stenosis	3 (7.0)	1 (7.7)	
Moderate stenosis	0 (0)	1 (7.7)	
Severe stenosis	4 (9.3)	2 (15.4)	0.219
Renal artery score	**Not on Loop diuretic (%)**	**On Loop diuretic (%)**	**p value**
Normal	2 1(84.0)	24 (77.4)	
Mild stenosis	1 (4.0)	3 (9.7)	
Moderate stenosis	1 (4.0)	0 (0)	
Severe stenosis	2 (8.0)	4 (12.9)	0.623
Renal artery score	**Not on Thiazide**	**On Thiazide**	**p value**
Normal	40 (83.3)	5 (62.5)	
Mild stenosis	3 (6.3)	1 (12.5)	
Moderate stenosis	1 (2.1)	0 (0)	
Severe stenosis	4 (8.3)	2 (25.0)	0.305

7 patients with moderate to severe renal artery stenosis were taking ACE-inhibitors or AIIRBs and 6 were taking a diuretic agent. 2 patients with mild renal artery stenosis and 1 patient with severe renal artery stenosis taking ACE inhibitors developed AKI. 1 patient with severe renal artery stenosis on an AIIRB developed AKI. All the patients on an ACE inhibitor or AIIRB with renal artery stenosis who developed AKI were also taking a diuretic.

4.2 The Mehran Risk Score

The Mehran risk score has been well validated in a general population undergoing coronary angiography and PCI procedures (326) as described in Section 1.6, (Table 9). From this original general population data it was shown to have good discriminant power for identifying contrast induced AKI with a C statistic of 0.69. To the best of our knowledge it has not been formally assessed in a high risk patient subset such as ours, that is in patients with diabetes and CKD. We therefore sought to evaluate its performance in our study population. The results are summarised in Table 27.

Table 27: Mehran Risk Score Data

	No AKI Outcome	**AKI Outcome**	**p value**
Mehran Risk Score, mean (SD)	11.1 (3.8)	13.7 (4.2)	< 0.001
Risk of AKI %, median, IQR	14 (14, 26.1)	26.1 (26.1, 57.3)	< 0.001
Risk of dialysis %, median, IQR	0.12 (0.12, 1.09)	1.09 (1.09, 12.6)	< 0.001

The mean Mehran risk score was significantly higher in the AKI group ($p < 0.001$). The incidence of AKI in our study population was 18.8%. No patients required haemodialysis. Overall the Mehran risk score had good discriminant power in our study population for identifying contrast induced AKI with a C statistic = 0.69.

4.3 The SYNTAX Score and Clinical SYNTAX Score

The SYNTAX score is an anatomical score of severity of coronary artery disease and is described in detail in Section 2.1. We aimed to establish if there was a relationship between severity of coronary artery disease and development of contrast induced AKI (Table 28).

Table 28: The SYNTAX score and Clinical SYNTAX Score data

	No AKI Outcome	AKI Outcome	p value
SYNTAX Score, mean (SD)	19.1 (16.5)	25.0 (20.1)	0.091
Clinical SYNTAX Score, median (IQR)	35.4 (12.7, 101.2)	82.7 (26.0, 158.9)	0.036

There was no significant difference between severity of coronary disease, assessed by the SYNTAX score between the 2 groups. However the Clinical SYNTAX Score (CSS) (described in detail in section 2.12.2) was significantly higher in the AKI group 35.4 vs. 82.7 ($p = 0.036$). The CSS takes into account clinical factors including Age, eGFR and LV function in addition to the anatomical scoring of the SYNTAX Score. It is likely that this difference is driven by the more severe LV dysfunction and renal function observed in the AKI group which we have discussed already. The CSS had reasonable accuracy for predicting development of contrast induced AKI with a C statistic of 0.64.

4.4 Discussion

4.4.1 Renal artery stenosis, ACE inhibitor prescription and AKI Development

The association between accelerated renal failure in patients with CKD and renal artery stenosis receiving ACE-inhibitors or AIIRBs is well described in the medical literature (468-473). The majority of these data comes from case reports, small case series and retrospective studies and have suggested that such failure is reversible following drug withdrawal. The overall conclusion has been that it is necessary for an additional precipitating risk factor to be present in order for patients with renal artery stenosis treated with RAAS blockade to develop AKI (285;468-474). Risk factors that have been identified include other therapeutic agents such as diuretics and NSAIDs, described as 'the triple whammy effect' (475). In the study presented here all of the patients with renal artery stenosis who developed AKI were also taking diuretic therapy. It has also been suggested that bilateral renal artery disease must be present in patients with dual kidneys for RAAS blockade to result in worsening renal failure. However it is possible for microvascular renal arteriolar narrowing to occur that is not visible either on direct renal angiography or magnetic resonance angiography (MRA) and can be identified only on renal artery biopsy (473). This microvascular renal narrowing can stimulate increased angiotensin II production in a similar manner to macroscopic renal artery stenosis (455;473). How patients with renal artery stenosis should be managed remains an area of debate.

Topol and Nissen coined the phrase ‘the oculo-stenotic’ reflex to describe the at the time widely held view that an angiographically severe stenosis must cause ischaemia and therefore revascularisation is necessary (476). Although quantitative angiography improves the accuracy of stenosis severity it does not improve the accuracy of diagnosing ischaemia (477). New technological developments such as Fractional Flow Reserve (FFR) have shown that > 1/3 of angiographically severe coronary stenoses are haemodynamically not significant and results correlate well with ischaemic findings on myocardial perfusion imaging (478). Renal artery stenosis severity has also been shown to poorly correlate with haemodynamic significance (479).

A number of randomised trials (480-484) have shown that renal artery stenting based solely on angiographic severity of stenoses is not associated with improvement in either blood pressure or renal function or clinical events compared to medical therapy alone. Potential explanations for these findings include treatment of patients with anatomic stenosis but no renal ischaemia, baseline parenchymal renal disease precluding later improvement in hypertension and renal function and essential hypertension without a renovascular component (485).

A recent study by Mangiacapra *et al* (486) has suggested that invasive assessment of renal ischaemia by measuring trans-lesional pressure gradient following administration of intra-arterial dopamine may predict blood pressure response following renal artery stenting and hence would identify patients who might benefit from this procedure. The study enrolled 53 hypertensive patients with unilateral renal artery stenosis. A dopamine induced mean pressure gradient of ≥ 20 mmHg before revascularisation was the only independent predictor of blood pressure improvement. The C statistic on ROC

analysis for predicting a decrease in systolic BP ≥ 20 mmHg at 3 months was 0.77, consistent with good accuracy. Mean number of medications per patient was reduced from 3.2 to 2.8 following stenting. However 18% of non-responders had a dopamine induced mean pressure gradient ≥ 20 mmHg, suggesting considerable heterogeneity in blood pressure response.

There are a number of possible explanations for this variable blood pressure response, even when renal ischaemia is present. Patients with atherosclerotic renal artery stenosis may have indistinguishable levels of renin activity compared to hypertensive patients without renal artery stenosis (487;488). This suggests that most patients with hypertension and renal artery stenosis do not have renovascular hypertension. Possible aetiologies include essential hypertension associated with sympathetic/cerebral nervous system activation, vasoactive oxygen species, abnormalities in endothelial dependent relaxation or ischaemic and hypertensive intra-renal injury (489-491). Therefore patients with haemodynamically significant renal artery stenosis may not have renovascular hypertension so expecting that hypertension will be cured following stenting is probably unrealistic.

Although marked clinical benefits may be achieved from even modest reductions in blood pressure (492), further studies in more patients are needed to assess suitability of patients with renal artery stenosis for revascularisation. Other methods available to assess the physiological impact of renal artery stenosis include nuclear scintigraphy, direct GFR measurement, FFR as well as translesional pressure gradients (477;479;486;493). This will allow objective assessment of renal ischaemia, confirm the

absence of irreversible renal injury and therefore proper assessment of the appropriateness for revascularisation to occur.

The number of renal angiograms performed in this study was small and no physiological assessment of lesion severity was made at the time of procedure. There are a number of reasons for this: (i) It is difficult to catheterise the renal arteries from the radial route as catheters are a set length and in general they do not reach around the aortic arch to the appropriate level in the abdominal aorta. Approximately 50% of procedures in this study were performed by the radial route. (ii) Few operators in the cardiac catheter laboratories at our institution are familiar with this technique and therefore were not willing to perform peripheral arterial cannulation. (iii) This was not an essential part of the study protocol and was performed at the operator's discretion. The study identified 7 patients with moderate-severe renal artery stenosis and these patients were referred to our nephrology colleagues for further assessment.

4.4.2 The Mehran risk score to predict development of contrast induced AKI

Radiographic contrast media are responsible for 11% of cases of hospital acquired renal insufficiency and are the third most common cause of renal failure after impaired renal perfusion and nephrotoxic drugs (266). Out of all the procedures which utilise contrast media for imaging purposes coronary angiography and PCI are associated with the highest rates of contrast induced AKI (266). Patients with diabetes and CKD have increased cardiovascular morbidity and mortality (7;13;443) as described in sections 1.2 and 1.3. These patients therefore frequently need to undergo coronary angiography

and PCI procedures. As mentioned before (section 1.6.4.1), when renal function is already impaired by pathological processes the kidney is far less able to tolerate the stress of excreting a contrast load (281). Therefore patients with diabetes and CKD are at particular risk for developing contrast induced AKI.

It would be extremely useful to identify those patients most at risk early, to allow possible therapeutic interventions to be initiated promptly. Possible interventions include more intensive i.v. hydration regimes, use of vasodilators such as dopamine, fenoldopam, endothelin receptor antagonists and aminophylline (329). Such attempts to date have been limited as the current 'gold standard' biomarker of renal injury, serum creatinine does not rise until 48-72 hours post the injury event (265). For the reasons previously mentioned (section 1.8), an alternative marker of renal injury than creatinine is required.

The Mehran risk score is a simple model that allows identification of patients at high risk for contrast induced AKI at the time of procedure and therefore could be incorporated into routine clinical practice (326). The score is described in detail in section 1.6.4.7. Briefly, 8357 patients from a database at the cardiac catheterisation laboratory at Columbia University Medical Centre in New York were randomly assigned to a development and validation dataset. The baseline clinical and procedural characteristics of 5571 patients in the development dataset were considered as candidate univariate predictors of contrast induced AKI. Multivariate logistic regression was then used to identify independent predictors of contrast induced AKI with a p value < 0.0001. Based on the odds ratio, 8 variables were identified (hypotension, intra-aortic balloon pump, congestive cardiac failure, CKD, diabetes mellitus, age > 75 years, anaemia and

volume of contrast). Each of these variables was assigned a weighted integer, the sum of the integers being the total risk score for each patient. This allows the patients to be stratified into a low risk (≤ 5), low-medium (6–10), medium-high (11-16) and high risk (≥ 16) group (326). From this score, % risk of contrast induced AKI and risk of dialysis can be ascertained (Table 9) at time of procedure. The score had a good discriminative ability in identifying contrast induced AKI in a general population undergoing coronary angiography and PCI with a C statistic of 0.69.

The score performed well in the study population of patients with diabetes and CKD described in this thesis. This was a medium-high risk group with mean risk score of 11.1 in the patients who did not develop AKI and 13.7 in those who developed AKI. The rate of AKI in this population was 18.8%, which is within the range (14 - 26.1%) that the score identifies. However it would appear that the score over-estimates the risk of contrast induced AKI (Table 27) requiring dialysis as we had no such cases in our study population of 208 patients. The C statistic for the score in patients with diabetes and CKD was 0.69, again showing good discriminant accuracy. Identification of high risk patients early allows continuation of supportive care such as i.v. fluids and also permits closely tailored monitoring in appropriate patients whilst also facilitating prompt discharge of those at lower risk. This will create a more efficient work flow within cardiology departments, both in terms of cardiac catheter laboratory time but also with hospital bed management. Care can therefore be tailored on an individual basis based on the patient's perceived risk.

It would also be possible to use the scoring system in a pre-admission setting to select cases that might require senior (experienced) operators. A standard volume of

contrast could be included in this calculation such as 50 ml for a diagnostic angiogram and 200 ml for a PCI. Since currently treatment options for contrast induced AKI are rather limited and development of this complication is associated with a prolonged hospital stay and unfavourable in-hospital and 1 year outcomes, early identification of those at high risk with options such as increasing pre-procedure hydration by admitting these patients the night before procedure. This might also allow the allocation of limited resources such as haemofiltration to those most at risk. A single centre study reported that peri-PCI haemofiltration starting prior to PCI and extending for 24 h post PCI was effective in decreasing the incidence of contrast induced AKI in high risk patients (serum creatinine > 2 mg/dl or 176.8 µmol/l). Contrast induced AKI (defined as a rise from baseline serum creatinine of >25%) developed in 5% of patients who underwent haemofiltration compared to 50% in the control group who received i.v. hydration ($p < 0.001$). Due to the high cost of haemofiltration compared to that of a saline infusion this approach has not become established in clinical practice (361).

The Mehran risk score has a number of limitations. The inclusion of 2 procedural characteristics (IABP and contrast volume) limits the ability to apply the score prior to procedure. A volume of contrast can be estimated (as described above) depending on the type of procedure and use of IABP is rare in the elective setting so an assessment of the 'minimum' risk can be made. However the score does not include any data on i.v. hydration which is the one factor that has been shown to conclusively decrease rates of contrast induced AKI (section 1.7.1), nor does it include other important data such as presence of proteinuria, urine output and nephrotoxic medications. These are areas that could perhaps be investigated in further studies and might result in improved accuracy in

identifying high risk patients.

4.4.3 The SYNTAX Score and Clinical SYNTAX score

The SYNTAX score allows objective quantification of complexity of coronary artery disease, taking into account not just the number of significant lesions and their location but also the complexity of each lesion independently. Higher SYNTAX scores, indicative of more complex disease, are thought to present a bigger therapeutic challenge and to potentially herald a worse prognosis (421). The scoring algorithm is described in detail in section 2.12. The score has been divided into terciles to guide optimal revascularisation strategy with a low score (0 – 22) favouring PCI, a medium score (23 – 32) suggesting either PCI or CABG are reasonable choices and a high score (≥ 33) favouring CABG (26). The patients in this study who did not develop AKI had a mean SYNTAX score of 19.1 whereas those who developed AKI had a mean score of 25.0 suggesting that their disease was more complex overall. Anatomical lesion scoring systems have been shown to have a lower ability to predict mortality than scoring systems that use clinical characteristics (424) and this led to the development of the Clinical SYNTAX Score (CSS) which was recently reported (431). The median CSS was 35.4 in the patients who did not develop AKI and 82.7 ($p = 0.036$) in those who did develop AKI. This difference is driven by the more severe LV dysfunction and baseline renal function of the patients who developed AKI described in chapter 3. The CSS classifies patients into terciles as described in section 2.12.2 and this would put the patients studied here into CSS_{HIGH} (>27.5). It has been shown that such patients have higher mortality and revascularisation rates at 1 year (431). Although patients in this study were only followed up to day 3 and mortality and revascularisation rates were not assessed, further

projects could address this. Limitations of both scores are that it is extremely time consuming even using the online calculator to complete the algorithm, taking approximately 30 min for the average patient. There is also a definite ‘learning curve’ that needs to be overcome in order to be consistent with result reporting and even then there is only moderate intra-observer reproducibility as has been reported in the literature (494;495). These limitations are likely to limit the adoption of these scores into routine clinical practice.

Chapter Five

Materials and Methods for Biomarker

ELISA Assays

Chapter 5: Materials and Methods for Biomarker ELISA Assays

5.1 NGAL Assay

(DuoSet ELISA Development System, R and D Systems, Oxford, United Kingdom; Catalogue Number DY1757)

The DuoSet kit contains the basic components required for the development of sandwich ELISAs to measure natural or recombinant NGAL. The ELISA technique has been described in detail in section 2.9. Briefly, a monoclonal antibody specific for NGAL is coated onto a microtitre plate and left overnight. The following day the plate is washed with buffer and standards and samples are pipetted into the wells. Any NGAL present is bound by the immobilised antibody. After washing away any unbound substances, an enzyme linked monoclonal antibody specific for NGAL is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the quantity of NGAL bound in the initial step. Colour development is stopped and the intensity of colour is measured.

5.1.1 Solutions and Antibodies

Phosphate Buffered Saline (PBS)

Make up 2 litres of PBS with 2 sachets of Sigma PBS 7.4 (P3813) which consists of 137 mM Na Cl, 2.7 mm K Cl, 8.1 mM Na_2 HPO_4, 1.5 mm KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered.

Reagent Buffer *(R and D Systems Catalogue # DY 995)*

To 500 ml of Reagent Buffer add 5 g of Fraction V Bovine Serum Albumin (A-3294) to make a final solution which consists of 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered

Wash Buffer *(R and D Systems Catalogue # WA 126)*

To 2 litres of PBS add 1 ml of neat Tween 20 (=0.05%)

Capture Antibody *(rat anti-human Lipocalin-2)*

To lyophilised antibody add 1 ml of PBS = 360 µg/ml. Then dilute 55 µl into 10 ml PBS to give working reagent of 2 µg/ml

Standards/Calibrators *(recombinant human Lipocalin-2)*

Make up stock calibrator: add 0.5 ml of reagent diluent to the supplied lyophilised calibrant = 90 ng/ml

Dilate stock calibrator 18 fold: 50 µl + 850 µl of reagent buffer = 5000 pg/ml. Then serial dilute down 1:2 to give calibrate equal to: 0, 78, 156, 313, 625, 1250, 2500, 5000 pg/ml.

Detection Antibody *(biotinylated goat anti-human Lipocalin-2)*

Add 1 ml of reagent diluents to lyophilised antibody. Add 55 µl of this to 10 ml of reagent diluent to give 100 ng/ml final concentration

Streptavidin-HRP

1 ml of streptavidin conjugated to horseradish-peroxidase supplied. Dilute 1 in 200 in Reagent Diluent – 50 microlitres in 10 ml. Protect from light

Substrate Solution: 3,3',5,5' Tetramethylbenzidine (TMB) Liquid Substrate System *(Sigma, Dorset, United Kingdom)*

The product is supplied as a ready to use peroxidase substrate containing 3,3',5,5' TMB in a mildly acidic buffer. Prior to reaction with peroxidase the substrate should be a colourless to light bluish-green solution. The substrate is a light sensitive and should be protected from direct sunlight or UV sources and stored at 2-8 °C. It should be brought to room temperature (~ 22-25°C) before use.

Stop Solution *(Sigma, Dorset, United Kingdom; Catalogue # 320501)*

2.5 mM Sulphuric acid (H_2SO4)

5.1.2 NGAL General ELISA Protocol

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µl/well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.

2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 400 µl of Wash Buffer using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each stage is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Block the plates by adding 300 µl of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 h.

4. Repeat the aspiration/wash as in step 2. The plates are now ready for a sample addition.

Assay Procedure

1. Add 100 µl of sample or standards in Reagent Diluent, or an appropriate diluent/well. Cover with an adhesive strip and incubate at room temperature for 2 h.

2. Repeat the aspiration/wash as in step 2 of Plate Preparation.

3. Add 100 µl of the Detection Antibody, diluted in Reagent Diluent to each well. Cover with a new adhesive strip and incubate for 2 h at room temperature.

4. Repeat the aspiration/wash as in step 2 of Plate Preparation.

5. Add 100 µl of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 min at room temperature. Avoid placing the plate in direct light.

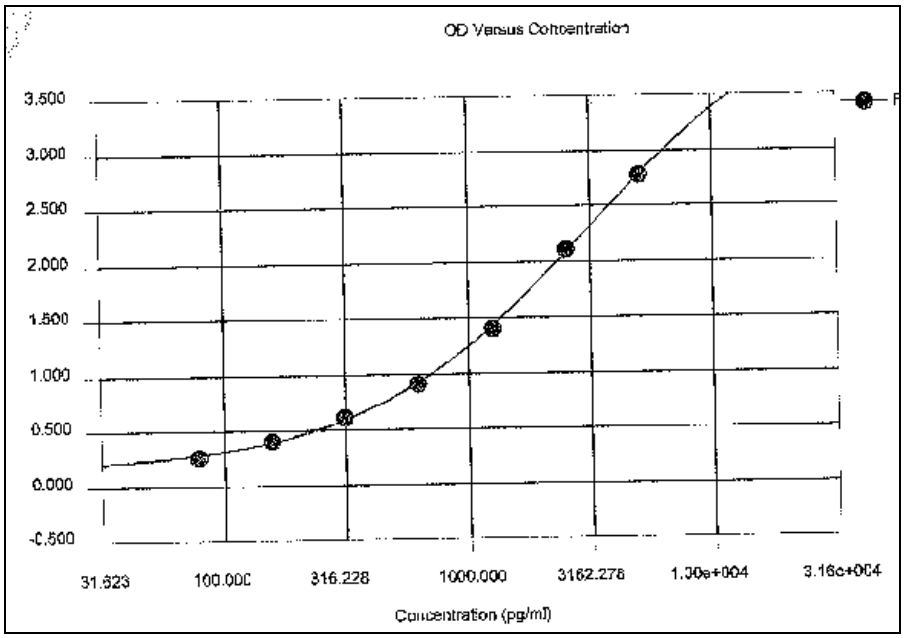
6. Repeat the aspiration/wash as in step 2 of Plate Preparation.

7. Add 100 µl of Substrate Solution to each well. Incubate for 20 min at room temperature. Avoid placing the plate in direct light. Following reaction with peroxidase, a blue reaction product forms.

8. Add 50 µl of Stop Solution to each well, yielding a yellow end product. Gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well immediately using a microplate reader set to 450 nm. Wavelength correction was set to 650 nm

Figure 19: Standard Curve for NGAL Assays



5.1.3 NGAL Assay Titrations

Our study population only included diabetic patients with CKD. Serum and urine NGAL concentrations have not been evaluated in such a population post contrast administration and therefore we had to titrate the samples so that we could identify the most appropriate dilutions for performing sample analysis. We identified a dilution for serum samples of 1 in 200 and for urine of 1 in 20 as the most appropriate for our study population. All samples from an individual patient were measured in a single assay. The co-efficient of variation for intra-assay reproducibility for NGAL concentration was 7.2% in our laboratory and corresponds to that reported by the kit manufacturer. Sample assays were repeated across several different plates on different days to ensure

reproducibility of results although formal interassay variation was not calculated.

5.2 Interleukin-18 Assay

(DuoSet ELISA Development System, R and D Systems, Oxford, United Kingdom; Catalogue Number DY119)

The DuoSet kit contains the basic components required for the development of sandwich ELISAs to measure natural or recombinant IL-18. The ELISA technique has been described in detail in section 2.9. Briefly, a monoclonal antibody specific for IL-18 is coated onto a microtitre plate and left overnight. The following day the plate is washed with buffer and standards and samples are pipetted into the wells. Any IL-18 present is bound by the immobilised antibody. After washing away any unbound substances, an enzyme linked monoclonal antibody specific for IL-18 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the quantity of IL-18 bound in the initial step. Colour development is stopped and the intensity of colour is measured.

5.2.1 Solutions and Antibodies

PBS

Make up 2 litres of PBS with 2 sachets of Sigma PBS 7.4 (P3813) which consists of: 137 mM Na Cl, 2.7 mm K Cl, 8.1 mM Na_2 HPO_4, 1.5 mm KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered.

Reagent Buffer *(R and D Systems Catalogue # DY 995)*

To 500 ml Reagent Buffer add 5 g of Fraction V BSA (A-3294) to make a final solution which consists of 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered

Wash Buffer *(R and D Systems Catalogue # WA 126)*

To 2 litres of PBS add 1 ml of neat Tween 20 (= 0.05%), pH 7.2-7.4

Capture Antibody *(mouse anti-human IL-18)*

To lyophilised antibody add 1 ml of PBS = 360 µg/ml. Then dilute 55 µl into 10 ml PBS to give working reagent of 2 µg/ml

Calibrators/Standards *(recombinant human IL-18)*

Make up stock calibrators: add 0.5 ml of reagent diluent to the supplied lyophilised calibrant = 250 ng/ml. Store as 50 µl aliquots.

Take 24 µl of stock calibrator and dilute with 976 µl of reagent buffer to give a high standard of 6000 pg/ml. Then serial dilute this 1:2 to give calibrants equal to: 0, 93.75, 187.5, 375, 750, 1500, 3000, 6000 pg/ml.

Detection Antibody *(biotinylated goat anti-human Il-18)*

Add 1 ml of reagent diluent to lyophilised antibody. Add 55 µl of this to 10 ml of reagent diluent to give 200 ng/ml final concentration.

Streptavidin-HRP

1 ml of streptavidin conjugated to horseradish-peroxidase supplied. Dilute 1 in 200 in Reagent Diluent – 50 µl in 10 ml and protect from light.

Substrate Solution: 3,3',5,5' Tetramethylbenzidine (TMB) Liquid Substrate System *(Sigma, Dorset, UK)*

The product is supplied as a ready to use peroxidase substrate containing 3,3',5,5' TMB in a mildly acidic buffer. Prior to reaction with peroxidase the substrate should be a colourless to light bluish-green solution. The substrate is a light sensitive and should be protected from direct sunlight or UV sources and stored at 2-8 °C. It should be brought to room temperature (~ 22-25°C) before use.

Stop Solution *(Sigma, Dorset, UK; Catalogue # 320501)*

2.5 mM H_2SO4

5.2.2 Interleukin-18 General ELISA Protocol

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µl/well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.

2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 400 µl of Wash Buffer using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each stage is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Block the plates by adding 300 µl of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 h.

4. Repeat the aspiration/wash as in step 2. The plates are now ready for a sample addition.

Assay Procedure

1. Add 100 µl of sample or standards in Reagent Diluent, or an appropriate diluent/well. Cover with an adhesive strip and incubate at room temperature for 2 h.

2. Repeat the aspiration/wash as in step 2 of Plate Preparation.

3. Add 100 µl of the Detection Antibody, diluted in Reagent Diluent to each well. Cover with a new adhesive strip and incubate for 2 h at room temperature.

4. Repeat the aspiration/wash as in step 2 of Plate Preparation.

5. Add 100 µl of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 min at room temperature. Avoid placing the plate in direct light.

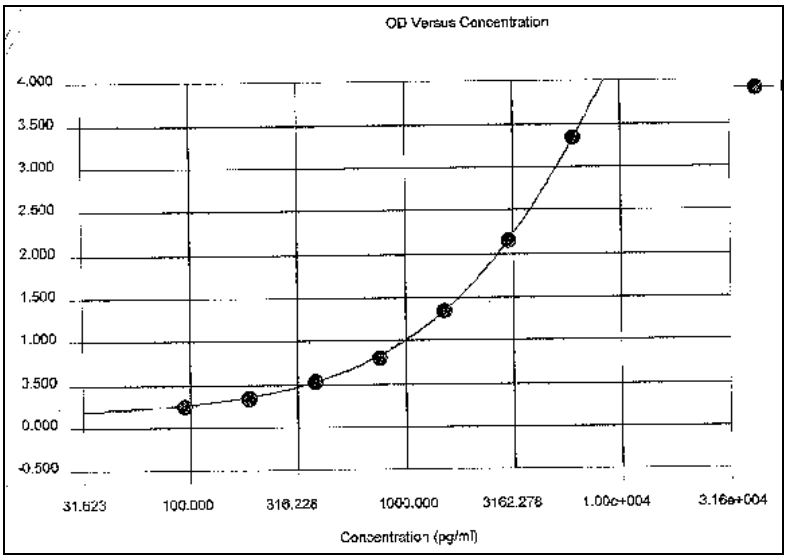
6. Repeat the aspiration/wash as in step 2 of Plate Preparation.

7. Add 100 µl of Substrate Solution to each well. Incubate for 20 min at room temperature. Avoid placing the plate in direct light.

8. Add 50 µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well immediately using a microplate reader set to 450nm. Wavelength correction was set at 650 nm.

Figure: 20: Standard Curve for IL-18 assay



5.2.3 Interleukin 18 Assay Titrations

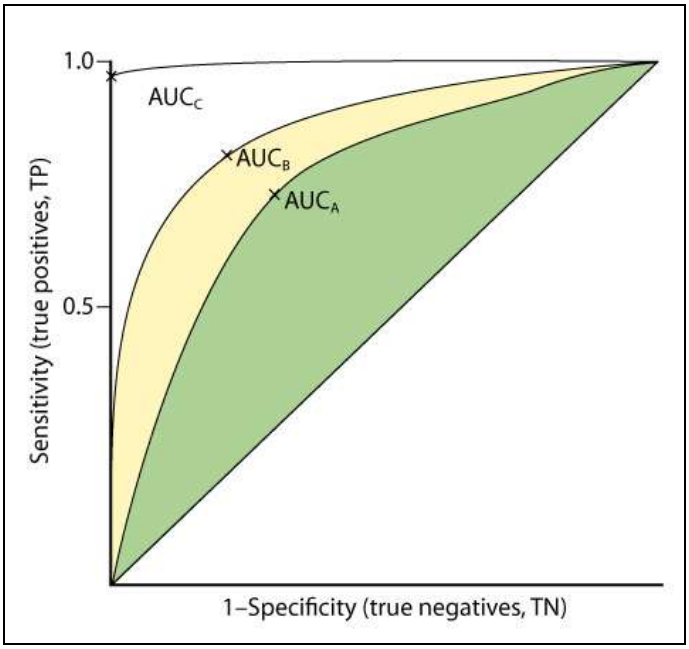
Our study population only included diabetic patients with CKD. Serum and urine IL-18 concentrations have not been evaluated in such a population post contrast administration and therefore we had to titrate the commercial kits so that we could identify the most appropriate dilutions for performing sample analysis. We identified a dilution for serum samples of 1 in 200 and for urine of 1 in 5 or 1 in 50 as the most appropriate for our study population. All samples from an individual patient were measured in a single assay. The co-efficient of variation for intra-assay reproducibility for IL-18 concentration was 11.9% in our laboratory and corresponds to that reported by the kit manufacturer. Sample assays were repeated across several different plates on different days to ensure reproducibility of results although formal interassay variation

was not calculated.

5.3 Receiver Operating Characteristic Curve Analysis

Receiver Operating Characteristic (ROC) curves provide a statistical method of assessing the diagnostic accuracy of a test (or biomarker) that has a continuous spectrum of test results. The ROC curve is a graphical display of the 'trade-off' between the true-positive rate (sensitivity) and false positive rate (1-specificity) corresponding to all possible binary tests that can be formed from this continuous biomarker (496). Every classification rule or cut-off level generates a point on the curve. The closer the curve follows the left hand border and then the top border of the ROC space, the more accurate the test. The closer the curve is to the 45° diagonal, the less accurate the test is (497). This is shown in Figure 21.

Figure 21: Receiver Operating Characteristic Curves



Adapted from Soreide J Clin Pathol 2009 (497). 3 curve plots and their respective area under the curve (AUC) are shown. The diagnostic accuracy of marker C (white area) is better than that of B and A as the AUC of C > B > A. X represents the best cut-off point for each biomarker.

The traditional ROC curve arises when a continuous value is measured in each subject. The classification is positive if the value is above a threshold. As the threshold varies, a new classification rule is created and the resulting plot is a single curve. The optimal ROC curve is therefore the line connecting the points highest and farthest to the left upper corner and captures the trade-off between sensitivity and specificity over a continuous range (497).

5.3.1 Area under the ROC curve

The area under the curve (AUC) is also known as the C statistic and can range in value from 0.5 – 1. A C statistic of 0.5 (refers to the 45° line in the ROC plot) is no better than random chance and therefore the marker has no predictive ability. A C statistic of 1 refers to perfect discrimination/accuracy.

The AUC is a measure of the overall diagnostic accuracy of a test and the cut-off value providing the highest sensitivity and specificity is calculated (Figure 21).

Chapter Six

Biomarker Panel Assays and ROC Analysis

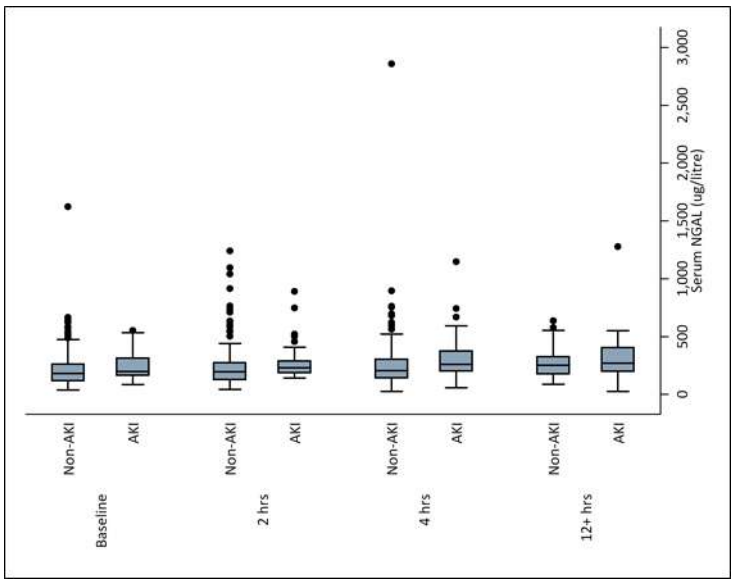
Chapter 6: Biomarker Panel Assays and ROC Analysis

6.1 NGAL as a biomarker of AKI in the Study Population

6.1.1 Serum NGAL results

We measured our biomarker panel at baseline, 2 h post procedure, 4 h post procedure and 12-24 h post procedure. The results for serum NGAL (units = µg/litre) are shown in Figure 22.

Figure 22: Box-plot of serum NGAL results as baseline, 2 hours, 4 hours and 12-24 hours showing development of AKI versus no AKI.



In the AKI group a significant absolute rise in serum NGAL is seen as early as 2 h post procedure compared to baseline (p = 0.023), and this persists at 4 h (p = 0.005) and 12-24 h (p = 0.017). However there is little evidence of an association between an increase in the absolute value of serum NGAL in the study population as a whole as evidenced by the odds ratio (Table 29). The large range of values and the wide variability within the study population even at baseline mean that a small absolute change in the levels of the biomarker may be lost due to a 'high signal to noise' ratio and therefore we looked at % rises from baseline in our biomarkers rather than absolute values (Table 30).

Table 29: Odds ratio for risk of developing contrast AKI for serum NGAL

Biomarker		**OR**	**95% CI**		**p value**
Serum NGAL Baseline (per 100 units)	207	1.07	0.89	1.29	0.458
Serum NGAL 2h (per 100 units)	198	1.09	0.92	1.28	0.342
Serum NGAL 4h (per 100 units)	182	1.06	0.94	1.20	0.348
Serum NGAL 12-24h (per 100 units)	71	1.23	0.91	1.67	0.186

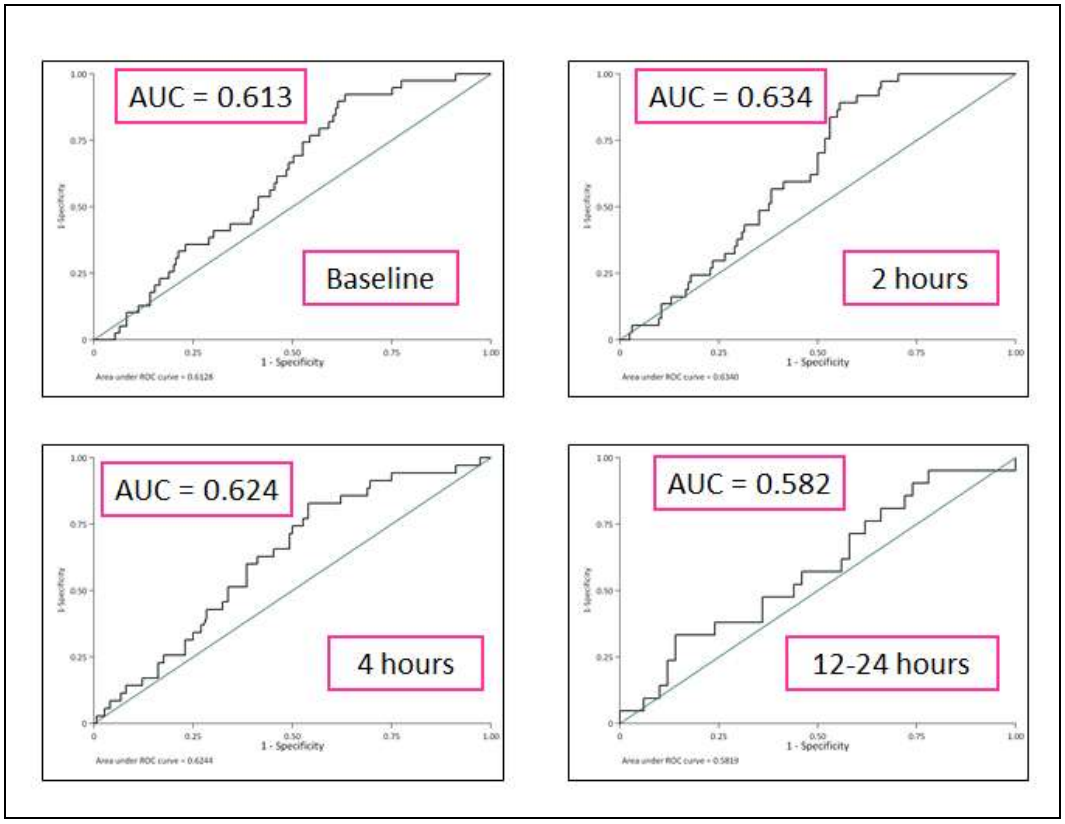
Table 30: Absolute and Percentage changes from baseline in serum NGAL

Table 30a) Serum NGAL in AKI patients				
Baseline Median (IQR)	**2 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
198 (167, 316)	230 (189, 292)	0.023	14.6 (-3.4, 28.7)	0.004
Baseline Median (IQR)	**4 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
198 (167, 316)	261 (202, 379)	0.005	22.0 (6.8, 52.4)	0.001
Baseline Median (IQR)	**12-24 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
198 (167, 316)	270 (200, 407)	0.017	26.1 (-4.0, 79.4)	0.019

Table 30b) Serum NGAL in non-AKI patients				
Baseline Median (IQR)	**2 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
180 (120, 266)	197 (129, 278)	0.008	7.4 (-15.1, 38.6)	0.001
Baseline Median (IQR)	**4 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
180 (120, 266)	207 (144, 307)	0.002	12.1 (-13.7, 43.4)	<0.001
Baseline Median (IQR)	**12-24 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
180 (120, 266)	252 (178, 329)	0.018	17.0 (-7.6, 41.4)	0.001

Receiver Operating Characteristic Curves were constructed to assess how discriminant serum NGAL concentrations at different time-points are for the development of AKI. The results are summarised in Figure 23.

Figure 23: Receiver Operating Characteristic Curves for serum NGAL



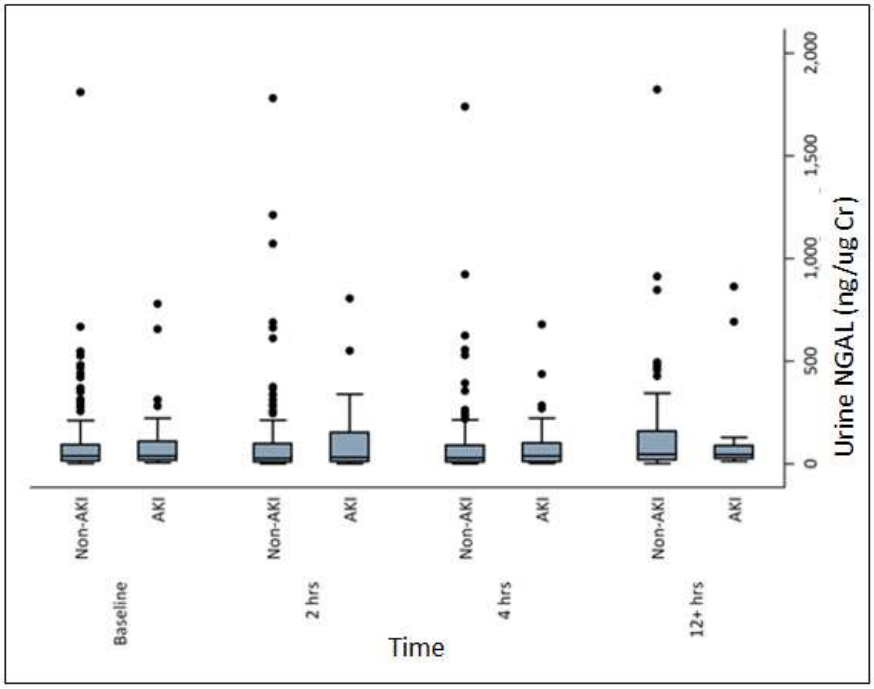
The respective C statistics for serum NGAL concentrations at baseline, 2 h, 4 h and 12-24 h are 0.6128, 0.6340, 0.6244, 0.5819.

6.12 Urine NGAL results

The box-plot results for urine NGAL concentrations at the different time-points are summarized in Figure 24.

Figure 24: Box-plot of urine NGAL results at baseline, 2 hours, 4 hours and 12-24 hours showing development of AKI versus no AKI.* (NGAL units =ng/µg creatinine)

*These results are corrected for urine creatinine excretion



Urine NGAL concentrations did not change significantly in the study population during the first 24 hours. The odd ratio data are shown in Table 31.

Table 31: Odds ratio for risk of developing contrast AKI for urine NGAL

Biomarker		**OR**	**95% CI**		**p value**
Urine NGAL Baseline (per 100 units)	202	0.99	0.81	1.21	0.934
Urine NGAL 2h (per 100 units)	188	1.01	0.85	1.20	0.936
Urine NGAL 4h (per 100 units)	181	1.02	0.83	1.25	0.866
Urine NGAL 12-24h (per 100 units)	74	0.95	0.76	1.19	0.667

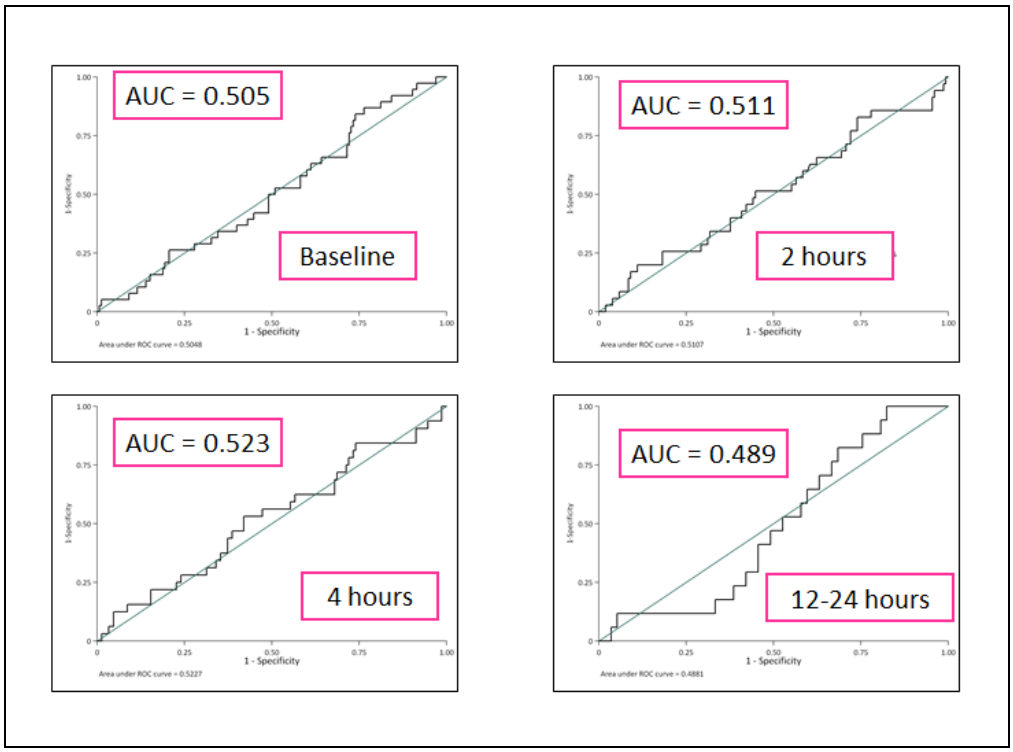
The absolute and percentage changes in urine NGAL are shown in Table 32.

Table 32 Absolute and Percentage changes from baseline in urine NGAL

Table 32a) Urine NGAL in AKI patients				
Baseline Median (IQR)	**2 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
39.1 (18.2, 113.2)	31.9 (11.9, 154.9)	0.675	-23.8 (-65.5, 69.4)	0.912
Baseline Median (IQR)	**4 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
39.1 (18.2, 113.2)	37.8 (12.7, 104.4)	0.347	-16.1 (-54.3, 67.2)	0.891
Baseline Median (IQR)	**12-24 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
39.1 (18.2, 113.2)	44.7 (27.6, 91.1)	0.177	49.6 (-25.1, 85.3)	0.102
Table 32b) Urine NGAL in non AKI patients				
Baseline Median (IQR)	**2 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
38.8 (16.0, 95.3)	26.4 (9.8, 101.1)	<0.001	-27.8 (59.4, 19.9)	0.007
Baseline Median (IQR)	**4 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
38.8 (16.0, 95.3)	28.7 (10.6, 92.7)	0.001	-29.3 (-64.7, 39.3)	0.079
Baseline Median (IQR)	**12+ hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
38.8 (16.0, 95.3)	47.2 (20.9, 162.1)	0.845	-5.1 (-51.4, 71.4)	0.254

Receiver Operating Characteristic Curves were constructed to assess how discriminant urine NGAL concentrations at different time-points are for the development of AKI. The results are summarised in Figure 25.

Figure 25: Receiver Operating Characteristic Curves for Urine NGAL



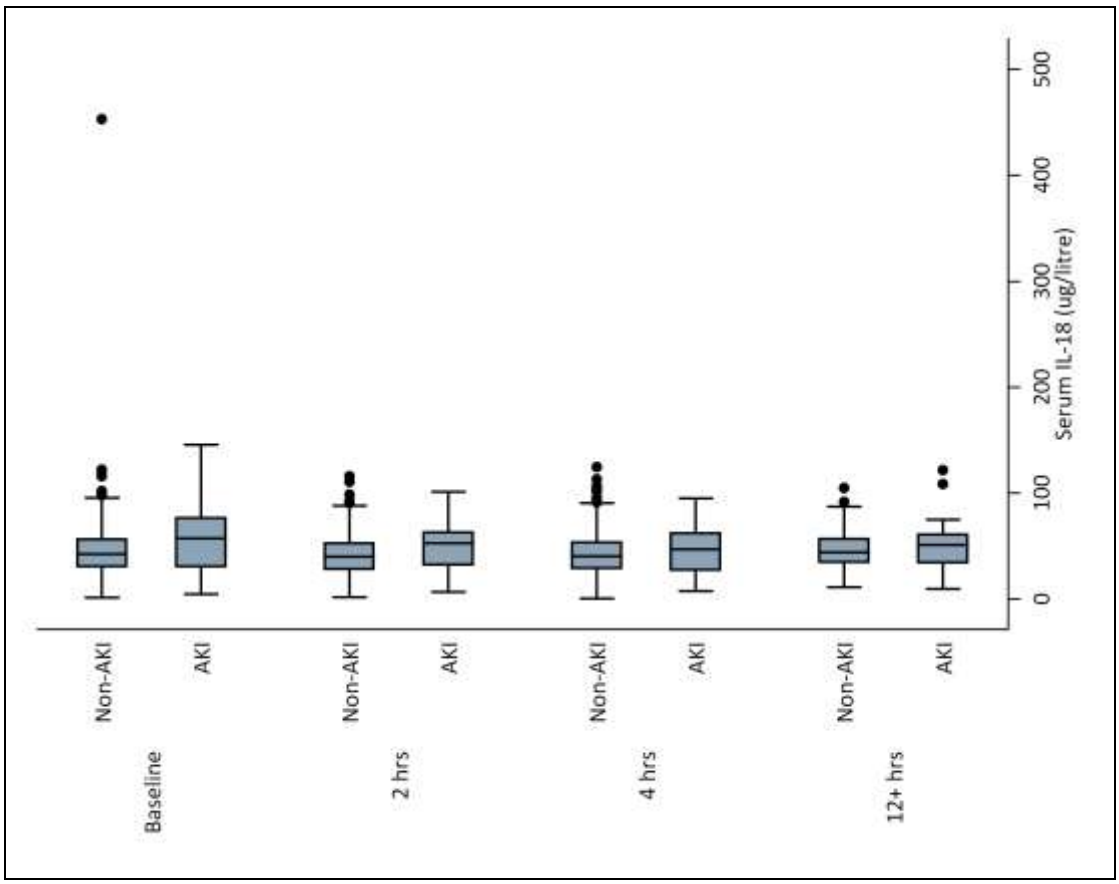
The respective C statistics for urine NGAL at baseline, 2 h, 4 h and 12-24 h are 0.5048, 0.5107, 0.5277, 0.4881.

6.2 Interleukin-18 as a biomarker of AKI in the study population

6.2.1 Serum IL-18 results

The results for serum IL-18 are shown on a box-plot in Figure 26.

Figure 26: Box plot of serum IL-18 results as baseline, 2 hours, 4 hours and 12-24 hours showing development of AKI versus no AKI. (units = µg/litre)



In the AKI group a significant absolute rise in serum IL-18 is seen as early as 2 h post procedure compared to baseline ($p < 0.001$), and this persists at 4 h ($p = 0.042$) but is not seen at 12-24 h ($p = 0.485$). However there is little evidence of an association between an increase in the absolute value of serum IL-18 in the study population as a whole as evidenced by the odds ratio (Table 33).

Table 33: Odds ratio for risk of developing contrast AKI for serum IL-18

Biomarker	n	OR	95% CI	p value	
Baseline serum IL-18	206	1.01	1.00	1.01	0.159
Serum IL-18 2 h	201	1.01	1.00	1.03	0.069
Serum IL-18 4 h	182	1.00	0.99	1.02	0.619
Serum IL-18 12 -24 h	75	1.01	0.99	1.03	0.460

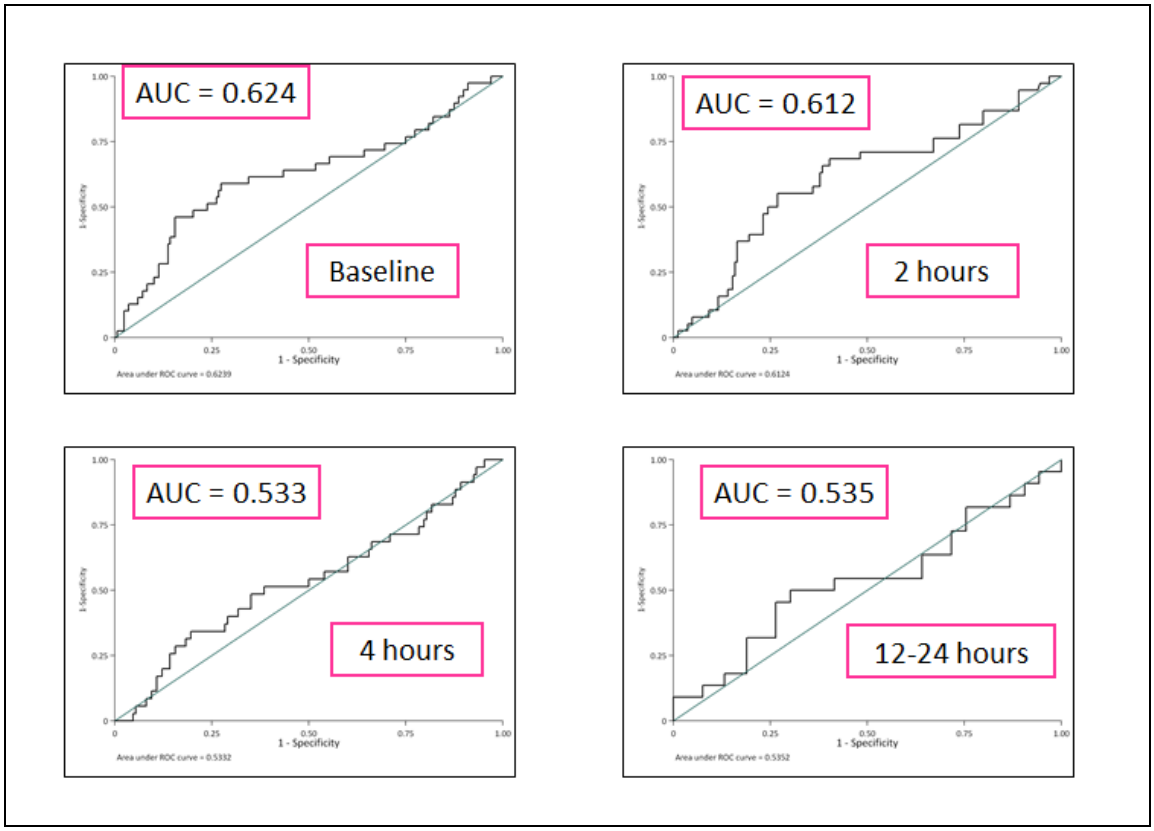
Again the large range of values and the wide variability within the study population even at baseline mean that a small absolute change in the levels of the biomarker may be lost due to a 'high signal to noise' ratio and therefore as previously described (section 6.1) we looked at % rises from baseline in our biomarkers rather than absolute values (Table 34).

Table 34: Absolute and Percentage changes from baseline in serum IL-18.

Table 30a) Serum IL-18 in AKI patients				
Baseline Median (IQR)	**2 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
57.3 (30.9, 77.2)	52.9 (32.5, 63.5)	<0.001	-6.9 (-16.9, 2.1)	0.022
Baseline Median (IQR)	**4 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
57.3 (30.9, 77.2)	47.0 (27.6, 62.6)	0.042	-6.7 (-18.8, 10.3)	0.265
Baseline Median (IQR)	**12+ hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
57.3 (30.9, 77.2)	51.3 (34.9, 61.2)	0.485	4.4 (-4.6, 23.3)	0.199
Table 30b) Serum IL-18				
Baseline Median (IQR)	**2 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
42.4 (30.8, 56.9)	39.9 (28.5, 53.1)	<0.001	-5.8 (-15.2, 5.5)	<0.001
Baseline Median (IQR)	**4 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
42.4 (30.8, 56.9)	40.6 (29.5, 54.0)	0.01	-6.3 (-15.7, 7.9)	0.034
Baseline Median (IQR)	**12+ hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
42.4 (30.8, 56.9)	44.4 (34.9, 57.4)	0.113	3.1 (-5.5, 22.0)	0.061

Receiver Operating Characteristic Curves were constructed to assess how discriminant serum IL-18 concentrations at different time-points are for the development of AKI. The results are summarised in Figure 27.

Figure 27: Receiver Operating Characteristic curves for serum IL-18

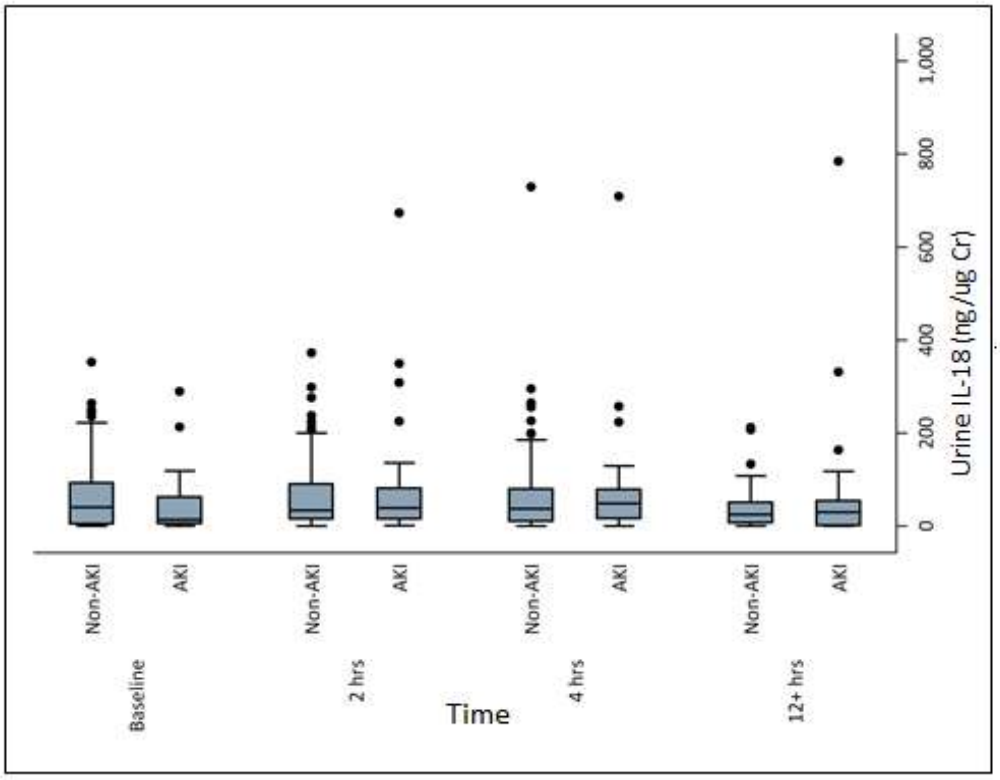


The respective C statistics for serum IL-18 at baseline, 2 h, 4 h and 12-24 h are: 0.624, 0.612, 0.533 and 0.535.

6.2.3 Urine IL-18 results

The box-plot results for urine IL-18 concentrations at the different time-points are summarized in Figure 28.

Figure 28: Box-plot of urine IL-18 results at baseline, 2 h, 4 h and 12-24 h showing development of AKI versus no AKI.



(units = ng/µg Cr– results are corrected for urine creatinine excretion

When we looked at absolute values in the AKI group there was no significant change from baseline at any time-point. However when we looked at percentage rise from

baseline within these patients, a significant rise was seen at 2 h ($p < 0.001$), and this persisted at 4 h ($p = 0.02$) and 12-24 h ($p = 0.049$). There was no significant change in urine IL-18 concentrations in the whole study population over 24 hours. The odds ratio data for urine IL-18 are summarised in Table 35.

Table 35: Odds ratio for urine IL-18 and risk of AKI in the study population

Biomarker	n	OR	95% CI	p value	
Baseline IL-18 urine	200	0.63	0.33	1.21	0.164
IL-18 urine 2 h	195	0.98	0.87	1.12	0.807
IL-18 urine 4 h	182	0.97	0.84	1.12	0.684
IL-18 urine 12-24 h	80	1.01	0.84	1.21	0.932

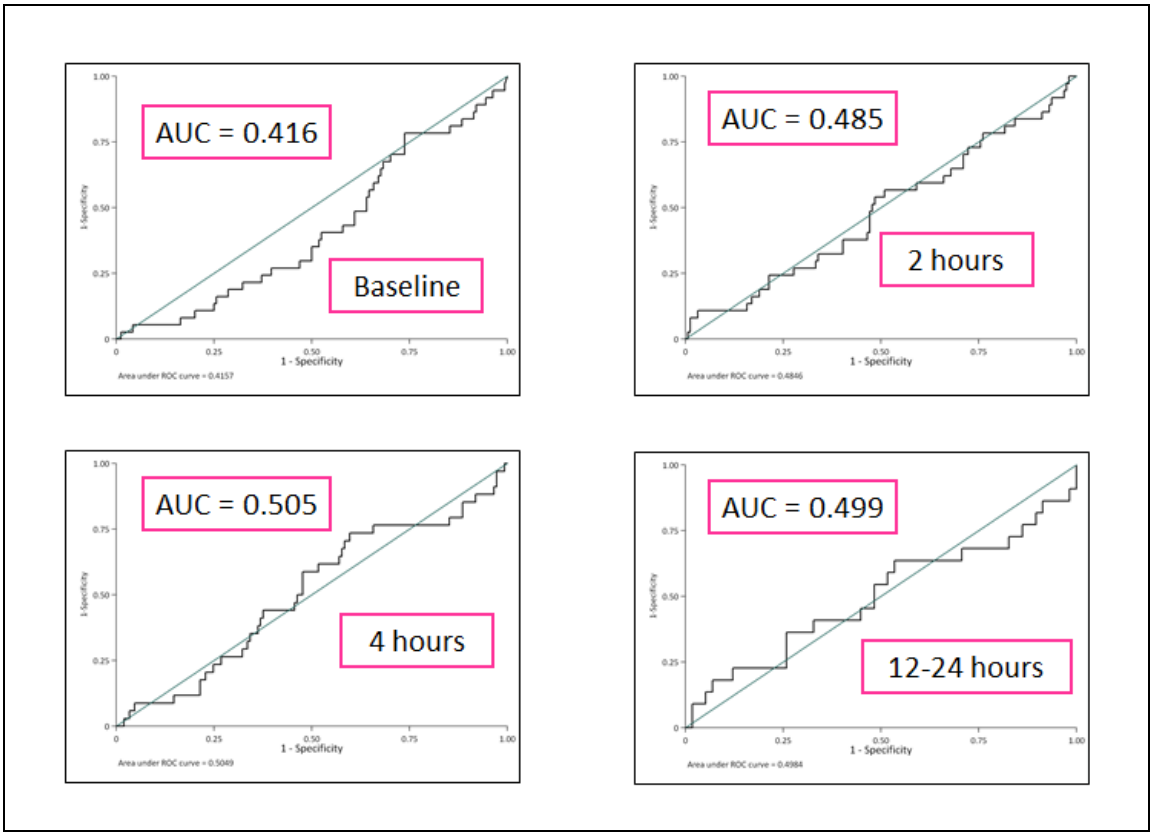
Absolute and percentage changes in urine IL-18 from baseline are summarised in Table 36.

Table 36: Absolute and Percentage Changes from Baseline in Urine IL-18

Table 36a) Urine IL-18 in AKI patients				
Baseline Median (IQR)	**2 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
14.3 (6.1, 64.0)	39.0 (15.8, 82.2)	0.116	119.4 (-17.5, 387.5)	<0.001
Baseline Median (IQR)	**4 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
14.3 (6.1, 64.0)	48.8 (17.0, 80.4)	0.278	24.5 (-31.8, 394.6)	0.02
Baseline Median (IQR)	**12-24 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
14.3 (6.1, 64.0)	29.6 (2.2, 55.6)	0.277	28.9 (-24.3, 222.1)	0.049
Table 36b) Urine IL-18 in non-AKI patients				
Baseline Median (IQR)	**2 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
41.7 (5.1, 95.0)	34.9 (16.0, 92.2)	0.081	19.5 (-28.6, 221.0)	<0.001
Baseline Median (IQR)	**4 hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
41.7 (5.1, 95.0)	37.9 (11.2, 85.3)	0.233	20.7 (-28.3, 175.2)	<0.001
Baseline Median (IQR)	**12+ hours Median (IQR)**	**p value**	**% Change Median (IQR)**	**p value**
41.7 (5.1, 95.0)	25.7 (7.7, 56.3)	0.156	-21.9 (-57.4, 115.7)	0.425

Receiver Operating Characteristic Curves were constructed to assess how discriminant urine IL-18 concentrations at different time-points are for the development of AKI. The results are summarised in Figure 29.

Figure 29: Receiver Operating Characteristic Curves for urine IL-18



The respective C statistics for urine IL-18 at baseline, 2 h, 4 h and 12-24 h are: 0.416, 0.485, 0.505 and 0.498.

6.3 Exploratory analysis

We had found that the Mehran risk score had a C statistic = 0.69 in our study population for predicting the development of AKI. We therefore aimed to determine if a combination of our biomarkers could improve this further. An exploratory analysis was performed

looking at different % rises in our biomarkers at different time points and calculating an odds ratio for development of contrast induced AKI. The marker time –points with the highest odds ratios are shown in Table 37.

Table 37: Exploratory analysis for % rises in biomarkers

Variable	OR	95% CI		p value
Serum NGAL 4 h 5% change	2.60	1.11	6.11	0.028
IL-18 urine 2 h 20% change	2.21	1.01	4.82	0.046
IL-18 urine 2 h 30% change	2.10	0.98	4.51	0.058
IL-18 urine 12 h 10% change	2.41	0.87	6.64	0.09
Serum NGAL 4 h 20% change	1.88	0.89	3.96	0.097
IL-18 urine 2h 5% change	1.90	0.86	4.23	0.114
IL-18 urine 12 h 5% change	2.24	0.81	6.18	0.119
IL-18 urine 2 h 10% change	1.75	0.80	3.82	0.16
Serum NGAL 4 h 10% change	1.70	0.79	3.66	0.179
Serum NGAL 2 h 10% change	1.62	0.79	3.34	0.188
Urine NGAL 2 h 20% change	1.68	0.76	3.72	0.201

We then combined these combinations with the Mehran risk score to see if it improved the C statistic further and the results are shown in Table 38.

Table 38: Improvement in C statistic on combining The Mehran risk score and our biomarker profiles.

	N	AUC	95% CI	
Mehran risk score only	207	0.65	0.60	0.78
IL-18 urine 2h 5% & urine NGAL 2h 20% + Mehran risk score	181	0.76	0.67	0.85
IL-18 urine 2h 20% & urine NGAL 2h 20% + Mehran risk score	181	0.77	0.68	0.86
IL-18 urine 12h 5% & serum NGAL 4h 10% + Mehran risk score	74	0.74	0.59	0.88
IL-18 urine 12h 5% & serum NGAL 4h 20% + Mehran risk score	74	0.74	0.60	0.88

Next we added in albumin : creatinine ratio and protein : creatinine ratio data in addition to our biomarker results and the Mehran risk score and this resulted in a further improvement in the C statistic (Table 39).

Table 39: Further improvement in C statistic on combining The Mehran risk score and our biomarker profiles.

Biomarkers	N	AUC	95% CI	
IL-18 urine 2h 5% & NGAL urine 2h 20% + Mehran risk score plus albumin:creatinine ratio & protein: creatinine ratio	180	0.79	0.70	0.87
IL-18 urine 2h 20% & NGAL urine 2h 20% + CIN score plus albumin:creatinine ratio & protein: creatinine ratio	180	0.79	0.71	0.87

The Clinical SYNTAX Score had a C statistic of 0.64 for predicting contrast induced AKI in our study population. The results of a similar exploratory analysis combining the CSS with our biomarker data are shown in Table 40.

Table 40: The Clinical Syntax Score combined with biomarker data

Biomarker	N	AUC	95% CI	
Clinical SYNTAX score	136	0.64	0.51	0.78
IL-18 urine 2h 5% & NGAL urine 2h 20% + CSS score plus albumin:creatinine and protein: creatinine ratio	117	0.76	0.65	0.88
IL-18 urine 2h 20% & NGAL urine 2h 20% + CSS score plus albumin:creatinine and protein: creatinine ratio	117	0.78	0.66	0.89

The addition of the CSS to the biomarker data resulted in further modest improvement in the C statistic.

6.4 Discussion

The current Western World epidemics of obesity, diabetes and hypertension are key players in the epidemic of combined CKD and cardiovascular disease (1;2;4;498). The marked increase in cardiovascular mortality in patients with CKD is often attributed to an increased burden of traditional cardiac risk factors such as diabetes, hypertension, smoking and dyslipidaemia (105;118). However, patients with CKD have higher rates of cardiovascular morbidity and mortality than are predicted by the Framingham model of cardiovascular risk (116;117). Other cardiovascular risk factors that have been identified to occur more frequently in patients with CKD than in the general population include anaemia, abnormal metabolism of calcium and phosphorous, inflammation and oxidative stress (103;107). Patients with diabetes and CKD are a particular challenge as they have a higher incidence of cardiovascular co-morbidities such as CHD and PVD than any other patient group with CKD (128;129). There is therefore frequently a need for patients with diabetes and CKD to undergo coronary angiography and PCI procedures. These patients are at high risk for developing contrast induced AKI as when renal function is already impaired, the kidney is much less able to tolerate the additional stress of excreting a contrast load (281).

It would be extremely useful to identify those patients most at risk early, to allow possible therapeutic interventions to be instituted promptly. Possible interventions include more intensive i.v. hydration regimes, use of vasodilators such as dopamine, fenoldopam, endothelin receptor antagonists and aminophylline (329). To date such attempts have been limited as the current ‘gold standard’ biomarker serum creatinine does not start to rise until 48-72 hours post the injury event (265). Additionally,

serum creatinine levels are influenced by a number of other factors including age, muscle mass, hydration and nutritional status (383). Furthermore a rise in serum creatinine may not be clinically detectable until almost 50% of renal function has been lost. Serum creatinine concentrations are not a reflection of GFR change in the acute setting as it takes a period of hours-days to elapse before equilibrium is achieved between steady-state production and decreased creatinine excretion (380). A biomarker of AKI that is easily measured, unaffected by other biological variables and capable of both early detection and possibly also risk stratification would therefore be a major clinical advance in improving the care of these patients.

A review of the literature was performed to identify novel biomarkers that might meet these parameters as discussed in sections 1.8 and 1.9. NGAL, IL-18 which are markers of structural injury and cystatin C which is a marker of functional renal injury were eventually selected. The aim was to identify a 'renal injury risk panel' in a similar manner to the well-established panel for identifying myocardial injury of creatinine kinase, creatinine kinase-MB and troponin.

These 3 renal injury markers were chosen for a number of reasons. The available data suggested that all these markers rise rapidly following renal injury and therefore might facilitate earlier identification of patients at risk prior to hospital discharge, rather than having to bring patients back 48 - 72 hours following the procedure as is current practice. NGAL has been shown to rise within 2 hours of renal injury, interleukin-18 within 4 hours and cystatin C within 8 - 24 hours (401). NGAL has been evaluated as a marker of AKI in a wide number of clinical settings including both children and adults post cardiac surgery, in the critical care unit and post contrast administration with

high accuracy (C statistic 0.78 – 0.99) (401;499;500). However there is very little data post contrast administration in patients with CKD (501). Interleukin-18 has been shown to be more specific for ischaemic AKI and other forms of acute tubular necrosis and does not appear to be affected by pre-renal azotaemia or CKD (380;406;407). From review of the medical literature it would appear that IL-18 had not been formally evaluated post contrast administration at the time the study commenced enrolment. Cystatin C has demonstrated excellent accuracy for predicting AKI early, prior to a clinical diagnosis being established (sensitivity 0.82, specificity 0.95), but the majority of studies have not reported C statistic data (401). Furthermore for both NGAL and cystatin C large scale immunoassays which can be performed in clinical laboratories have been developed, potentially allowing rapid translation of research methodologies into clinical practice (444;502).

208 patients with diabetes and CKD undergoing coronary angiography or PCI at The London Chest Hospital between 14th August 2009 and 26th July 2010 were recruited. 39 patients developed AKI at day 3. One patient required haemodynamic support with inotropes but no patients required renal replacement therapy in the form of dialysis or filtration.

ROC curve analysis does not suggest that either NGAL or IL-18 are particularly beneficial markers in isolation, for identifying contrast induced AKI in a population with diabetes and CKD as our best C statistic for accurately detecting AKI was from serum NGAL 0.634 on a serum sample 2 h post procedure. Although a number of studies have reported on both serum and urine NGAL as a biomarker post contrast administration with higher accuracy (up to a C statistic of 0.92), these have been in very

homogeneous populations such as children undergoing coronary angiography (420;503). More heterogeneous populations similar to that described in this thesis have been evaluated such as adults undergoing cardiac surgery. Wagener *et al* showed that urine NGAL had a sensitivity of 0.68 and specificity of 0.69 in identifying ischaemic renal injury in patients post bypass surgery (400). Nickolas *et al* reported that in an adult population presenting to the emergency department, urine NGAL had a sensitivity of 0.87 and specificity of 0.97 for identifying development of AKI (504). I was unable to identify any other studies in the medical literature reporting a C statistic for IL-18 post contrast administration.

Very few studies in the literature have evaluated NGAL as a marker post contrast induced AKI in adult populations. These have all been very small studies and 3 of the 4 come from a single research group in Poland. Of the Polish studies there are some methodological limitations and these are described below.

The first study of 35 patients undergoing elective PCI excluded patients with CKD but included 12 patients with diabetes (505). Serum and urine NGAL were measured at baseline, 2 hours, 4 hours, 12 hours, 24 hours and 48 hours following PCI. The authors reported a significant rise in serum NGAL 2 hours and 4 hours following PCI and in urine NGAL 4 hours and 12 hours following PCI. They also found that baseline serum NGAL levels were significantly higher in patients with diabetes than in non-diabetics. However none of the patients in this study developed contrast induced AKI, that is to say the rise in NGAL concentrations was not associated with a rise in creatinine. The study used 2 different contrast agents, one a LOCM (n = 28) and one HOCM (n =7) with no explanation given and the actual agents used were not identified.

The second study (n = 92), was described as evaluating NGAL in a population with CKD (501) although diabetic patients were excluded. However 52 of the patients included had an eGFR ≥ 60 ml/min, with only 15 patients included with an eGFR < 30 ml/min. The authors showed a relationship between baseline NGAL levels and serum creatinine concentrations. The patients included were all due to undergo coronary angiography but no procedural data are given and no data on rates of contrast induced AKI.

The third study (n = 140) included patients with diabetes (n = 70) but excluded patients with CKD (414). 2 different contrast agents were used in this study IOCM, iodixanol in 24 patients and LOCM, Iopromide in 116 patients. There are also conflicting data about the cessation of nephrotoxic drugs. It is stated early in the manuscript that all nephrotoxic medications were discontinued for 1 week prior to the procedure. Subsequently the authors state that agents such as metformin, diuretics and NSAIDs were discontinued 24 h prior to procedure. ACE inhibitors were either withdrawn at this time (if blood pressure permitted) or dosage halved. All patients received 2 litres of i.v. hydration in the peri-procedure period although the exact regime is not specified. The authors defined contrast induced AKI as a rise in serum creatinine of 25% from baseline and reported prevalence of 10% in non-diabetics and 14% in diabetics. Serum NGAL levels correlated with baseline creatinine and eGFR and were significantly elevated 2 hours following contrast administration in patients who developed AKI. Urine NGAL was significantly higher in patients who developed AKI 4 hours following the procedure. The authors also evaluated a number of other biomarkers including serum cystatin C and serum and urine IL-18. A significant rise in serum cystatin C was seen at 8 hours and 24 hours following the procedure. IL-18 apparently followed a similar pattern to cystatin C although no data were given for separate serum and urine values. These data are

difficult to interpret as actual p values are not given and no data on how the biomarkers performed compared to creatinine are provided.

A final study from China has evaluated urine NGAL and IL-18 as markers of contrast induced AKI following coronary angiography procedures. 40 patients, of whom approximately 15% had diabetes were included in this study, all received LOCM, Iopamidol. A rate of contrast induced AKI (defined as a rise in serum creatinine of > 25% from baseline or an absolute rise of 44.2 μmol/l) of 8.7% was reported. The mean baseline eGFR was 91.8 ml/min in the patients who developed AKI compared to 113.8 ml/min in the patients who did not develop AKI. For coronary angiography the mean contrast dosage was high at 155.4 ml and 111.9 ml respectively. Urine IL-18 and NGAL levels were measured 24 hours post procedure and were shown to be significantly increased in the patients who developed AKI ($p < 0.05$). The C statistic for urine IL-18 was 0.749 and for urine NGAL 0.734, demonstrating good performance in diagnosing AKI at 24 hours post procedure. Patients were followed up for at least 17 months and assessed for major adverse cardiac events (MACE) including death and MI. Urine IL-18 was shown to be an independent predictor of late MACE, relative risk = 2.09 ($p < 0.01$).

The value of these markers probably best lies as part of a ‘renal injury risk panel’. From exploratory analysis we identified the highest odds ratios for predicting AKI and by combining with the Mehran risk score we were able to further improve the C statistic to 0.77. Combining these data with urine albumin : creatinine ratio and protein : creatinine ratio improved this further to 0.79. However it could be argued that the Mehran risk score alone has reasonable discriminatory ability to identify patients with AKI in the study population with a C statistic of 0.69 and therefore are our biomarkers truly

adding benefit? One of the limitations of ROC analysis is that even biomarkers which can be shown to be effective, as independent predictors of an outcome when using logistic regression may struggle to show much increase in the AUC or C statistic of the ROC curve when the AUC for the previously used measurement was already high. This is discussed in more detail in the next section along with suggestions for further work.

Limitations of the Study and Further Work

There are some important limitations of NGAL as a biomarker of renal injury in our study population. NGAL has been shown to be expressed in atherosclerotic plaques (506) which may influence serum measurements and possibly be a confounder in patients with coronary artery disease. Furthermore serum measurements may be influenced by a number of other variables including chronic hypertension, CKD and anaemia (402;444;501;507). As described above serum creatinine concentrations correlate with NGAL levels and therefore CKD may act as a confounding variable. Finally defining AKI based on serum creatinine has limitations as it sets the biomarker assay for lack of accuracy due to either false positives (true tubular injury but no change in serum creatinine) or false negatives (absence of tubular injury but elevation in serum creatinine caused by e.g. pre-renal effects).

Patients undergoing coronary angiography and PCI are not usually catheterised (unlike patients post CABG). There are therefore problems with the accuracy of timing of urine samples. Furthermore hydration status and use of medications such as diuretics may also influence biomarker concentration. Although we attempted to correct for the possibility of urine dilution by reporting urine NGAL and IL-18 samples adjusted for

urine creatinine concentration, this may be inaccurate in the AKI setting due to the abnormal creatinine kinetics known to occur in the early stages of AKI (508).

The use of ROC curves and logistic regression is well established when testing biomarkers for their relationship with disease status and clinical outcomes (509). However, there are limitations to this approach, especially when attempting to use a new biomarker measurement to complement an established diagnostic test (510).

One of these limitations is that even biomarkers which can be shown to be an effective, independent predictor of an outcome when using logistic regression may not show much increase in the AUC or C statistic of the ROC curve when the AUC for the previously used measurement was already high. In many ways this is not a surprising result, since a predictive model which performs well will generally be harder to improve upon (511).

Given this, other approaches have been suggested for determining how well a predictive model performs (510;511). One method is to check the calibration of the model with regards to the predicted and observed rates of disease or events. After running a logistic regression model, the predicted probability of the outcome can be calculated for each individual, based on the coefficients from the model and the observed values of those variables for the individual. A predicted probability of 0 would mean the logistic model believed that individual had no chance of experiencing the outcome event, whilst a probability of 1 would mean they were thought to be certain to experience it. To check the model calibration one can then compare the observed and predicted event rates in different subgroups of our population. A common method is to split the population up into quartiles or quintiles, based on predicted risk, and then compare the level of

predicted risk in this group with the observed event rate. If the two values are similar in each subgroup then the model can be thought to be well calibrated (509).

Another method that can be used are reclassification tables and the net reclassification improvement (NRI) (510;511). Using this technique, individuals are again put into groups based on their predicted probabilities, firstly using the existing diagnostic tool. The model is then rerun with the additional biomarker, with the grouping process repeated. It is then possible to calculate what percentage of individuals who did not have an event were reclassified into a lower risk group, and how many who did have an event had their risk group increased. The two percentages are then summed to give the NRI, with higher values indicating the new model was better as reclassifying individuals correctly. These methods are still relatively new, and not yet commonly seen in papers looking at risk prediction. However, they may present further opportunities for biomarker testing in this population in any future studies.

The risk model that was identified on ROC analysis, (namely combining the Mehran score with NGAL and IL-18 to predict development of AKI) will need to be re-evaluated in a separate cohort prospectively to support the conclusions I have made from the development phase. Time constraints did not allow this to be performed as part of the body of work presented in this thesis but this can be validated by other members of the department.

Due to time constraints on the study the cystatin C assays have not yet been performed. However it is intended that this work will be completed in our department in the next few months. Furthermore this work was planned as a long term programme of clinical research and the original ethics application covered a number of other biomarkers

discussed in section 1.9 including L-FABP, KIM-1 and NAG. Analysis of all of these was outside the scope of this PhD thesis. We now have a library with multiple aliquots of plasma, serum and urine for each patient, stored at -80 °C. As further putative biomarkers are identified in the medical literature we have an available library on which to assess their accuracy in patients with diabetes and CKD.

Chapter Seven

Discussion

Chapter 7: General Discussion

The Western World is in the middle of a worldwide epidemic of diabetes (1;2;512). Diabetes is one of the main risk factors for the development of coronary artery disease. Once coronary artery disease has developed these patients are then at high risk for developing ACS (17-19). From the Framingham study (10) it is well recognised that even after adjusting for other factors, diabetic patients have higher mortality rates and also a higher incidence of reinfarction in both the acute and post-infarct setting. Patients with diabetes and CKD are a particular challenge as they have a higher incidence of cardiovascular morbidities and mortalities than any other patient group and as a consequence of this, frequently need to undergo coronary angiography and PCI procedures (7;13;443). However when renal function is impaired by pathological processes, the kidney is far less able to tolerate the additional stress of excreting a contrast load (281), rendering these patients at particular risk for developing contrast induced AKI.

Contrast induced AKI has received increasing recognition in the last few years as a result of increased understanding of its pathogenesis, possible new innovations in prevention and recognition that development of contrast induced AKI is associated with long term adverse events (268;269;278;283). It is the third most common cause of hospital acquired renal insufficiency after impaired renal perfusion and use of nephrotoxic medications (266). Coronary angiography and PCI are associated with the

highest rates of contrast induced AKI out of all procedures utilising contrast media (266).

The definition of contrast induced AKI requires 3 necessary components (310): (i) an absolute or relative increase in serum creatinine compared to baseline values, (ii) a temporal relationship between the rise in serum creatinine and exposure to a contrast agent and (iii) exclusion of an alternative explanation for renal impairment. The definition most widely used in the literature was used in this study: that is a relative increase in serum creatinine of 25% or more from baseline or an absolute rise of 0.5 mg/dl (44.5 µmol/l) (273). As discussed in section 1.4.2, the use of different definitions of contrast induced AKI has led to difficulties in comparing the results of studies in the literature. The advent of new proposed classifications of AKI such as the RIFLE and AKIN criteria (242;243;513) may complicate this further, so it will be important that any future studies incorporate standardised definitions as well to facilitate comparison of data.

The increased incidence of adverse events following contrast induced AKI has primarily been derived from retrospective analyses of large databases or observational studies of patients who have undergone coronary angiography or PCI (268;278;281). 'A cause and effect' relationship cannot be derived from non-randomised studies. Patients with multiple cardiovascular risk factors prior to exposure to contrast media may be more likely to develop contrast induced AKI and independent of this may have more long term adverse events. The converse may also be true – that is to say the occurrence of contrast induced AKI may in some as yet undefined way alter the likelihood of adverse events. In other words, contrast induced AKI is on a pathophysiologic pathway that results in the development of adverse events (232).

Prospective randomised clinical trials allow the assessment of causal relationships. If there exists a causal association between long term adverse events and contrast induced AKI then a strategy that prevents AKI should reduce long term adverse events, as long as the strategy itself does not alter any other risk factors for those adverse events. In a randomised trial of 2 different treatments such as 2 different contrast agents or i.v. fluids vs. haemofiltration, the assumption is that the baseline risk factors for long term adverse events will be equally distributed between the 2 treatments being tested. Differences in the incidence of contrast induced AKI, if paralleled by differences in long term adverse events, would suggest that contrast induced AKI is part of a pathophysiologic pathway that leads to the development of those adverse events.

Marenzi *et al* assessed 2 different haemofiltration protocols compared to i.v. fluid therapy in 92 patients with CKD undergoing coronary angiography. They showed that a strategy of pre and post procedure haemofiltration was not only associated with a significantly lower rate of contrast induced AKI (3% vs. 40% in the control group, $p = 0.013$) but also significantly lower in-hospital mortality (0% vs. 20% in controls, $p = 0.03$). The possibility that contrast induced AKI is associated with increased cardiovascular and renal events is further supported by recent data from Solomon *et al* (232;291). 294 patients with moderate to severe CKD undergoing coronary angiography were randomised to 2 different contrast media: LOCM non-ionic Iopamidol or IOCM non-ionic iodixanol. Rates of long term adverse events were significantly higher in patients who developed AKI. Both these studies used a definition of AKI of an increase in serum creatinine > 25% from baseline. Further data from trials which are currently enrolling should provide further evidence (514).

It would be extremely useful to identify patients at high risk for the development of contrast induced AKI early, to allow possible therapeutic interventions to be initiated promptly. Possible interventions include more intensive i.v. hydration regimes, use of vasodilators such as dopamine, fenoldopam, endothelin receptor antagonists and aminophylline (329). Such attempts to date have been limited as the current 'gold standard' biomarker serum creatinine does not start to rise until 48-72 hours post the injury event (265). Furthermore serum creatinine concentrations are influenced by a number of other factors including age, muscle mass, hydration and nutritional status (383). Additionally a rise in serum creatinine may not be clinically detectable until almost 50% of renal function has been lost and serum creatinine concentration may not be a reflection of acute GFR change in the acute setting since a period of hours-days must elapse before equilibrium between steady-state production and decreased creatinine excretion is achieved (380). A biomarker of AKI that is easily measured, unaffected by other biological variables and capable of both early detection and also risk stratification would therefore be a major clinical advance in improving the care of such patients. This concept led to the development of the study described in this thesis assessing novel biomarkers in patients with diabetes and CKD. This population was chosen because as discussed in section 1.6.4 they are an extremely high risk population for the development of contrast induced AKI (286).

The rationale for the choice of biomarkers has already been described in sections 1.9 and 6.4. Briefly, a literature review was performed to identify novel biomarkers that might meet the criteria described above. From this NGAL and interleukin-18 which are markers of structural renal injury and cystatin C which is a marker of functional renal injury were identified as promising candidates. These 3 markers were chosen for a

number of reasons. The available data at the time suggested that these markers all rose rapidly following renal injury and therefore might facilitate identification of patients at risk of contrast induced AKI prior to hospital discharge rather than having to bring back patients at 48-72 hours following the procedure as is current practice. NGAL had been shown to rise within 2 hours of renal injury, interleukin-18 within 4 hours and cystatin C within 8-24 hours (401). NGAL has been evaluated as a marker of kidney injury in a wide number of clinical settings including post cardiac surgery, critical care and post contrast administration and has been shown to have high accuracy for detecting AKI (383;499;500). The data post contrast administration have involved very small studies with some methodological concerns (414;420;501;505). Interleukin-18 had been shown to be more specific for ischaemic AKI and other forms of acute tubular necrosis and did not appear to be affected by pre-renal azotaemia or CKD (245;380;515). 2 small studies had evaluated IL-18 post contrast administration with some promising data although not in a population with CKD (414;420). Cystatin C had demonstrated excellent accuracy for predicting AKI early prior to a clinical diagnosis being established (409). Interpretation as to its accuracy as a biomarker of AKI was limited as the majority of studies had not reported c statistic data(401). The availability of large scale immunoassays for NGAL and cystatin C meant that the potential existed for rapid translation of research methodologies into clinical practice (444;502).

Chapter 3 described the baseline demographics and procedural data of the population studied in detail. 208 patients with diabetes and CKD undergoing coronary angiography or PCI at The London Chest Hospital were recruited between 14th August 2009 and 26th July 2010. 39 patients (18.8%) developed AKI. One patient required haemodynamic support with inotropes but no patients required renal replacement therapy in the form

of dialysis or filtration. The study included a high proportion of patients of South Asian origin reflective of the demographics of the local population. These patients are known to be at high risk for development of diabetes, CKD and CHD with these conditions often co-existing in the same patients (433-436).

Rates of use of ACE inhibitors and AIIRBs was high in the study population (Table 19), as would be expected. At the time of the study the hospital guidelines suggested that continuation of these agents should be considered on a case by case basis (331) (see Appendix). A detailed discussion of the available literature was performed in section 3.8 and overall would appear to favour cessation of these agents at least 2 days prior to procedures involving the use of iodinated contrast media (315;460-464) . However it is recognised that the majority of these data came from small case series and post hoc analyses and therefore there is a need for large scale randomised clinical trials to address this definitively.

Both symptomatic (NYHA Class, section 3.2) and objective data from imaging (section 3.5) showed that there was a higher proportion of patients with moderate to severe LV dysfunction who developed AKI. Whilst not unexpected this highlighted parts of clinical practice that might need to be reviewed such as usage of ACE inhibitors/AIIRBs at time of procedure and also the ‘routine’ performance of LV grams in patients at high risk for contrast induced AKI. These have been incorporated into my recommendations as to the optimal management of these patients when undergoing coronary angiography or PCI described later in this chapter.

The study provided further supporting evidence that co-prescription of diuretics with ACE inhibitors or AIIRBs in patients with renal artery stenosis should be avoided.

Although the data were limited as no physiological assessment of lesion severity was performed, it did provide further data supporting the discontinuation of ACE inhibitors/AIIRBs prior to procedure. It also identified 7 patients with moderate to severe renal artery stenosis who were referred to our nephrology colleagues for further assessment and optimisation.

Although our ROC curve analysis did not suggest that either NGAL or IL-18 are particularly accurate markers in isolation for predicting AKI (Chapter 6), it was extremely interesting to see how well simple scoring systems can work in identifying patients at high risk for contrast induced AKI. The Mehran risk score described in 1.6.4.7 is a simple model that can be easily calculated at the time of procedure and had a good accuracy in identifying high risk patients with a C statistic of 0.69 in our study population. Advantages of this scoring system are that it takes minutes to perform and does not require any specialist training. It can also be adapted to allow use in the pre-admission setting by either using a fixed 'average' volume of contrast such as 100 ml for a coronary angiogram and 200 ml for a PCI; or alternatively estimating the volume of contrast used from the baseline GFR as described in section 1.6.4.4 since it has been suggested that these patients should not have a contrast volume used in their procedure that is greater than twice the value of the baseline eGFR (307). The Clinical SYNTAX Score performed similarly well in identifying patients at high risk for contrast induced AKI with a C statistic of 0.64. This has not been reported in the literature previously and although this scoring system is more complex and time consuming, with definite training required and intra-observer variability (as described in section 4.4.3), it provides additional useful information on the complexity of a patients coronary artery disease.

Whilst the CSS may be limited in clinical practice due to the problems outlined previously it will remain a useful research tool for risk - stratifying patients.

Possible amendments to the current hospital guidelines for patients at risk of contrast induced acute kidney injury

From the new data available in the literature and from the work described in this thesis I have identified a number of areas to be considered for inclusion at the next review of the hospital guidelines for prevention of contrast induced kidney injury and these are listed below:

1. i.v. hydration and oral N-acetylcysteine to be prescribed to all patients with eGFR < 60 ml/min. i.v. hydration with 0.9% sodium chloride for a minimum of 2 hours prior to procedure. Careful evaluation of oral intake and urine output post procedure with a low threshold for further post-procedure hydration. N-acetylcysteine 600 mg po bd for 24 hours prior and 24 hours post procedure.

2. Nephrotoxic medications such as ACE inhibitors, AIIRBs and diuretics to be discontinued at least 48 hours prior to procedure. Metformin can be discontinued at the same time point. If necessary for blood pressure control, consider increasing other medication dosage temporarily or prescribing an additional medication such as a calcium channel blocker, doxazosin or hydralazine. Medications to be restarted following a satisfactory day 3 serum creatinine level.

3. The Mehran risk score to be calculated at pre-admission for elective patients or included in the appropriate pro-formas for hospital transfer/unstable patients.

High risk patients (Mehran risk score ≥ 16) to have a minimum of 6 h i.v. hydration and case to be performed by a senior operator.

4. LV gram to be avoided in these patients unless absolutely necessary. Reason for performing the LV gram to be stated in the report / included in the cardiac catheter lab database.

Limitations of the Study and Further Work

Limitations of the study and suggestions for further work have been described in detail at the end of each results chapter (Chapter 3, Chapter 4 and Chapter 6). A few important points are mentioned here for completeness.

This was a single-centre study in a highly selected population (diabetes and CKD) undergoing coronary angiography and PCI. All procedures were performed in a tertiary referral unit at a teaching hospital and therefore there may have been an element of selection bias with a tendency to more complex cases being referred from peripheral hospitals. The study excluded patients with STEMI. To be truly reflective of real-world clinical practice it would be important for these patients to be included in further studies.

A pragmatic approach to i.v. hydration has been taken with the current guidelines in order to ensure efficient use of both hospital beds and laboratory time and work flow. If the suggested recommendations to the current renal guidelines were incorporated it would be interesting to audit the average length of stay of patients. It may be that by risk stratifying the patients, with those at most high risk having more intensive i.v. hydration with possibly pre-admission the night before that this significantly decreases the

rates of contrast induced kidney injury. This may be reflected in overall shorter admissions with lower rates of readmission.

Although all patients were invited back to The London Chest Hospital for a day 3 blood test, a number were not able to attend for logistical reasons and therefore their results were obtained from their local hospital or GP. This may have led to lack of correct identification of all cases of contrast induced AKI.

Patients in this study were only formally followed-up until Day 3 post procedure. Since contrast induced AKI has been shown to be associated with other adverse events, it would be extremely interesting to follow up these patients for a longer time period to further characterise this high risk population.

The Mehran risk score has a number of limitations including in particular the lack of any data on hydration (discussed in section 4.4.2). Other parameters that could be investigated for inclusion in further studies include the presence of proteinuria, urine output and nephrotoxic medications.

The NGAL and Interleukin-18 biomarker data were perhaps disappointing given the interest there has been in these in the medical literature. However if markers cannot identify accurately high risk patients then their usage in clinical practice is going to be severely limited. It may be that combination of these tubular markers with a marker of glomerular injury such as cystatin C may improve the diagnostic performance. It is intended that the cystatin C assays will be completed in the department in the next few months. Finally the model developed in the work presented in this thesis for predicting AKI (urine NGAL, IL-18 and the Mehran risk score) requires prospective validation in

a separate cohort. From the work in this thesis a library with multiple aliquots of plasma, serum and urine has been collated and stored at -80 °C for each patient. As further putative biomarkers are identified in the medical literature this will facilitate rapid assessment of their accuracy in a cohort of patients with diabetes and CKD.

Appendix

Appendix contents

NYHA Classification

The New York Heart Association (NYHA) Functional Classification provides a simple means of classifying the extent of heart failure (516). It places patients into one of four categories based on the degree of limitation during physical activity; the limitations/symptoms are in regards to normal breathing and varying degrees in shortness of breath and or angina pain and are classified as follows:

NYHA I No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.

NYHA II Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.

NYHA III Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.

NYHA IV Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.

CCS Classification

The Canadian Cardiovascular Society (CCS) Angina grading scale (517) is commonly used to classify the severity of angina symptoms:

Class I – Angina only during strenuous or prolonged physical activity
Class II – Slight limitation, with angina only during vigorous physical activity
Class III – Symptoms with everyday living activities, i.e., moderate limitation

Class IV – Inability to perform any activity without angina or angina at rest, i.e., severe limitation

Haemoglobin A1c conversion

To convert from the well-known Diabetes Control and Complications Trials (DCCT) units of % to the International Federation of Clinical Chemistry (IFCC) units of mmol/mol use the following equation (438):

$$\text{IFCC-HbA}_{1c}\ (\text{mmol/mol}) = [\text{DCCT-HbA}_{1c}\ (\%) - 2.15] \times 10.929$$

For simplicity some common values are tabulated below:

DCCT HbA1c (%)	IFCC HbA1c (mmol/mol)
4	20
5	31
6	42
6.5	48
7	53
7.5	59
8	64
9	75
10	86

Cockcroft Gault Formula

This estimates Creatinine clearance (eC_{Cr}) in ml/min as follows:

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times Constant}{\text{Serum Creatinine (in } \mu\text{mol/L)}}$$

Where *Constant* is *1.23* for men and *1.04* for women (220)

Modification of Diet in Renal Disease (MDRD) Formula

The most commonly used formula is the '4-variable MDRD,' which estimates GFR using four variables: serum creatinine, age, race, and gender. This uses the following equation (creatinine µmol/l)(221):

eGFR = 32788 X Serum creatinine$^{-1.154}$ x Age$^{-0.203}$ x [1.212 if Black] x [0.742 if Female]

Barts and The London Heart Centre Guidelines for the Prevention of Contrast Nephropathy in Patients Undergoing Coronary Angiography or Angioplasty

The guidelines are included overleaf.

Barts and The London NHS
NHS Trust

Barts and the London Heart Centre

Guidelines for the Prevention of Contrast Nephropathy in Patients Undergoing Coronary Angiography or Angioplasty

Charles Knight
August 2005

Authors:
Charles Knight
Matthew Ginks
Magdi Yaqoob
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Introduction

Patients undergoing exposure to intravenous contrast are at risk of developing contrast nephropathy. Patients with contrast nephropathy have an adverse prognosis and in those that go on to require dialysis the prognosis is very poor. The main risk factors for contrast nephropathy are:

- Renal impairment (creatinine clearance <50ml/min)
- Dose of intravenous contrast
- Diabetes mellitus + renal impairment (diabetes with normal renal function is not a risk)
- Age >70 years
- Co-administration of nephrotoxic drugs
- Volume depletion

The principal risk factor is the degree of renal impairment. Patients with a creatinine clearance of >50 ml/min do not require special measures and can be treated with free clear oral fluids prior to the procedure. There is evidence that patients with creatinine clearance ≤ 50 ml/min benefit from specific measures to reduce the risk of contrast nephropathy. These are:

- Intravenous hydration therapy with normal saline or sodium bicarbonate (most protocols state 12 hours pre and post – shorter durations have not been studied)
- The use of iso-osmolar contrast agents
- N Acetyl cysteine (evidence of benefit is weaker and controversy surrounds its use, but it is well tolerated and non-toxic)

The evidence base for all of these measures is not of the highest quality and we have interpreted the evidence, in collaboration with our renal colleagues, to maximise both patient protection and operational effectiveness of the unit.

Iso-osmolar contrast agents should be used in all patients

Before Procedure

The creatinine clearance for all patients undergoing angiography or angioplasty must be known

This will normally be provided direct from the biochemistry laboratory. If it is not, then it can be calculated using the Cockcroft-Gault formula (see appendix 1). If for any reason only the serum creatinine is available then a threshold for treatment of 120 can be used (this is NOT ideal and the degree of renal impairment may be underestimated in, for example, small elderly females with apparently 'normal' serum creatinine).

n.b. Estimation of renal function is not necessary prior to true emergency procedures such as primary PCI for acute STEMI

Patients with Creatinine Clearance > 50ml/min

- No special measures. Free clear fluids until procedure.

Patients with Creatinine Clearance ≤ 50ml/min

- If the patient is under the care of a renal physician then they should be consulted for advice before the procedure if possible

- Stop potentially nephrotoxic drugs if possible (e.g. NSAID's, ACE-inhibitors)

- IV fluid hydration

  1 litre of 0.9% sodium chloride starting at least 2 hours prior to the procedure, over an 8 hour period. In addition, free access to clear oral fluids should be available before and after the procedure.

- Oral N-Acetylcysteine

  600mg orally twice daily for a total of 4 doses. The first dose should be taken at least 2 hours before the procedure. N-Acetylcysteine (NAC) should be administered orally as sachets. These must be dissolved in water before administration and three sachets are needed for each 600mg dose (200mg per sachet). At the time of writing, NAC sachets are unlicensed in England and obtained as a "named patient" item, so the name of each patient receiving them must be recorded. Drug interactions, contraindications, precautions and adverse effects of NAC are listed in appendix 2.

During Procedure

Patients with Creatinine Clearance > 50ml/min

- No special measures.

Patients with Creatinine Clearance ≤ 50ml/min

- Use contralateral femoral artery if renal transplant in situ
- Consider carefully the need for left ventriculography
- Limit contrast dose as far as possible

After Procedure

Patients with Creatinine Clearance > 50ml/min

- No special measures.

Patients with Creatinine Clearance ≤ 50ml/min

- Continue intravenous hydration as above for a total of 8 hours
- Ensure N-Acetylcysteine is administered as above
- Arrange for repeat creatinine clearance (or serum creatinine) to be measured in 4 days and check result.
- Repeat administration of contrast agents should be delayed for at least 10 days unless required in an emergency.

Appendix 1 The Cockroft Gault formula

For Males:

$$\frac{1.23 \times (140 - \text{age}) \times \text{Weight (kg)}}{\text{Creatinine } (\mu\text{mol/l})}$$

For Females:

$$\frac{1.04 \times (140 - \text{age}) \times \text{Weight (kg)}}{\text{Creatinine } (\mu\text{mol/l})}$$

The creatinine clearance can be calculated easily on-line using the website www.nephron.com. Other formulas are also available which do not require the body weight (e.g. MDRD).

Appendix 2 – N-Acetylcysteine

Drug Interactions

- *Carbamazepine* - Decreased levels (rarely)
- *Glyceryl Trinitrate* - Risk of hypotension and increased temporal artery dilation.
- *Tetracycline* – NAC impairs absorption. Take at least 2 hours apart.
- *Antitussives* – Dangerous congestion of secretions may occur if cough reflex is impaired.

Contraindications and precautions

- NAC is contraindicated in patients with *hypersensitivity* or previous anaphylactic reaction to NAC.
- Use with caution in asthmatic patients.
- Use with caution in patients with a history of *peptic ulcereration* (because drug induced nausea and vomiting may increase risk of GI haemorrhage)
- Use with caution in *pregnancy and lactation*. For further information contact a specialist physician or Medicines Information on extension 14-7487/7489.
- In the case of patients with the hereditary metabolic disease phenylketonuria, it should be noted that NAC sachets contain aspartame (25mg in each sachet – equivalent to 14mg phenylalanine).
- Patients who suffer from the rare hereditary fructose intolerance should not take this medicine, as it contains sorbitol, and sorbitol is converted to fructose.
- When treating diabetic patients, it should be noted that about 0.7g carbohydrate is supplied as sorbitol per sachet of granules.

Possible Adverse Effects

- Nausea, vomiting and other GI symptoms.
- Hypersensitivity reactions including bronchospasm, pruritis, rashes, angioedema, hypertension and hypotension
- Rarely:
  - Flushing
  - Sweating
  - Liver function disturbances
  - Syncope
  - Blurred vision
  - Seizures
  - Tachycardia
  - Mild fever
  - Arthralgia
  - Acidosis
  - Cardiac or respiratory
  - arrest
  - Generalised urticaria
  - Stomatitis
  - Tinnitus

Corrected Calcium

The amount of total calcium varies with the level of serum albumin, a protein to which calcium binds. The biologic effect of calcium is determined by the amount of *ionized calcium*, rather than the total calcium. When albumin levels are abnormal a corrected calcium can be derived:

Corrected calcium (mmol/L) = measured total calcium (mmol/L) + 0.02 (40 - serum albumin [g/L]),

where 40 represents the average albumin level in g/L

i.e. each 1 g/L decrease of albumin, will decrease 0.02 mmol/L in measured serum calcium and thus 0.02 must be added to the measured value to take this into account and get a corrected calcium. When there is hypoalbuminaemia (a lower than normal albumin), the corrected calcium level is higher than the total calcium. Most clinical laboratories in the United Kingdom report the corrected value alongside the serum calcium automatically.

Syntax Score Algorithm Definitions

Definitions:

Dominance: a) Right dominance: the posterior descending coronary artery is a branch of the right coronary artery (segment 4). b) Left dominance: the posterior descending artery is a branch of the left coronary artery (segment 15). Co-dominance does not exist as an option at the SYNTAX score.

Total occlusion: TIMI 0 flow: no perfusion; no antegrade flow beyond the point of occlusion

Bridging collaterals: Small channels running in parallel to the vessel and connecting proximal vessel to distal and being responsible for the ipsilateral collateralization

Trifurcation: A junction of three branches, one main vessel and two side-branches. Trifurcations are only scored for the following segment junctions: 3/4/16/16a, 5/6/11/12, 11/12a/12b/13, 6/7/9/9a and 7/8/10/10a

Bifurcation: A junction of a main vessel and a side branch of at least 1.5mm in diameter. Bifurcations are only scored for the following segment junctions: 5/6/11, 6/7/9, 7/8/10, 11/13/12a, 13/14/14a, 3/4/16 and 13/14/15. Bifurcation lesions may involve one segment (types A, B and E), two segments (types C, F and G) or three segments (type D).

Aorto ostial: A lesion is classified as aorto-ostial when it is located immediately at the origin of the coronary vessels from the aorta (applies only to segments 1 and 5, or to 6 and 11 in case of double ostium of the LCA).

Severe tortuosity: One or more bends of 90° or more, or three or more bends of 45° to 90° proximal of the diseased segment.

Length >20mm: Estimation of the length of that portion of the stenosis that has ≥ 50% reduction in luminal diameter in the projection where the lesion appears to be the longest. (In case of a bifurcation lesion at least one of the branches has a lesion length of >20mm).

Heavy calcification: Multiple persisting opacifications of the coronary wall visible in more than one projection surrounding the complete lumen of the coronary artery at the site of the lesion.

Thrombus: Spheric, ovoid or irregular intraluminal filling defect or lucency surrounded on three sides by contrast medium seen just distal or within the coronary stenosis in multiple projections or a visible embolization of intraluminal material downstream.

Diffuse disease/small vessels: More than 75% of the length of the segment has a vessel diameter of 2mm, irrespective of the presence or absence of a lesion.

(Adapted from Sianos *et al* EuroIntervention 2005 (421)).

Patient CRF and Patient Information Sheet

These are included overleaf:

PATIENT DETAILS

1	Patient's name:	
2	Hospital / Unit number:	
3	Address: Street:	
4	City:	
5	Postal code:	
6	Country:	
7	Telephone number:	
8	NHS Number:	

DETAILS OF NEXT OF KIN

9	Relative or friend name:	
10	Address: Street:	
11	City:	
12	Postal code:	
13	Country:	
14	Telephone number:	

FOLLOW-UP SCHEDULE

15	Date of consent	__ __ / __ __ /__ __ __ __	*Done*
16	Date of procedure	__ __ / __ __ /__ __ __ __	[ ]
17	Date of planned 3 day follow-up	__ __ / __ __ /__ __ __ __	[ ]

Instructions, definitions and reminders:

opposite page: 2

♥① **Ethnicity**

1 Caucasian
2 South Asian
3 East Asian
4 Afro Caribbean
5 African
6 Other

♥② **Smoking history**

1. Current smoker (within past 3 months)
2. Ex-smoker (stopped smoking for greater than 3 months)
3. Never smoked

♥③ **History of Hypertension: -** Blood pressure > 130/85 untreated

♥④ **History of Hyperlipidaemia: -** Total cholesterol > 5.0 untreated

♥⑤ **History of Renal Failure: -** eGFR < 60 ml/min

Baseline Demographics and Medical history

1	Date of assessment (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
2	Date of birth (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
3	Gender	Male [ ] Female [ ]
4	Ethnicity (Enter code) ♥①	

DIABETES STATUS

5	Type of diabetes	Type 1 [ ] Type 2 [ ]
6	How is the diabetes treated (tick all that apply)	[ ] Insulin [ ] Oral hypoglycaemic medication [ ] Diet controlled
7	Date of diagnosis of diabetes (mm/yyyy)	__ __ /__ __ __ __
8	Diagnosis made on BM on arrival (AMI only)	Yes [ ] No [ ]
9	Smoking history ♥②	Current [ ] Ex [ ] Never [ ]
10	Family history of ischaemic heart disease	Yes [ ] No [ ]
11	History of hypertension ♥③	Yes [ ] No [ ]
12	If Yes, hypertension treated with medication?	Yes [ ] No [ ] NA [ ]
13	History of hyperlipidaemia ♥④	Yes [ ] No [ ]
14	If Yes, hyperlipidaemia treated?	Yes [ ] No [ ] NA [ ]
15	History of renal failure ♥⑤	Yes [ ] No [ ]
16	If Yes, on haemodialysis?	Yes [ ] No [ ] NA [ ]
17	History of cerebrovascular disease	Yes [ ] No [ ]
18	History of carotid artery disease	Yes [ ] No [ ]
19	History of peripheral vascular disease	Yes [ ] No [ ]
20	History of chronic lung disease	Yes [ ] No [ ]
21	Angina	Yes [ ] No [ ]
22	Previous Myocardial Infarction (mm/yyyy)	Yes [ ] __ __ /__ __ __ __ No [ ]
23	Valvular Heart Disease	Yes [ ] No [ ]

Instructions, definitions and reminders:

opposite page: 3

♥① Type of PCI procedure (enter code):

1. Elective
2. Urgent
3. Emergency

♥② CCS Class

0. No chest pain: No limitation of physical activity by pain.
I. Pain on moderate exertion: Ordinary physical activity, such as walking or climbing stairs does not cause angina. Pain with strenuous, rapid or prolonged exertion.
II. Pain limitation of normal daily activities: Comfortable at rest, but ordinary physical activity, such as walking rapidly or climbing stairs, exercise after meals, in wind or cold weather causes anginal pain.
III. Marked pain limitation of ordinary physical activity: Pain on walking on the level or climbing one flight of stairs.
IVa. Unstable: Pain on any activity or rest pain: Symptom deterioration now controlled on additional oral medical therapy.
IVb. Unstable: Pain on any activity or rest pain: Continued pain symptoms despite maximal oral medical therapy.
IVc. Unstable: Pain on any activity or rest pain: Continued pain symptoms despite IV therapy.

♥③ NYHA Class

I. No objective evidence of cardiovascular disease: Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnoea or anginal pain.
II. Objective evidence of minimal cardiovascular disease: Patients with cardiac disease resulting in slight limitation of physical activity. Comfortable at rest, ordinary physical activity results in fatigue, palpitation, dyspnoea or anginal pain.
III. Objective evidence of moderately severe cardiovascular disease: Patients with cardiac disease resulting in marked limitation of physical activity. Comfortable at rest, less than ordinary physical activity causes fatigue, palpitation, dyspnoea or anginal pain.
IV. Objective evidence of severe cardiovascular disease: Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

CLINICAL EXAMINATION AT BASELINE

1	Height (cm)	__ __ __
2	Weight (kg)	__ __ __ . __
3	Systolic blood pressure (mmHg)	__ __ __
4	Diastolic blood pressure (mmHg)	__ __ __
5	Pulse (bpm)	__ __ __
6	Temperature (°C)	__ __. __
7	Clinical evidence of heart failure	Yes [ ] No [ ]

MODE OF PRESENTATION

8	Type of PCI procedure ♥①	
9	Silent ischaemia	Yes [ ] No [ ]
10	Previous PCI	Yes [ ] No [ ]
11	Previous CABG	Yes [ ] No [ ]
12	CCS class ♥②	
13	NYHA class ♥③	

Notes

Instructions, definitions and reminders:

opposite page: 4

♥① **Medications at Baseline:** Please include all medication taken regularly by the patient prior to this admission to hospital

♥② **Heparin:** LMWH – Low molecular weight heparin; UFH – Unfractionated heparin

♥③ **Dihydropyridine calcium antagonists:** e.g.Nifedipine, Amlodopine

♥④ **Non - Dihydropyridine calcium antagonists:** e.g. Diltiazem, Verapamil

♥⑤ **Potassium channel activators:** e.g. Nicorandil

♥⑥ **Loop diuretics:** e.g.Furosemide, Bumetanide

♥⑦ **Thiazide diuretics:** e.g. Bendrofluazide, Metolazone

♥⑧ **Sulphonylureas:** e.g. Glibenclamide, Gliclazide, Tolbutamide

♥⑨ **Thiazolidinediones:** e.g. Pioglitazone, Rosiglitazone

MEDICATIONS AT BASELINE♥①

1	Aspirin	Yes [ ] No [ ]
2	Clopidogrel	Yes [ ] No [ ]
3	Warfarin	Yes [ ] No [ ]
4	Other anticoagulant, please specify	Yes [ ] _____ No [ ]
5	Heparin ♥②	LMWH [ ] UFH [ ] No [ ]
6	Beta-blockers	Yes [ ] No [ ]
7	Dihydropyridine calcium-channel antagonists ♥③	Yes [ ] No [ ]
8	Non – dihydropyridine calcium-channel antagonists ♥④	Yes [ ] No [ ]
9	Nitrates	Yes [ ] No [ ]
10	Potassium channel activators ♥⑤	Yes [ ] No [ ]
11	Lipid lowering agent (tick all that apply)	Statins [ ] Other [ ] No [ ]
12	ACE inhibitors	Yes [ ] No [ ]
13	Angiotensin-II antagonists	Yes [ ] No [ ]
14	Loop diuretics ♥⑥	Yes [ ] No [ ]
15	Thiazide diuretics ♥⑦	Yes [ ] No [ ]
16	Digoxin	Yes [ ] No [ ]
17	Amiodarone	Yes [ ] No [ ]
18	Thyroxine	Yes [ ] No [ ]
19	Metformin	Yes [ ] No [ ]
20	Sulphonylureas ♥⑧	Yes [ ] No [ ]
21	Thiazolidinediones ♥⑨	Yes [ ] No [ ]
22	Insulin	Yes [ ] No [ ]
23	Any other cardiovascular medication	NA [ ]
24	Any other diabetic medication	NA [ ]

Instructions, definitions and reminders:

opposite page: 5

BLOOD RESULTS AT BASELINE

1	Random glucose	Yes [ ]	__ __ . __ __mmol/L	NA [ ]
2	Fasting glucose	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
3	HbA1C (units %)	Yes [ ]	__ __ . __ __ %	NA [ ]
4	HbA1C (units mmol/mol)	Yes [ ]	__ __ . __ __ mmol/mol	NA [ ]
5	Haemoglobin	Yes [ ]	__ __ . __ g/dL	NA [ ]
6	White cell count	Yes [ ]	__ __ . __ x 10^9/L	NA [ ]
7	Platelets	Yes [ ]	__ __ __ . __ x 10^9/L	NA [ ]
8	INR	Yes [ ]	__ __ . __ __	NA [ ]
9	CRP	Yes [ ]	__ __ __ . __ __ mg/L	NA [ ]
10	Sodium	Yes [ ]	__ __ __ mmol/L	NA [ ]
11	Potassium	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
12	Urea	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
13	Creatinine	Yes [ ]	__ __ __µmol/L	NA [ ]
14	Corrected Calcium	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
15	Phosphate	Yes [ ]	__ . __ __ mmol/L	NA [ ]
16	Albumin	Yes [ ]	__ __ mmol/L	NA [ ]
17	ALP	Yes [ ]	__ __ __ Units	NA [ ]
18	ALT	Yes [ ]	__ __ __ Units	NA [ ]
19	Creatinine Kinase	Yes [ ]	__ __ __ __	NA [ ]
20	Troponin T	Yes [ ]	__ __ __	NA [ ]
21	Cholesterol	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
22	LDL	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
23	HDL	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
24	Triglyceride	Yes [ ]	__ . __ __ mmol/L	NA [ ]
25	Haematocrit	Yes [ ]	__ __ . __ %	NA [ ]
26	Screening Creatinine	Yes [ ]	__ __ __µmol/L	NA [ ]
27	Screening GFR	Yes [ ]	__ __. __ ml/min	NA [ ]

Instructions, definitions and reminders:

opposite page: 6

TIMING OF BASELINE SAMPLES

1	Date of sample (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
2	Time sample taken (hh:mm)	__ __ : __ __

URINE RESULTS AT BASELINE

16	Urine : albumin	Yes [ ]	__ __ __ . __mg/L	NA [ ]
3	Albumin : Creatinine ratio	Yes [ ]	__ __ . __ __mmol/L	NA [ ]
4	Urine : Creatinine	Yes [ ]	__ __ . __ __mmol/L	NA [ ]
5	Urine : Protein	Yes [ ]	__ __ . __ __g/L	NA [ ]
6	Protein : Creatinine ratio	Yes [ ]	__ __ . __ __mg/mmol	NA [ ]
7	eGFR	Yes [ ]	__ __ . __ __ml/min	NA [ ]

BIOMARKER RESULTS AT BASELINE

SERUM AND PLASMA RESULTS AT BASELINE

		SERUM	PLASMA
8	NGAL	Yes [ ] _ _ . _ _ NA [ ]	Yes [ ] _ _ . _ _ NA [ ]
9	Cystatin C	Yes [ ] _ _ . _ _ NA [ ]	Yes [ ] _ _ . _ _ NA [ ]

URINE BIOMARKERS AT BASELINE

10	NGAL	Yes [ ]	__ __ . __ _	NA [ ]
11	Cystatin C	Yes [ ]	__ __ . __ __	NA [ ]
12	KIM -1	Yes [ ]	__ __ . __ _	NA [ ]
13	Interleukin-18	Yes [ ]	__ __ . __ _	NA [ ]
14	NAG	Yes [ ]	__ __ . __ _	NA [ ]
15	FAB-P	Yes [ ]	__ __ . __ __	NA [ ]

Instructions, definitions and reminders:

opposite page: 7

♥① LV function

1 Normal
2 Mild Impairment
3 Moderate Impairment
4 Severe Impairment

♥② Appearance of renal arteries

1 No objective evidence of atherosclerotic disease
2 Mild atherosclerotic disease
3 Moderate atherosclerotic disease
4 Severe atherosclerotic disease

♥③ Type of contrast used

1 Visipaque
2 Ultravist
3 Other

PROCEDURE DETAILS

1	Date of procedure (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
2	AMI	Yes [ ] No [ ]
3	STEMI	Yes [ ] No [ ]
4	Staged procedure	Yes [ ] No [ ]
5	Point of access	Radial [ ] Femoral [ ]
6	LV gram performed	Yes [ ] No [ ]
7	Echo performed	Yes [ ] No [ ]
8	If Yes, date of echo (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
9	If Yes, Ejection fraction (%)	__ __
10	LV function ♥①	
11	Renal angiogram	Yes [ ] No [ ]
12	Appearance of renal arteries ♥②	
13	N-acetylcysteine pre-procedure	Yes [ ] No [ ]
14	Intravenous fluids pre-procedure	Yes [ ] No [ ]
15	Total procedure time (minutes)	__ __ __
16	Fluoro time (minutes)	__ __ __
17	Type of contrast used ♥③	
18	Volume of contrast used (ml)	__ __ __
19	IABP inserted	Yes [ ] No [ ]

RISK SCORING

20	SYNTAX Score	
21	CIN Score	
22	Risk CIN	__ __ %
23	Risk of haemodialysis	__ __ . __ __ %
24	Procedure performed	Angio [ ] PCI [ ] Both [ ]

Instructions, definitions and reminders:

opposite page: 8

♥① SYNTAX SCORE segment numbers

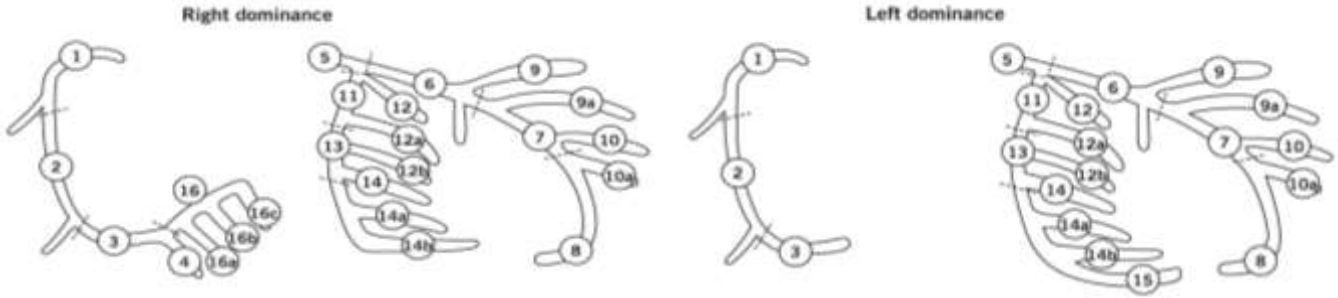

Figure 1. Definition of the coronary tree segments

1. *RCA proximal: From the ostium to one half the distance to the acute margin of the heart.*
2. *RCA mid: From the end of first segment to acute margin of heart.*
3. *RCA distal: From the acute margin of the heart to the origin of the posterior descending artery.*
4. *Posterior descending artery: Running in the posterior interventricular groove.*
16. *Posterolateral branch from RCA: Posterolateral branch originating from the distal coronary artery distal to the crux.*
16a. *Posterolateral branch from RCA: First posterolateral branch from segment 16.*
16b. *Posterolateral branch from RCA: Second posterolateral branch from segment 16.*
16c. *Posterolateral branch from RCA: Third posterolateral branch from segment 16.*
5. *Left main: From the ostium of the LCA through bifurcation into left anterior descending and left circumflex branches.*
6. *LAD proximal: Proximal to and including first major septal branch.*
7. *LAD mid: LAD immediately distal to origin of first septal branch and extending to the point where LAD forms an angle (RAO view). If this angle is not identifiable this segment ends at one half the distance from the first septal to the apex of the heart.*
8. *LAD apical: Terminal portion of LAD, beginning at the end of previous segment and extending to or beyond the apex.*
9. *First diagonal: The first diagonal originating from segment 6 or 7.*
9a. *First diagonal a: Additional first diagonal originating from segment 6 or 7, before segment 8.*
10. *Second diagonal: Originating from segment 8 or the transition between segment 7 and 8.*
10a. *Second diagonal a: Additional second diagonal originating from segment 8.*
11. *Proximal circumflex artery: Main stem of circumflex from its origin of left main and including origin of first obtuse marginal branch.*
12. *Intermediate/anterolateral artery: Branch from trifurcating left main other than proximal LAD or LCX. It belongs to the circumflex territory.*
12a. *Obtuse marginal a: First side branch of circumflex running in general to the area of obtuse margin of the heart.*
12b. *Obtuse marginal b: Second additional branch of circumflex running in the same direction as 12.*
13. *Distal circumflex artery: The stem of the circumflex distal to the origin of the most distal obtuse marginal branch, and running along the posterior left atrioventricular groove. Caliber may be small or artery absent.*
14. *Left posterolateral: Running to the posterolateral surface of the left ventricle. May be absent or a division of obtuse marginal branch.*
14a. *Left posterolateral a: Distal from 14 and running in the same direction.*
14b. *Left posterolateral b: Distal from 14 and 14 a and running in the same direction.*
15. *Posterior descending: Most distal part of dominant left circumflex when present. It gives origin to septal branches. When this artery is present, segment 4 is usually absent.*

♥② Give the TIMI flow at the beginning and end of PCI procedure – select one only (Enter code)

Contrast Flow

0 No flow
1 Small amount of flow, incomplete opacification of distal vessel
2 Slow filling but complete opacification of distal vessel
3 Prompt antegrade flow and rapid clearing

♥③ Estimate the stenosis at the beginning and end of the PCI procedure e.g. 50%, 75%

♥④ Type of stent implanted – select one only (Enter code)

1. Cypher
2. Endeavour
3. Taxus
4. Promus
5. Other

♥⑤ PCI procedure performed – select one only (Enter code)

1. Attempted but failed to succeed at lesion
2. PTCA only (includes cutting balloon)
3. Bare metal stent only implanted
4. Drug eluting stent only implanted
5. Bare metal stent and drug eluting stent implanted

♥⑥ PCI procedural success – select one only (Enter code)

1. Good
2. Failure
3. Sub-optimal
4. Not treated

TARGET LESIONS

		Segment number ♥①	TIMI pre-procedure ♥②	Stenosis pre procedure ♥③	TIMI post-procedure ♥②	Stent implanted (Yes/No)	Type of stent implanted ♥④	Stent Length (mm)	Stent Diameter (mm)	Stenosis post procedure ♥③	Procedure performed ♥⑤	Procedural success ♥⑥
1	Lesion 1											
2	Lesion 2											
3	Lesion 3											
4	Lesion 4											
5	Lesion 5											
6	Lesion 6											

Instructions, definitions and reminders:

opposite page: 9

TIMING OF SAMPLES TAKEN AT 2 HOURS

1	Date of sample (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
2	Time sample taken (hh:mm)	__ __ : __ __

BIOMARKER RESULTS AT 2 Hours

SERUM AND PLASMA RESULTS AT 2 Hours

		SERUM	PLASMA
3	NGAL	Yes [ ] _ _ . _ _ NA [ ]	Yes [ ] _ _ . _ _ NA [ ]
4	Cystatin C	Yes [ ] _ _ . _ _ NA [ ]	Yes [ ] _ _ . _ _ NA [ ]

URINE BIOMARKERS AT 2 Hours

5	NGAL	Yes [ ] __ __ . __ _	NA [ ]
6	Cystatin C	Yes [ ] __ __ . __ __	NA [ ]
7	KIM -1	Yes [ ] __ __ . __ _	NA [ ]
8	Interleukin-18	Yes [ ] __ __ . __ _	NA [ ]
9	NAG	Yes [ ] __ __ . __ _	NA [ ]
10	FAB-P	Yes [ ] __ __ . __ __	NA [ ]

TIMING OF SAMPLES TAKEN AT 4 HOURS

11	Date of sample (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
12	Time sample taken (hh:mm)	__ __ : __ __

BIOMARKER RESULTS AT 4 Hours

SERUM AND PLASMA RESULTS AT 4 Hours

		SERUM	PLASMA
13	NGAL	Yes [ ] _ _ . _ _ NA [ ]	Yes [ ] _ _ . _ _ NA [ ]
14	Cystatin C	Yes [ ] _ _ . _ _ NA [ ]	Yes [ ] _ _ . _ _ NA [ ]

URINE BIOMARKERS AT 4 Hours

15	NGAL	Yes [ ] __ __ . __ _	NA [ ]
16	Cystatin C	Yes [ ] __ __ . __ __	NA [ ]
17	KIM -1	Yes [ ] __ __ . __ _	NA [ ]
18	Interleukin-18	Yes [ ] __ __ . __ _	NA [ ]
19	NAG	Yes [ ] __ __ . __ _	NA [ ]
20	FAB-P	Yes [ ] __ __ . __ __	NA [ ]

Instructions, definitions and reminders:

opposite page: 10

BIOMARKERS **Pt ID: __ __ __ __ __ __**

TIMING OF SAMPLES TAKEN AT PRE-DISCHARGE

1	Date of sample (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
2	Time sample taken (hh:mm)	__ __ : __ __

BIOMARKER RESULTS AT PRE-DISCHARGE

SERUM RESULTS AT PRE-DISCHARGE

		SERUM	PLASMA
3	NGAL	Yes [ ] _ _ . _ _ NA [ ]	Yes [ ] _ _ . _ _ NA [ ]
4	Cystatin C	Yes [ ] _ _ . _ _ NA [ ]	Yes [ ] _ _ . _ _ NA [ ]

URINE BIOMARKERS AT PRE-DISCHARGE

5	NGAL	Yes [ ]	__ __ . __ _	NA [ ]
6	Cystatin C	Yes [ ]	__ __ . __ __	NA [ ]
7	KIM -1	Yes [ ]	__ __ . __ _	NA [ ]
8	Interleukin-18	Yes [ ]	__ __ . __ _	NA [ ]
9	NAG	Yes [ ]	__ __ . __ _	NA [ ]
10	FAB-P	Yes [ ]	__ __ . __ __	NA [ ]

BLOOD RESULTS PRE-DISCHARGE

11	Haemoglobin	Yes [ ]	__ __ . __ g/dL	NA [ ]
12	White cell count	Yes [ ]	__ __ . __ x 10^9/L	NA [ ]
13	Platelets	Yes [ ]	__ __ __ . __ x 10^9/L	NA [ ]
14	Sodium	Yes [ ]	__ __ __ mmol/L	NA [ ]
15	Potassium	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
16	Urea	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
17	Creatinine	Yes [ ]	__ __ µmol/L	NA [ ]
18	Creatinine Kinase	Yes [ ]	__ __ __ __	NA [ ]
19	Troponin	Yes [ ]	__ __ __	NA [ ]
20	eGFR	Yes [ ]	__ __ . __ ml/min	NA [ ]

Instructions, definitions and reminders:

opposite page: 11

♥① Complications

Please complete an SAE form if the patient has any event requiring hospitalisation or prolonging length of stay during hospitalisation at time of index procedure.

DISCHARGE DETAILS

1	Referral for further PCI	Yes [ ] No [ ]
2	Referral for CABG	Yes [ ] No [ ]
3	Did the patient have any complications ♥①	Yes [ ] → Complete SAE No [ ]
4	Status of patient on leaving hospital	Alive [ ] Dead [ ]

INVESTIGATOR'S DECLARATION

By signing below, I declare that the information presented in this Case Record Form accurately reflects the medical records on the date specified.

5	Date form completed (dd/mm/yyyy)	__ __ / __ __ / __ __ __ __
6	Name of person completing form (capitals)	
7	Signature of person completing form	

♥① Contrast Induced Nephropathy

Contrast Induced Nephropathy is defined as a creatinine increase of ≥ 44µmol/l from baseline.

♥② Other Serious Adverse Event (SAE)

Please complete an SAE form if the patient has any event requiring hospitalisation or prolonging length of stay during hospitalisation at time of index procedure.

BIOMARKERS	Pt ID: __ __ __ __ __ __

3 DAY FOLLOW UP

1	Date of follow up (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __

SERIOUS ADVERSE EVENTS SINCE DISCHARGE

2	Contrast Nephropathy event ♥①	Yes [ ] Complete SAE report	No [ ]
3	Other SAE event ♥②	Yes [ ] Complete SAE report	No [ ]

TIMING OF SAMPLES TAKEN AT 3 DAY FOLLOW UP

4	Date of sample (dd/mm/yyyy)	__ __ / __ __ /__ __ __ __
5	Time sample taken (hh:mm)	__ __ : __ __

BLOOD RESULTS AT 3 DAY FOLLOW UP

6	Haemoglobin	Yes [ ]	__ __ . __ g/dL	NA [ ]
7	White cell count	Yes [ ]	__ __ . __ x 10^9/L	NA [ ]
8	Platelets	Yes [ ]	__ __ __ . __ x 10^9/L	NA [ ]
9	Sodium	Yes [ ]	__ __ __ mmol/L	NA [ ]
10	Potassium	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
11	Urea	Yes [ ]	__ __ . __ __ mmol/L	NA [ ]
12	Creatinine	Yes [ ]	__ __ __ µmol/L	NA [ ]
16	eGFR	Yes [ ]	__ __ . __ ml/min	NA [ ]

INVESTIGATOR'S DECLARATION

By signing below, I declare that the information presented in this Case Record Form accurately reflects the medical records on the date specified.

13	Date form completed (dd/mm/yyyy)	__ __ / __ __ / __ __ __ __
14	Name of person completing form (capitals)	
15	Signature of person completing form	

Development of a biomarker panel for the earlier prediction of contrast nephropathy post percutaneous coronary intervention in patients with diabetes mellitus

Patient Information Sheet

You are being asked to take part in this study because you are an adult who is about to undergo a planned procedure on your heart blood vessels (percutaneous coronary intervention - angioplasty and coronary stenting)

Part 1

Invitation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time and read the following information carefully. Talk to others about the study if you wish.

- **Part 1 Tells you about the purpose of this study and what will happen to you if you take part.**
- **Part 2 This gives you more detailed information about the conduct of the study**

Ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.
Barts and The London NHS Trust (BLT) is the Sponsor of this research.

What is the purpose of the study?

Patients living with diabetes mellitus have double the risk of kidney failure compared to patients without diabetes following use of dye in many x-rays and procedures to diagnose and treat narrowing of the arteries (blood vessels) in the heart that can lead to angina or a heart attack. Heart disease is the commonest cause of death in patients with diabetes. People with diabetes are more likely to need these tests/treatments. By identifying those at greater risk of kidney complications we may be able to make these

tests/treatments safer and offer them to more patients with diabetes

Why have I been chosen?
You have been asked to take part in this study because you are an adult who is about to undergo a **planned** procedure (percutaneous coronary intervention – angioplasty and coronary stenting) to look at the blood vessels (arteries) of the heart and treat any narrowings found.

The percutaneous coronary intervention procedure that you are undergoing as part of your **planned** care requires the use of x-rays to look at your blood vessels and as such there is a risk arising from the use of ionising radiation. The dose from the x-ray exposure for the whole procedure is about the same dose that you will get from 5 years of natural background radiation and is considered to be of **low** risk.

Do I have to take part?
No – it is up to you to decide whether or not you want to take part. If you do, you will be given this information sheet to keep and be asked to sign the agreement to research section on the consent form. You are still free to withdraw at any time and without giving a reason. Your involvement in the study will not affect your planned procedure in any way. Nor will it influence the standard of care that you will receive in hospital.

Before you can begin the study
You may read the full study protocol as well as this Information Sheet which gives you many details about the study. The recruiting investigator will tell you about the study and any risks. You will be told exactly what the study involves and what will be required of you. You are encouraged to ask questions of the investigators conducting the recruitment interview until you are satisfied that you fully understand the nature of the study and the requirements.

What do I have to do?
If you agree to take part and to sign the research section of your Consent Form, we will review your medical records and collect data relating to your health, diagnosis and planned treatment.

In relation to the study, we only request that you agree to us taking some blood and urine samples before / during and at time intervals after (2 hours, 4 hours, 8-12 hours, pre-discharge) the procedure.

These samples will have no impact on your planned treatment or health. 3 days after the procedure we request that you come back to The London Chest Hospital for a final blood test. The total blood volume will not exceed 145ml and the total urine volume will not exceed 500ml. Having this amount of blood taken is not associated with any medical problems.

What are the alternatives for diagnosis or treatment?
Your doctor will have already discussed your treatment with you. If you have any concerns about your planned treatment please discuss them with the medical team.

What are the other possible disadvantages or risks of taking part?
We do not believe that there are any disadvantages or risks in you taking part.

Expenses and Payments
There will be no payment in exchange for your participation in this study or for the donation of your blood and urine samples.

What are the possible benefits of taking part?
None although you will be contributing to our understanding of the reasons for kidney problems developing in patients with diabetes and this knowledge may help other patients, like yourself in the future.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2. If you have a complaint please contact the following in the first instance:
Dr Katie Qureshi, Specialist Registrar in Cardiology
Dr Akhil Kapur, Consultant Cardiologist

Will my taking part in the study be kept confidential?
Yes. All information about your participation in this study will be kept confidential

Contact Details:
If you require any further information please contact:

Dr Katie Qureshi,
Specialist Registrar,
Department of Cardiology,
St. James' Building,
The London Chest Hospital,
Barts and The London NHS Trust,
Bonner Road,
London,
E2 9JX
Tel: 0208 983 2477
Mob: 07592 745217
Fax: 0208 983 2381
E-mail: drkatiequreshi@yahoo.co.uk
E-mail: katie.qureshi@bartsandthelondon.nhs.uk

Dr Akhil Kapur,
Consultant Cardiologist,
Department of Cardiology,
St. James' Building
The London Chest Hospital,
Barts and The London NHS Trust,
Bonner Road,
London,
E2 9JX
0208 983 2413

0208 983 2381
akhil.kapur@bartsandthelondon.nhs.uk

This completes **Part 1** of the Information Sheet. If the information in Part1 has interested you and you are considering participation, please continue to read the information in **Part 2** before making any decision.

Part 2

What will happen if I don't want to carry on with the study?

If you withdraw from the study we will need to use the data collected up to your withdrawal. Any blood and urine samples that can still be identified as yours will be destroyed if you wish.

What if there is a problem?

Barts and The London NHS Trust has agreed that if you are harmed as a result of your participation in the study, you will be compensated, provided that, on the balance of probabilities, an injury was caused as a direct result of the intervention or procedures you received during the course of the study. These special compensation arrangements apply where an injury is caused to you that would not have occurred if you were not in the trial. These arrangements do not affect your right to pursue a claim through legal action.

Complaints:

If you have a concern about any aspect of this study, you should ask to speak to the Research Team who will do their best to answer your questions (Dr Katie Qureshi 07592 745217, 0208 983 2477, Dr Akhil Kapur 0208 983 2477).
If you remain unhappy and wish to complain formally you can do this by contacting : The Patient Advisory Liaison Service (PALS) if

you have any concerns regarding the care you have received, or as an initial point of contact if you have a complaint. Please telephone 0207 377 6335, minicom 0207 943 1350, or email pals@bartsandthelondon.nhs.uk . You can also visit the PALS office by asking at any hospital reception.

Will my taking part in this study be kept confidential?
All the information obtained about you in the course of the study is confidential and will be kept in a secure, locked room. The investigators performing the study and a study monitor will have access to the data collected in this study. They may also be looked at by representatives of regulatory authorities and by authorised people from Bart's and The London NHS Trust to check that the study is being performed correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

What will happen to any samples I give?
All your blood and urine samples will be analysed at the William Harvey Research Institute at Barts and The London School of Medicine. Some will be tested immediately, while others will be stored for later use in the project. Once the results have been obtained, if there is any left, it will be destroyed.

Will any genetic tests be done?
No genetic tests will be performed on your samples

What will happen to the results of the research study?
Results from this study may be published or presented at meetings and in scientific journals.

Who has reviewed the study?
This study has been given a favourable ethical opinion for conduct in the NHS by East London and The City Research Ethics Committee 1 (NRES 09/H0703/29).

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Declaration

I declare that this thesis has been composed by myself and that the work of which it is a record has been done by myself. Any results which were generated in collaboration with others is fully acknowledged. It has not been previously presented to any institution for a higher degree.

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