

AN INVESTIGATION INTO FACTORS PREDICTING PATENCY AND  
MATURATION OF ARTERIOVENOUS FISTULAE USED FOR  
HAEMODIALYSIS IN ENDSTAGE RENAL DISEASE

By

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## ABSTRACT

Arteriovenous fistulae (AVF) are the preferred access for haemodialysis in endstage renal disease, but have a considerable failure rate.

In this thesis we investigated factors which may affect patency and maturation of new AVF: (1) ethnicity, (2) native vein histology, (3) pre-operative vascular mapping with ultrasound, and (4) post-operative ultrasound of AVF.

Ethnicity did not affect AVF outcomes in our large retrospective study, although diabetes was associated with AVF failure and more common among non-Caucasians. Native vein disease, specifically vein media fibrosis, was associated with immediate AVF failure in our small cohort study, possibly reflecting poor vein dilatation. In our randomised trial we showed that routine pre-operative ultrasound mapping of native vessels prior to AVF formation was effective at improving immediate and assisted primary AVF patency. Early post-operative assessment with ultrasound predicted subsequent AVF failure in our cohort study.

In conclusion, we recommend routine pre-operative ultrasound vascular mapping to improve AVF outcomes. A randomised prospective trial should be considered to evaluate whether early post-operative ultrasound helps to improve AVF patency. The significance of native vein media fibrosis needs to be confirmed in a larger study.

## DEDICATION

To my parents, wife and children

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## LIST OF ABBREVIATIONS

AVF = arteriovenous fistula

AVG = arteriovenous graft

CVC = central venous catheter

EDV = end-diastolic velocity

IMT = intima media thickness

IQR = interquartile ratio

MMP = matrix metalloproteinase

NIH = neo-intimal hyperplasia

NPV = negative predictive value

PPV = positive predictive value

PSV = peak systolic velocity

RI = resistive index

ROC = receiver operating curve

TIMP = tissue inhibitor of metalloproteinase

## **A) Thesis Introduction**

### **1. The importance of vascular access for haemodialysis in endstage renal disease**

Treatment options for endstage renal disease include kidney transplantation, haemodialysis and peritoneal dialysis. There has been increasing demand for haemodialysis(1). In 2005, 694 patients per million population were treated by haemodialysis in the United Kingdom(1).

The haemodialysis population tends to be older with increased comorbidity, including diabetes and vascular disease (2).The median age for haemodialysis patients was 65 years, for peritoneal dialysis patients 59 years and for transplant recipients 50 years (1).

“Uraemic toxins” accumulate in endstage renal disease and are cleared from the blood by intermittent haemodialysis. Vascular access is necessary to ensure delivery of a sufficiently large blood volume to the dialyser so as to allow adequate clearance of uraemic toxins within a 4 hour treatment session. Adequate clearance is associated with improved patient survival (3).

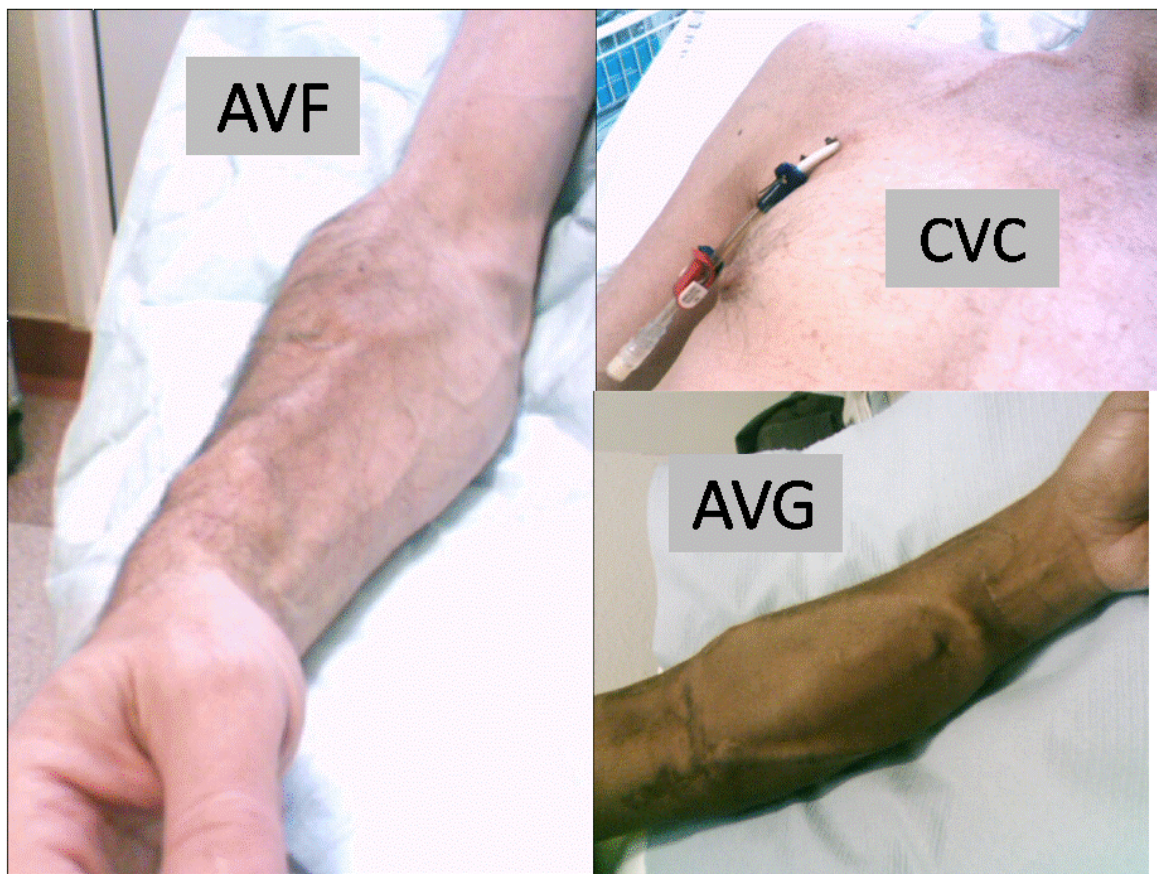
#### **1.1. Vascular access: types, properties and recommendations**

Three types of vascular access are in common use (Figure 1). Arteriovenous fistulae (AVF) are a surgical connection between a superficial vein and an artery on the patient’s arm, resulting in increased blood flow through the vein which can then be accessed for haemodialysis.

Arteriovenous grafts (AVG) usually consist of synthetic material and are surgically placed to connect a superficial vein to an artery. Central venous catheters (CVC) are paired synthetic

tubes placed into a large central vein and are usually tunnelled subcutaneously; the extra-cutaneous catheter portion can be connected to the dialyser circuit (4).

Figure 1: Three common forms of vascular access for haemodialysis (AVF = arteriovenous fistula: here a fistula between radial artery and cephalic vein at the right wrist; CVC = central venous catheter: here a tunnelled catheter inserted into the right internal jugular vein; AVG = arteriovenous graft: here a forearm loop graft bridging the left brachial artery to the upper arm cephalic vein)



Both CVCs and AVGs can be used for haemodialysis early after insertion, but failure due to thrombosis as well as complications from infection or central venous damage are associated with increased morbidity and mortality (5). A serious complication of CVC-related bacteraemia is infective endocarditis associated with a high mortality (6, 7).

By contrast, AVF haemodialysis use is delayed by several weeks because of AVF development (maturation) and also because of a considerable early failure rate. In comparison to CVC and AVG, AVF provide better and safer long-term use with a low risk of thrombosis and almost no infection (8, 9). American and European guidelines agree that AVF are the access of choice; when an AVF cannot be formed, AVGs are useful as a bridge between artery and vein, whilst CVC are access of last resort (10, 11).

### **1.2. Arteriovenous fistulae (AVF): common sites and maturation**

The distal forearm is the preferred site for AVF formation, connecting the radial artery with the cephalic vein (10, 11). When this is not possible, the upper arm brachial artery is used to connect to the upper arm cephalic or basilic vein in the antecubital fossa. The preference for forearm to upper arm AVF is based on the observation that the lower blood flow in the former results in less blood diversion and thus a lower risk of hand ischaemia or high output cardiac failure(10). AVF are usually made by direct connection of artery and vein through a single incision. If the vein is distant or lies too deep, like the basilic vein, the surgeon can transpose or superficialise the vein(12).

AVF surgery forms a direct connection from the high pressure arterial system to the low pressure, high compliance of the venous system draining ultimately into the right heart. The AVF haemodynamics and usability for haemodialysis depend on the functioning of this circuit: left heart – arterial system – fistulous connection – outflow vein – right heart. At the fistulous connection itself, there is a significant hydrostatic pressure drop, resulting in a jet of turbulent blood flow(13, 14). Blood flow increases dramatically through the AVF on the day of surgery and continues to rise further over the several weeks, due to an increase in

cardiac output and arterial dilation (13, 15). This process, together with dilation of the draining vein and strengthening of the venous wall, is termed AVF “maturation”(8).

AVF maturity for haemodialysis is often determined clinically but maturation periods may vary considerably between AVF. The “rule of 6” was recently introduced to aid recognition of a mature AVF ready for haemodialysis use: ultrasound measured flow greater than 600 ml/min, vein diameter of at least 6 mm, and vein depth less than 6 mm (16). Contrary to common belief, early AVF use for haemodialysis once clinically mature is not detrimental to AVF, if a minimum maturation of 2 weeks is observed (17).

### **1.3. Failure of AVF**

#### ***1.3.1 Clinical relevance***

AVF fail when they are not usable for haemodialysis. As a result, there is often delay until a functioning AVF is established, surgical workload and service costs increase, and CVC are often needed for interim haemodialysis (18). AVF failure is an important problem, both in terms of establishing vascular access initially (“primary failure”), as well as maintaining functional access (“secondary failure”) for ongoing haemodialysis use (8, 19, 20). This thesis focuses on the former, which is more common and occurs 20-50% of new AVF (8).

At a clinical level, AVF failure most commonly presents as patency failure (thrombotic occlusion) or maturation failure (insufficient blood flow usually due to AVF stenosis); the latter may occur at one or more focal points of the feeding artery, the fistula connection or

the draining vein (19). Less common causes for AVF failure include a generally narrowed feeding artery or oedema of the AVF arm due to central vein injury and obstruction from prior CVC use. Furthermore, an AVF may not be used for haemodialysis because of cannulation difficulties of the AVF either due to a short or deep vein or due to inexperience of dialysis staff, or uncommonly hand ischaemia (19, 21, 22).

### *1.3.2. Pathogenesis*

The pathogenesis of AVF failure is poorly understood and probably diverse. Haemodynamic problems at local vascular (insufficient feeding artery) and systemic (hypotension, heart failure) level have been associated with AVF failure (19, 23). Venous damage from excessive venopuncture or cannulation prior to AVF formation as well as traumatic vein manipulation during surgery have also been implicated (24). Recently, the vascular response to shear stress and the development of neo-intimal hyperplasia after AVF formation have been attributed to the development of flow-limiting stenosis which leads to AVF thrombosis (18, 24-26). Furthermore, AVF failure is more likely in forearm compared to upper arm AVF as the smaller calibre radial artery results in lower blood flow (8, 27, 28).

### *1.3.3. Definitions of AVF outcomes*

AVF failure definitions in the literature are rather diverse; AVF failure is classified by time occurrence after surgery, or by AVF use for haemodialysis.

The term “early failure” has been used for any AVF failure in the first 6-12 weeks after surgery (19, 29-32). Others distinguish immediate failure on the day of surgery, early thrombosis within 8 weeks or failure to mature within 6 months of AVF formation (30).

Primary AVF failure defined by haemodialysis use is a pragmatic but clinically relevant definition useful to describe short-term AVF outcome(8). Longer term AVF outcome is described by survival analysis of AVF patency over time (= the opposite of AVF failure). Sidawy et al have standardised AVF patency reporting (33). They distinguish primary patency (all AVF used for dialysis until failure), assisted primary patency (all AVF used for dialysis until thrombosis, including patency time gained through successful AVF stenosis repair), and secondary patency (all AVF used for dialysis until finally abandoned, including patency time gained through any successful AVF salvage including thrombectomy). Haemodialysis use of AVF is often not well defined in the literature but a definition describing two needle AVF cannulation and adequate dialysis has been described (28).

#### *1.3.4. AVF failure epidemiology*

The literature reports moderate 1 year primary patency rates for AVF at 50-80% (8). A meta-analysis specifically for forearm (radiocephalic) AVF showed primary patency of only 63% and secondary patency of 66% at 1 year (31). There is a perception that primary failure and AVF survival were better in publications before 1990 than after (8, 31). Because this parallels a change in the dialysis population to include older patients with more cardiovascular disease and diabetes, established vascular disease has been attributed to worse AVF outcomes (2, 8, 31). The work in this thesis therefore will focus on vascular assessment as a means to reduce the likelihood of AVF failure.



## **2. Risk factors for AVF failure**

AVF failure has been associated with several risk factors, including certain patient characteristics, local vascular disease, aspects of surgery and the development of a new stenosis after AVF formation.

### **2.1. Risk associated with patient characteristics**

Several authors have investigated associations between AVF outcome and patient characteristics such as age, gender, ethnicity, obesity, diabetes and cardiovascular disease. Direct comparison of the studies is hindered by different AVF outcome definitions; however, broadly three main AVF outcomes were considered, including AVF prevalence, AVF failure and AVF survival; the studies are summarised in table 1 (28, 34-40). AVF prevalence describes the rate of AVF relative to other forms of vascular access, and is a topic of great interest in the United States where traditionally there has been a preference for AVG rather than AVF formation, until the recent work of the “Fistula First Initiative” (8, 36). Both studies with AVF prevalence have considerably larger patient numbers and show that older age, female gender, non-caucasian ethnicity, obesity and vascular comorbidity are associated with lower likelihood of AVF formation. AVF prevalence, however, is a less useful measure as it is largely affected by surgeons’ practice patterns. Pisoni et al demonstrated this by showing that AVF prevalence was lower in the United States compared to Europe, even after adjusting for patient characteristics and comorbidity (36).

Table 1: Association of patient characteristics on AVF outcome

Study	AVF outcome	n	Age	Gen-der	Eth-nic	Obese	DM	IHD	PVD	CVD
Allon 2000(34)	Prevalence	1824	+	+	+	+	-	-	+	.
Pisoni 2002(36)	Prevalence	6479	+	+	+	+	+	+	+	.
Feldman 2003(38)	Failure	348	+	.	.	.	.	.	.	+
Miller 1999 (28)	Failure	101	+	+	-	+	+	.	.	.
Konner 2002(40)	Survival	748	-	+	.	.	+	.	.	.
Weale 2008 (41)	Survival	658	-	+	-	.	+	.	.	.
Puskar 2002(39)	Survival	463	-	-	.	.	+	-	.	-
Lok 2005(37)	Survival	444	-	+	+	.	.	+	.	.
Prischl 1995 (42)	Survival	139	+	-	.	.	+	.	.	.
Golledge 1999 (35)	Survival	107	-	+	.	.	+	.	.	.

Abbreviations: DM = Diabetes mellitus, IHD = ischaemic heart disease, PVD = peripheral vascular disease, CVD = cerebrovascular disease. AVF outcome is described as AVF prevalence, primary failure and AVF survival: (+) association shown, (-) association not shown, (.) association not tested

### 2.1.1. Age and AVF outcome

Age can be seen as a surrogate marker for cardiovascular disease including heart failure, arterial stiffness and atheroma; as a result one may expect haemodynamic compromise and worse AVF outcomes, perhaps particularly for forearm AVF. Age is an important factor in the face of an increasingly older dialysis population (2).

Table 2 summarises the reports from the literature. Many authors report worse AVF outcomes in older patients, but Weale et al recently described equivalent AVF survival with a

similar proportion of forearm AVF(41). A meta-analysis found a significantly shorter primary and secondary patency (odds ratio for failure 1.79 and 1.53, respectively) for forearm (radiocephalic) AVF in the elderly at 1 year; upper arm (brachiocephalic) AVF had significantly less failure (risk difference 12%) compared to forearm (radiocephalic) AVF (43). Therefore, radiocephalic AVF cannot be considered as routine default in the elderly, although careful individual assessment may allow AVF formation in some (41, 43).

Table 2: Age (above and below 65 years) and AVF failure or survival

Study	n	RC : all AVF		Primary Failure		1 year primary survival	
		<65	> 65	< 65	> 65	< 65	> 65
Konner 2002(40)	748					77%	76%
Weale 2008(41)	658	56%	56%			46%	46%
Lok 2005 (37)	444	56%	47%	34% #	49% #	65 %	65%
Feldman 2003(38)	348			32-49%	55%		
Prischl 1995(42)	139					55% §	19%§
Miller 1999 (28)	101			46% †	70% †		

Annotation: RC: all AVF = percentage of forearm radiocephalic among all AVF; (# ) = AVF failure to mature only; († ) = calculated from 100% - % AVF adequate for haemodialysis; (§) = reported for <40 and > 70 years

### 2.1.2. Gender and AVF outcome

Most studies observe worse AVF outcome among women compared to men, which remains evident in multivariate analysis (28, 34-37). Table 3 summarises reported AVF outcomes by gender. Particularly forearm (radiocephalic) AVF seem less successful in women, because failure rates are higher or relatively more upper arm AVF are constructed (28, 35, 41). Lok et al reported significantly lower cumulative AVF survival among women with a hazard ratio for male gender = 0.63, 95% confidence interval 0.44-0.91 (37). In the United States, AVF prevalence among women remains lower than men, even though the “Fistula First Initiative”

has achieved in a considerable increase of overall AVF prevalence from 24% to 47% in recent years (34, 36, 44).

Table 3: Gender and AVF failure or survival

<b>Study</b>	<b>n</b>	<b>RC : all AVF</b>		<b>Primary failure</b>		<b>1 year primary survival</b>	
		<b>m</b>	<b>f</b>	<b>m</b>	<b>f</b>	<b>m</b>	<b>f</b>
Allon 2000(34)	1824	70%	57%				
Konner 2002(40)	748					81%	71%
Feldman 2003(38)	348			42%	50%		
Miller 1999(28)	101	56%	40%	48% †	64% †		
Miller 2003(30)	189			50% †	68% †		

Annotation: RC: all AVF = percentage of forearm radiocephalic among all AVF; (†) = calculated from 100% - % AVF adequate for haemodialysis

Why women do worse is unknown. Women tend to have smaller pre-operative arterial diameters, which might result in lower AVF blood flow (30, 45, 46). However, despite selecting adequate vessels with ultrasound mapping prior to AVF surgery, women had a significantly higher primary failure rate (68% versus 50%) which included considerably more immediate AVF failure (13% versus 3%) among women (30). Almost twice as many women than men required AVF salvage, including a greater need (30% versus 8%) of vein superficialisation (30). Lomonte et al reported similar forearm AVF blood flow measurements in men and women during follow-up in the first month after surgery (15). These findings suggest that the causes of poorer AVF outcomes among women may be diverse and not haemodynamic. Hence, more research is needed to understand the pathogenesis (30). Practically, women with advanced renal disease may benefit from earlier AVF formation, as one would expect a greater need for additional intervention.

### *2.1.3. Ethnicity and AVF outcome*

Ethnicity is an interesting aspect because ethnic minorities are common in parts of the United Kingdom, the United States and Canada and have a greater likelihood of renal disease(1, 36). Furthermore, ethnic minorities of Indo-Asian and African background have a higher incidence of diabetes mellitus and hypertension, but tend to be younger compared to Caucasians (28, 36, 47). Two studies on African Americans report equivalent primary AVF failure and comparable AVF survival (28, 48). However, Lok et al reported a significantly less failure when analysing AVF survival (including time gained from AVF salvage) among Caucasians (hazard ratio 0.63) compared to non-Caucasians; these included African, Indo-Asian and Southeast Asian ethnicities (37). Little is known about AVF outcomes in Indo-Asian patients in the United Kingdom (49).

### *2.1.4. Obesity and AVF outcome*

A frequent issue with obese patients is a tendency towards deeper veins. The vein may be suitable for AVF but is not clinically appreciated and hence AVF may not be considered by the surgeon; this can be overcome by ultrasound vein mapping (50). Furthermore, post-operatively, there may be AVF cannulation difficulties due to the depth of the vein; this may be overcome by ultrasound-guided AVF cannulation or surgical superficialisation of the vein, but the latter may carry a risk of additional traumatic vein manipulation intra-operatively (12). In addition, effects on AVF outcomes in obese patients may be confounded by a higher proportion of women and diabetics (20).

AVF prevalence was reported lower among obese patients in large scale studies (34, 36).

Obese patients had a trend towards more primary AVF failure (65% versus 45%) at a single

centre. These findings may well reflect practice patterns including preferences of the surgeon and lack of pre-operative ultrasound mapping. With pre-operative ultrasound, AVF prevalence was equivalent (obese 44%, non-obese 37%) and primary failure was similar (obese 46%, non-obese 41%), despite a significantly greater proportion of women (20, 51). Hence, AVF outcomes appear similar among the obese with pre-operative mapping.

#### ***2.1.5. Diabetes and AVF outcome***

Diabetes is associated with cardiovascular disease as well as distal arterial disease; hence a poorer AVF outcome may be expected. Outcomes from the literature are summarised in table 4.

Table 4: Diabetes (DM) and AVF failure or survival

Study	n	RC : all AVF		Primary Failure		1 year primary survival	
		DM	no DM	DM	no DM	DM	no DM
Konner 2002(40)	748	24%	62%			75%	77%
Miller 1999(28)	101	48%	51%	65%	46%		
Feldman 2003(38)	348			51%	41%		

Annotation: RC: all AVF = percentage of forearm radiocephalic among all AVF;

Miller et al observed more primary AVF failure among diabetics. Konner et al reported equal AVF survival in diabetic patients but formed more upper arm AVF among diabetics; although uncommon, the risk of hand ischaemia through blood diversion in upper arm AVF was significantly higher (7 versus 0.6 events per100 patient-years) among diabetics (40). These findings support the assumption of worse distal arterial disease in diabetics. Hence pre-operative vascular mapping may be particularly important in diabetic patients.

#### *2.1.6. Cardiovascular disease and AVF outcome*

Cardiovascular disease, including cardiac disease, peripheral arterial disease and cerebrovascular disease, may be expected to result in poorer AVF outcome. Indeed, more primary AVF failure was reported in patients with cerebrovascular disease (38). Among patients with coronary artery disease AVF failure was twice as likely when AVF survival (including time gained from AVF salvage) was analysed (37). Puskar et al found no difference although more than a quarter of patients were lost to follow-up (39). Thus, cardiovascular disease is important for AVF outcome.

#### *2.1.7. Dependence on haemodialysis*

Patients dependent on dialysis at the time of AVF formation appear to have worse AVF outcome, compared to patients with advanced renal disease awaiting to start haemodialysis (38, 39, 41). Reasons for this observation may include CVC-related central venous obstruction, exhaustion of peripheral veins, or worsening arterial disease and stiffness in endstage renal disease.

In summary, AVF outcome appears to be worse for some patients, including women, diabetics, older age and cardiovascular disease. These associations are important to guide further research into the pathogenesis of AVF failure and optimal timing of AVF surgery for patient subgroups in advanced renal disease.

## 2.2. Risk associated with established native vascular disease: focus on histological assessment

### 2.2.1. *Native venous disease*

Pre-existing native vascular disease may well result in poorer AVF outcome, because an insufficient artery or vein may be unable to increase blood flow sufficiently and undergo adequate maturation. Vascular ultrasound assessment is a clinically useful option and will be presented in detail in section “Ultrasound vascular mapping prior to AVF formation”.

Furthermore, histological analysis may identify pre-existing vascular disease in more detail and this is presented here.

There is some analogy between AVF and vein grafts used for peripheral or coronary bypass surgery, because in both situations veins are exposed to and adapt to the arterial circulation. However, AVF are distinct from vein grafts because venous outflow remains in the venous rather than the arterial system; as a consequence, pressure in the AVF is low despite high flow (14).

#### 2.2.1.1. The histology of native saphenous veins

Most research on vein graft histology is focussed on post-operative changes and in particular the development of myointimal hyperplasia, whereas native venous disease before peripheral or coronary grafting has received less attention (52, 53).

In one study, native saphenous vein histology revealed a hyperplastic intima and medial sclerosis in at least 70% of veins, while only 7% of veins had no lesion; these changes were not related to patients' age (54). Others found focal or circumferential intimal hyperplasia in



all native saphenous veins (55). A further study found that severe intimal hyperplasia of native saphenous veins was associated with diabetes(56). Such structural changes could affect the outcome of the vein as bypass conduit (54-56).

Unlu et al reported measurements of saphenous vein samples in the proximal, middle and distal portions: The mean intima width was 66.6, 50.4, and 19.1  $\mu\text{m}$ , respectively, and mean media width 225.2, 150.4, and 123.8  $\mu\text{m}$ , respectively (57).

#### 2.2.1.2. The histology of native cephalic and other veins prior to AVF formation

Histology of the cephalic vein prior to AVF surgery has been described in detail by one research group (58-60). They examined veins from patients with endstage kidney disease (mean age 44 years) prior to AVF formation and compared these to veins from male patients without kidney disease (mean age 24 years) who required vascular reconstruction after arterial injury.

On light microscopy they described considerable native venous disease amongst renal patients which were not present among the trauma group. The abnormalities are summarised in table 5 and include focal or diffuse intimal hyperplasia and medial fibrosis.

Feinfeld et al harvested brachial veins at vascular access formation and quantified dimensions using image analysis software: wider mean intimal and medial widths were associated with longer term haemodialysis (61).

Two very recent studies from the United States examined native vein histology. Lee et al reported a detailed morphological study of native veins (50% cephalic) in endstage renal disease in 12 patients (62). They found neo-intimal hyperplasia with myofibroblasts to varying degree among 10 of those patients and concluded that neo-intimal hyperplasia

Table 5: Cephalic vein histology by light microscopy in patients with and without endstage kidney disease (58-60)

	No kidney disease	Endstage kidney disease
Intima	Regular smooth outline	Irregular outline due to focal / diffuse intimal hyperplasia, or focal / diffuse endothelial layer loss
Intimal hyperplasia	Absent	Present: loosely laid fibrous tissue, scattered smooth muscle cells, teleangiectasia (appearing as mucoid or myxoid degeneration)
Intimal smooth muscle cells	Palisade arrangement	Scattered
Lamina elastica interna	Regular, continuous	Partial or complete loss
Media	Normal	Thinner with increase in fibrous tissue
Intima:media ratio	1:5	5:1 (in focal areas)
Medial smooth muscle layer	Regular, circumferential layers separated by little extracellular matrix	Wide separation of smooth muscle cells by excessive fibrous tissue

prevalent even prior to AVF or AVG formation. In contrast, Allon et al found no intimal hyperplasia in forearm and upper arm veins in 50 patients (63). Hence the significance of pre-existing neo-intimal hyperplasia is currently unclear.

The relevance of such native venous changes to AVF outcomes is unknown, although one may speculate that severe structural changes might predispose to insufficient vein dilation or accelerated development of neo-intimal hyperplasia.

#### 2.2.1.3. Preparation and histological evaluation of native veins

In the following we will review the methods of vein specimen handling for native vein histology in published reports (54, 55, 57-61). All authors used 10% formalin for fixation.

Vein specimens were embedded in paraffin and then cut. Haematoxyline-Eosin staining was

generally used for light microscopy, as well as additional stains; some used an image analyser (table 6). Wali examined much shorter vein segments than other authors, which could lead to bias with regards to focal venous changes such as intimal hyperplasia (60).

Table 6: Native vein histology: Vein preparation and staining techniques

Author	Vein length	Sections	Stains
Feinfeld 1999(61)	1 mm	-	HE, evG
Milroy 1989(55)	0.5 – 8 cm	4 $\mu$ m	HE, evG, Mt
Thiene 1980(54)	-	10 $\mu$ m	HE, WvG, PAS
Unlu 2003(57)	72.4 cm	6 $\mu$ m	HE, evG
Wali 2006(60)	0.2-0.3 cm	Semi-thin	HE, Mt
Lee 2011 (62)	0.8 – 1 cm	5 $\mu$ m	HE, various immunochemical markers

Abbreviations: HE = Haematoxylin-Eosin, evG = elastic van Gieson, WvG = Weigert van Gieson, Mt = Masson Trichrome, PAS = periodic acid shift

All authors examined vein structure in cross-section. Most authors reported on vein histology by descriptive or semiquantitative means (54, 55, 60). Such subjective analysis may have disadvantages, such as poorer reproducibility and the potential for bias. Only two studies report quantitative changes obtained by computerised image analysis which may be more objective (57, 61). Table 7 summarises the methods of histological evaluation in the published literature.

Table 7: Native vein histological evaluation

Author	Image analyser	Reporting methods	Histological criteria studied
Feinfeld 1999	Yes	Quantitative	Intimal and medial width
Milroy 1989	No	Descriptive and semi-quantitative	Degrees of intimal and medial fibrosis (minimal, mild, moderate, severe)
Thiene 1980	No	Semi-quantitative	Degrees of intimal and medial fibrosis (minimal, mild, moderate, severe)
Unlu 2003	Yes	Quantitative	Lumen diameter, intima thickness, media thickness
Wali 2006	No	Descriptive	Intima-media ratio at maximum thickness of intimal hyperplasia
Lee 2011	Yes	Quantitative	Area and thickness of lumen, intima, media; ratio of area intima to media; ratio of average intima to media thickness; ratio of maximal intima to media thickness

In conclusion, native vein histology is altered in the majority of patients with renal, coronary artery and peripheral vascular disease; important changes include intimal hyperplasia and medial sclerosis. We currently do not know whether such changes, if severe, could translate into poorer AVF outcomes, and whether it could be related to patient characteristics. Also, a useful quantitative method for vein examination has not yet been established. These questions will be addressed by the research in this thesis

#### 2.2.1.4. Native arterial disease

In a functioning AVF, the artery dilates and elongates so as to deliver high blood flow into the AVF (13). Native arterial disease may prevent this process.

Samples of native radial arteries from patients undergoing coronary artery bypass graft surgery were taken for histological examination (64, 65). Among such patients with established ischaemic heart disease, radial arterial disease was almost universal: The distal radial artery was worst affected, showing intimal hyperplasia in approximately 90%, medial calcification in 5 % and arteriosclerosis in 6-13%. Intimal hyperplasia was associated with age, smoking, hypertension, Diabetes and peripheral vascular disease (64, 65), while medial calcification was also associated with CKD (65).

Kim et al harvested native radial artery samples at formation of radiocephalic AVF from renal patients in South-East Asia and performed histological evaluation (66). With an ellipsoid excision, a 10 mm long sample of the arterial wall was taken from the arteriotomy site. Intimal hyperplasia affected the majority (76%) of radial arteries, which was associated with 50% AVF failure within the first year, compared to no failure among those without intimal hyperplasia (66). Intimal hyperplasia was associated with age and Diabetes. Importantly, intimal hyperplasia can be directly assessed with ultrasound (67). In contrast, a recent study from the United States found no significant intimal hyperplasia; however, the results of the two studies may not be comparable: there are ethnic differences (the majority of the US patients were African American), only 1/3 of the arterial specimens in the US study were from the radial artery, and systematic pre-operative vascular ultrasound in the US study may have excluded patients with significant vascular disease.

In conclusion, native radial artery disease is very common in patients with endstage renal disease or cardiovascular disease. Pre-existing intimal hyperplasia may be important for early AVF failure and can be assessed in vivo with high-resolution ultrasound.

### **2.3. Risk associated with surgery**

Surgical experience and skill is considered a major determinant of AVF patency and maturation (12, 40). Particular surgical skills include careful vein manipulation to avoid trauma, as well as creating an appropriate anastomotic length to allow sufficient but not excessive AVF blood flow. Yet, vascular access surgery has traditionally attracted little interest and does not form part of the vascular surgical curriculum in the United Kingdom.

The original Brescia-Cimino fistula was the side-to-side AVF which was easier to construct but lead to venous hypertension of the hand; the more demanding end-to-side technique has become established and this might contribute to poorer AVF outcomes (68).

In one study, an experienced surgeon was able to form an AVF in all 748 consecutive patients who required vascular access for the first time, including women, the elderly, and diabetics; primary AVF survival at one year ranged from 51%-75% among subgroups of patients and compared favourably to other published reports (40). Direct comparison of surgeons in terms of AVF outcome has been attempted by two observational studies. Prischl et al reported that, when the surgeon as the only determinant of AVF outcome on multivariate analysis, there was significant variation in early AVF failure (0-39%) among seven surgeons who formed 108 first-time forearm (radiocephalic) AVF; also, 1-year AVF patency varied considerably (34-69%) between surgeons (42). This analysis may be limited

by small numbers, ranging from 6 to 28 AVF, per surgeon (42). Choi et al compared vascular access formation and outcome by two experienced surgeons, taking into account patient demographics, previous access surgery and pre-operative vascular mapping (69). There were significant differences between the two: One surgeon placed more AVF (98% versus 71%), had less primary access failure (5% versus 31%) and better 1 year access survival (85% versus 47%), despite a seemingly worse patient case-mix (69). These data show that surgical skill has considerable impact on AVF outcome.

Given such concerns, the work of surgical trainees has been under scrutiny: They need training opportunities but this needs to be delivered without worsening AVF outcome. Comparing trainees to consultant surgeons, an international study observed a preference for AVG placement among trainees (36). Two studies from the United Kingdom showed no significant difference in AVF formation or outcome, when trainees were compared to consultant surgeons (70, 71). While it is likely that the more “difficult” operations were carried out by consultants and that supervision was tailored to an individual trainee’s experience, these data illustrate that it is possible to have trainees operating with good outcomes.

### **3. Screening to identify vascular disease**

#### **3.1. Physical examination**

Physical examination allows a rapid bedside patient assessment to assess the suitability of blood vessels for AVF formation. Avoiding sites where blood vessels are poor is likely to result in better AVF outcomes.

The history is important, in particular to identify patients at risk of central venous damage related to a previous CVC or cardiac pacemaker, or those with severe heart failure and diabetes, because such patients are likely to require radiological vascular imaging (72).

Physical examination should include measurement of blood pressure in both arms to screen for proximal arterial stenosis, palpation of arterial pulses in the antecubital fossa and at the wrist, as well as palpating the cephalic vein under congestion with the help of a tourniquet, ideally in its entire course; some also advocate the Allen's test which examines whether the radial and ulnar arteries are able individually to perfuse the hand (72). Physical examination is subjective and therefore an experienced assessor is essential. A suitable site would be suggested by finding normal blood pressures in both arms, good pulses and a patent cephalic vein of good calibre throughout its course. An unsuitable site would be suggested by abnormal findings, such as a significantly lower blood pressure in one arm, absent or weak pulses, and a thin or abnormal vein. In many patients, particularly those who are obese, complete venous assessment is difficult (50). A clear weakness of the clinical examination is the arterial assessment, because a strong pulse may not be due to a good calibre artery but due to hypertension which is common in renal disease (73).

Thus, clinical examination is important as a baseline assessment but additional radiological imaging may be useful.



### 3.2. Ultrasound vascular mapping prior to AVF formation: review of the evidence

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Vascular ultrasound offers a detailed and non-invasive assessment of native vessels.

Selecting sites with good blood vessels may result in better AVF outcomes (8). In the following we review the evidence for ultrasound evaluation prior to AVF formation. The following areas are addressed:

- Pre-operative ultrasound technique
- Pre-operative ultrasound findings and AVF outcomes
- Comparison of pre-operative physical examination with ultrasound in terms of AVF outcomes

The published literature was searched on 29.10.2007 through Pubmed, Embase and the Cochrane library using the following keywords: (“arteriovenous fistula” or “vascular access”) and (“kidney disease” or “dialysis”) and (“ultrasound” or “vein mapping”). Relevant papers were reviewed and their references were also searched.

#### 3.2.1. *Pre-operative ultrasound technique*

A comprehensive review of ultrasound is beyond the scope of this article but we describe aspects relevant to pre-operative evaluation. The ultrasound scanner should allow examination with B-mode and Doppler mode. Linear array probes with a frequency of 7 MHz or higher for B-mode, and 5 MHz or higher for Doppler, are appropriate for most vessels. The ultrasound gel should be warmed and the patient in supine position (50, 74).

#### 3.2.1.1. Arterial scan

The arm arteries are followed longitudinally with directional colour Doppler, from the distal part of the subclavian artery to the radial and ulnar arteries; segments with abnormal colour Doppler are further assessed with B-mode and spectral Doppler to identify a stenosis or occlusion. A 50% narrowing of the arterial luminal diameter or a twofold increase of the peak systolic velocity in the narrowing may be considered as a haemodynamically significant stenosis (75). However, even a lesser stenosis may become significant after AVF formation when blood flow increases. Ultrasound accurately estimates stenosis severity compared to angiography (76). Ultrasound identifies anatomical variations, such as a proximal origin of the radial and ulnar arteries in the upper arm (77).

Successful use of AVF for dialysis requires adequate blood flow, ideally at least 500 ml/min. Ultrasound measurements of vessel diameter and time-averaged velocity allow calculation of blood flow. However, blood flow estimates in a small calibre radial artery are often inaccurate (27, 74). Therefore, the suitability of the radial artery for AVF formation is determined by other criteria, including the diameter, wall morphology, and the hyperaemic response (78).

##### *Arterial diameter*

The internal diameter is measured in longitudinal or transverse section in the radial artery at the wrist and in the distal forearm (50, 79). In longitudinal section, the probe is aligned to show the intimal layers at the near and far walls to measure the distance from intima to intima perpendicular to the arterial wall (see figure 2). In transverse section, the probe needs to be perpendicular to the skin surface and the long axis of the artery parallel to the

skin surface to avoid diameter overestimation. There is some systolic-diastolic diameter variation due to arterial pulsatility. With M-mode, a point of the artery may be insonated over time and the diameter may be measured at the desired point of the cardiac cycle, for example at peak systole, as shown in figure 3 (27, 29). M-mode measurement is useful when the small error due to arterial pulsatility could become relevant, for instance in small calibre arteries. The arterial diameter correlates well with the diameter measured at surgery (27).

#### *Arterial wall morphology*

During AVF maturation, AVF blood flow increases with dilatation of the feeding artery, but this may not occur in a diseased artery. Morphologic information on thickness and structure of the arterial wall (smoothness of the intima, wall thickening, calcification) can be obtained with B-mode (78). Using high resolution ultrasound, intima-media-thickness may be quantified on the far wall of a longitudinal section of the distal radial artery as shown in figure 2 (67, 78). Heavy calcification, identified in B-mode, may make surgery difficult (50).

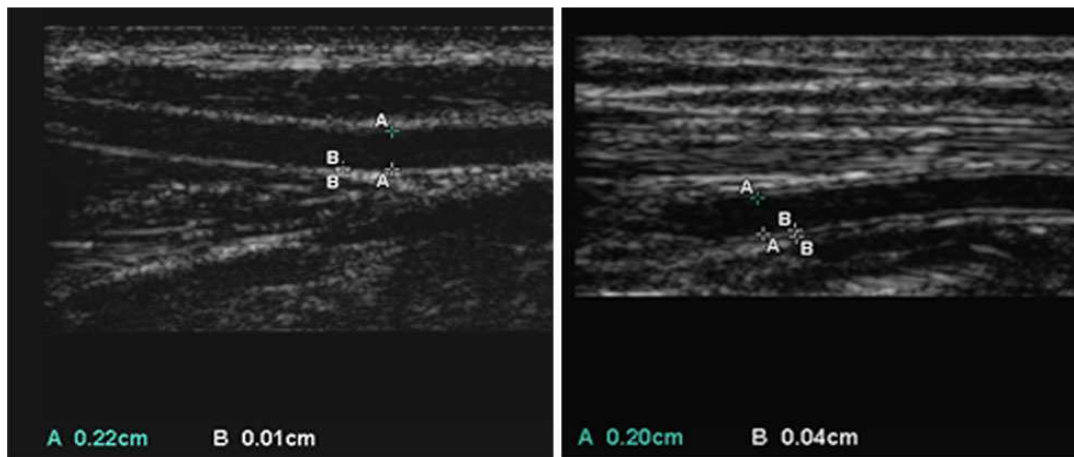
#### *Hyperaemic response*

A healthy artery responds to ischaemia with reactive hyperaemia, ie increased blood flow and dilation of downstream arterioles. This can be assessed with spectral duplex ultrasound (see figure 4). After ischaemia has been induced by clenching a fist or by placing an upper arm pressure cuff, reactive hyperaemia is observed immediately after release. Spectral Doppler in the distal radial artery shows a triphasic high-resistance waveform during ischaemia, which changes to a monophasic low-resistance waveform with overall increased velocity during reactive hyperaemia (50, 80, 81). The spectral waveform change may be

quantified by the resistive index or by the difference in peak systolic velocity (see figure 4).

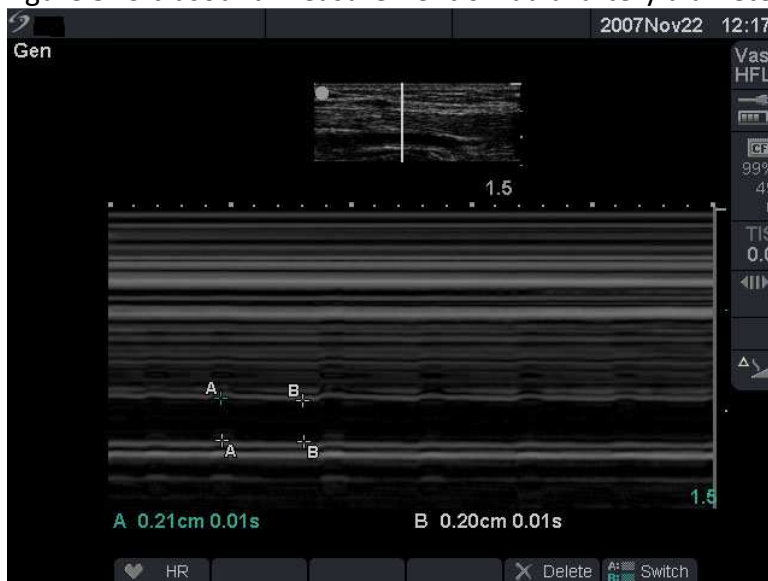
The hyperaemic response of the artery is greater the lower the resistive index (78) or the greater the peak velocity difference (80, 81). The resistive index is less prone to error because is less dependent on the Doppler angle (82).

Figure 2: Ultrasound measurement of radial artery diameter and intima-media-thickness in B-mode



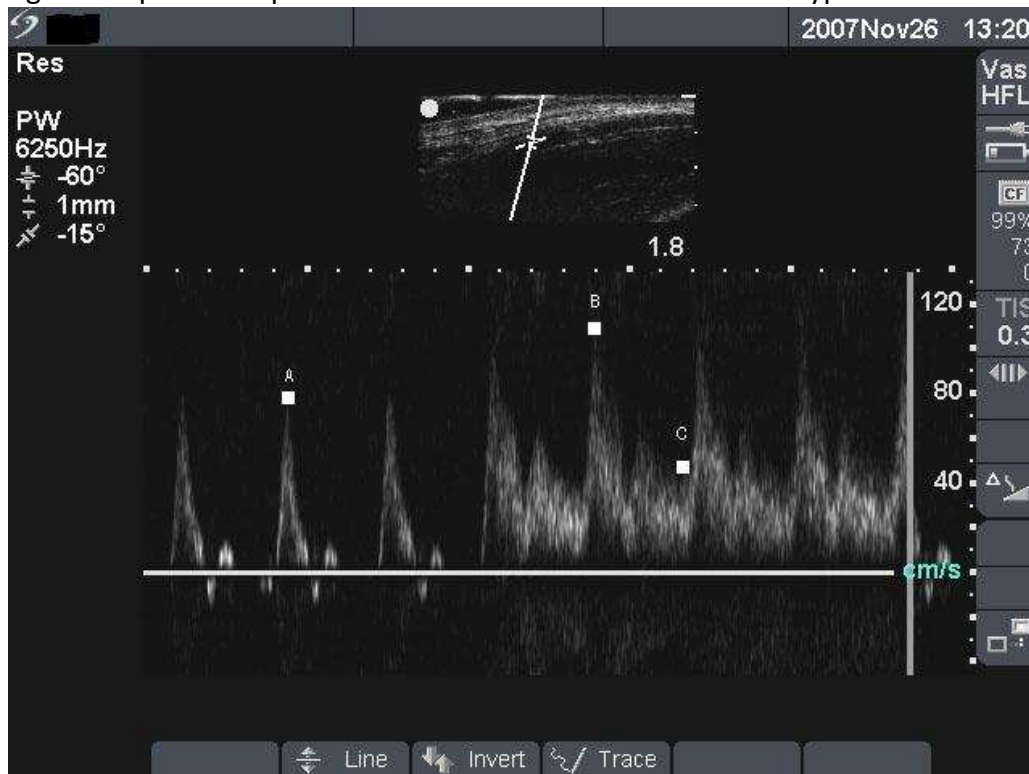
Radial arteries in longitudinal section (left: healthy individual, right: renal patient). A: the internal diameter, measured from near to far wall intima. B: intima-media-thickness. The intima is the bright line adjacent to the lumen and is prominent in the renal patient; the media appears as a dark line to the outer side of the intima.

Figure 3: Ultrasound measurement of radial artery diameter in M-mode



Radial artery of the same renal patient in M-mode. This allows to measure the diameter at peak systole (A) or in diastole (B).

Figure 4: Spectral duplex of the radial before and at reactive hyperaemia



Spectral Doppler waveform in a normal radial artery: During ischaemia (fist clenched), the waveform is triphasic due to high resistance (left part of the picture). As the fist is opened, there is monophasic flow continuing throughout diastole with systolic accentuation (right part of the picture).

The velocity scale in cm/s is shown on the right.

A: peak systolic velocity during ischaemia

B: peak systolic velocity at reactive hyperaemia

C: end-diastolic velocity.

The resistive index (RI) at reactive hyperaemia is defined as:

$$RI = (B - C) / B$$

The difference in peaks systolic velocity is defined as:

$$\Delta PSV = B - A$$

### 3.2.1.2. Venous scan

The forearm cephalic vein is distended by placing a tourniquet downstream. The cephalic vein is followed to the point of drainage into the deep venous system. Patency is assessed by frequent intermittent compression with the probe placed in transverse section (79, 83). If no

suitable cephalic vein is found, the basilic vein is examined. The deep venous system beyond the drain point should be followed to the subclavian vein. Often, the more central veins cannot be assessed directly (83). This scan yields an anatomic vein map and may identify an outflow obstruction which could result in AVF failure.

Criteria used to determine the suitability of the cephalic vein for AVF formation include appearance, diameter, distensibility, Doppler and suitability for cannulation.

#### *Venous appearance and suitability for cannulation*

A normal vein has a thin and smooth wall, an anechoic lumen, and is fully compressible (84). The vein considered for AVF formation should have sufficient length for future needle placement and should be less than 6 mm deep.(72).

#### *Venous diameter and distensibility*

The diameter and depth are measured at points throughout the upper limb but direct pressure on the vein must be avoided by using sufficient gel and resting the probe to the side of the vein. Diameters can be measured in longitudinal (50) or transverse section (79); in transverse section, the probe needs to be perpendicular to the skin surface and the long axis of the vein parallel to the skin surface to avoid diameter overestimation.

Venous diameters can be measured before and after 2 minutes application of a tourniquet, to assess the percentage increase in venous diameter, or venous distensibility (50):

Venous diameter measurements are reproducible between observers, and not different when distended by a tourniquet or pressure cuff (85). However, there is a considerable day-to-day variation (85), and change with patient position or immersion of the arm in a warm bath, which remains even after placement of a tourniquet (86). In order to minimise diameter errors, it is necessary to examine patients in a warm room, using warm gel, and in a standardised position (50).

### *Venous Doppler*

If in doubt, venous patency can be further assessed with Doppler (50, 78). To obtain a venous spectral Doppler waveform, the duplex settings need to be optimal (correct angle, low pulse repetition frequency). In a normal vein, spectral doppler shows spontaneous flow (84).

Central vein disease occurs with dialysis catheters. With ultrasound, the veins central to the subclavian vein are rarely visualised directly but the spectral Doppler waveforms in the subclavian and internal jugular veins allow an indirect assessment. A Doppler waveform changing with respiration and cardiac cycle suggests central venous patency, whereas a monophasic waveform indicates complete occlusion (87). Conventional contrast venography is used to identify a central vein stenosis (72).

### *3.2.2. Pre-operative ultrasound findings and AVF outcomes*

#### *3.2.2.1. Individual pre-operative ultrasound criteria and their relation to AVF outcome*

##### *Arterial criteria*

Arterial diameter has been studied in radiocephalic AVF. Immediate and early AVF failure are well recognised when very small calibre arteries < 1.6 mm are used for AVF construction: Malovrh (27) reported 55% immediate and 64% early failure rate for arteries of 1.5 mm diameter or below, compared to 8% and 17%, respectively, for arteries greater than 1.5 mm. Parmar et al (29) found a 46% early failure rate for arteries below 1.5 mm diameter, compared to 0% for arteries above 1.5 mm. Wong et al (32) reported early failure in all arteries of 1.6 mm diameter or below. Therefore, the larger the arterial diameter the more certain is AVF patency (27, 32, 78, 88). The ideal cut-off point for the arterial diameter in terms of AVF maturation and adequacy for dialysis is not known (50), probably because other factors such as the presence of arterial disease may also play a role. A minimum diameter of 2 mm was first suggested by Silva et al (89) who reported good AVF outcomes (8% early failure, 83% functional primary patency at 1 year). There are no diameter recommendations for the brachial artery, but because of its larger calibre diameter measurement may be less crucial for AVF outcome. However, ultrasound is still useful as it can identify a common anatomical variation, the upper arm division of the axillary or



proximal brachial artery into radial and ulnar arteries, which means that two smaller calibre arteries are found at the level of the elbow.

Radial artery wall changes due to arterial disease are common in patients with endstage renal disease and worse in patients with diabetes or renovascular disease (78). Ku et al (67) reported that measurement of the intima-media thickness (IMT) with ultrasound correlated significantly with histology ( $r = 0.786$ ,  $p < 0.001$ ). Furthermore, Ku et al (67) found a significant correlation between IMT and AVF failure due to thrombosis or dialysis inadequacy at 1 year ( $r = 0.358$ ,  $p = 0.027$ ). These studies show that pre-existing arterial disease is important for AVF outcome and this can be assessed by ultrasonography .

Three studies examined the pre-operative arterial response to reactive hyperaemia with ultrasound and related this to AVF outcome (78, 80, 81). Malovrh (78), who did not select radial arteries for AVF construction by diameter, found that lack of hyperaemic response (defined as a resistive index of  $> 0.7$  at reactive hyperaemia) predicted immediate AVF failure (5% for arteries with hyperaemic response compared to 61% without). For the AVF patent at 24 hours, Malovrh showed that blood flow had increased significantly more in AVF with pre-operative hyperaemic response (close to 500 ml/min) compared to those without (just above 300 ml/min) by 12 weeks. By contrast, Lockhart et al (81) found no difference in AVF outcome for hyperaemic response overall; only among women more AVF were adequate for dialysis with a pre-operative hyperaemic response, defined by the change in

systolic velocity ( $\Delta\text{PSV} \geq 0$ ). This study excluded radial arteries of  $< 2$  mm for AVF formation, but women still had smaller calibre arteries (81). Wall et al (80) found no difference in functional primary patency of access based on arteries with hyperaemic response (defined as  $\Delta\text{PSV} > 5$  cm/s), but a significantly better secondary patency after AVF revision, compared to those without. This was more pronounced for radial compared to brachial artery based access. These studies are heterogeneous and difficult to compare, but suggest that the hyperaemic response may be a useful adjunct in radial arteries of borderline quality or calibre.

#### *Venous criteria*

Three studies examined pre-operative venous diameter and AVF adequacy for dialysis (32, 90, 91). Wong et al (32) found no difference in average venous diameter at the wrist between failed and adequate AVF, but reported that all AVF failed if the diameter was 1.6 mm or less. No tourniquet was used in the studies by Brimble et al (90) and Mendes et al (91) who measured the cephalic vein at several points in the arm and used the smallest vein diameter to predict AVF outcome. Mendes (91) reported that 16% of AVF were adequate with a diameter of 2 mm or less, compared to 76% of those above 2 mm. Brimble et al (90) found a cut-off value of 2.6 mm, but the difference of venous diameter between failed and adequate AVF was only significant for women.

Venous distensibility of the forearm cephalic vein was examined in two studies. Malovrh (78) reported that venous distensibility predicted immediate AVF failure: Pre-operative vein

diameter increased by 12% in failed, compared to 48% in patent AVF. Lockhart et al (46) reported that dialysis adequacy of radiocephalic AVF was similar for cephalic veins of  $\geq 2.5$  mm pre-operative diameter compared to smaller veins that dilated to  $\geq 2.5$  mm only after tourniquet application.

While it is accepted that very small calibre veins will fail, there is no agreed minimum venous diameter to predict radiocephalic AVF maturation (50). A minimum diameter of 2.5 mm with tourniquet was first suggested by Silva who reported good AVF outcomes (8% early failure, 83% functional primary patency at 1 year) (89). Criteria for upper arm veins are not established but a diameter of at least 3 mm has been recommended (50). One should remember that vein diameters have a considerable day-to-day variation and depend on the examination conditions (ambient temperature and patient position) (85, 86). Therefore, veins should be evaluated under optimal conditions and venous distensibility tested in case of apparently small veins.

#### 3.2.2.2. Pre-operative ultrasound use and vascular access outcomes

Several US American studies were recently published which report good or improved AVF outcomes achieved with the use of pre-operative ultrasound. Almost all report a higher rate of AVF formation (in preference to AVG) and better primary patency AVF as outlined in table 8 (89, 92-99). Some authors compare their results achieved with ultrasound to historical controls.

However, a comparison to historical controls may not be appropriate because practice changes other than ultrasound may have contributed to the increase in AVF (100). The rates

of AVF-based access need to be interpreted in the context of USA practice patterns for vascular access which is very different from the European setting. AVF use among prevalent haemodialysis patients in the USA has been significantly lower than in Europe due to a preference for AVG (24% versus 80%), even after accounting for patient differences, which suggests a difference in practice patterns (36). The USA practice has been influenced by reimbursement issues of the USA healthcare system (8, 100). The vascular access guidelines of 1997 of the Dialysis Outcomes Quality Initiative (DOQI) and the 2000 update recognised the serious negative effects of AVG use and defined strategies and targets to increase AVF use on dialysis (50% for patients initiating and 40% for patients established on haemodialysis) (101). The 40% target for prevalent patients was met by 17 out of 18 renal networks in the USA by December 2006 (102). By contrast, AVF formation is predominant in European countries (40, 103). The report by Ngyuen et al (96) of a 98% AVF-based access in a USA renal network is exceptional and reflects his personal initiative in terms of promoting AVF use. This study supports the view that substantial increases in AVF rates can be achieved by changing established practice patterns.

Table 8: Vascular access outcomes before and after using pre-operative ultrasound

Author	n	Ultra-sound	% AVF-based access after - before ultrasound		1 year primary patency after - before ultrasound		% AVF in forearm
<i>Prospective</i>							
Patel 2003 (40)	256	selective	73%	61%	57%*	73%*	31%
Allon 2001 (39)	217	routine	64%	34%	54%*	46%*	54%
Silva 1998 (29)	172	routine	63%	14%	83%*	48%	-
Huber 2002 (41)	139	routine	90%		84%*		25%
Jungling 2003 (49)	51	routine	94%		71%*		85%
McGill 2005 (42)	-	routine	72%	32%	-	-	52%
Nguyen 2003 (43)	-	selective	98%		-	-	35%
<i>Retrospective</i>							
Konner 2002 (48)	748	selective	>95%		76%		53%
Asher 2000 (28)	267	selective	68%	5%	84%		-
Gibson 2001‡ (44)	187	selective	74%	41%	56%		-
Ackad 2005 (45)	111	routine	87%	49%	-	-	31%

NOTE:

(\*): proportion of AVF adequate for dialysis use

### 3.2.3. Comparison of pre-operative physical examination and ultrasound in terms of AVF outcomes

So far we have shown that pre-operative ultrasound is useful. In the following we examine how pre-operative ultrasound compares to physical examination which has been traditionally used. Physical examination can be carried out rapidly at the patient's bed-side

at no extra cost or equipment. Ultrasound, in turn, allows non-invasive and safe imaging, but requires some time for the scan as well as skill and experience of the operator.

In general, physical examination usually yields more information from venous than arterial assessment: If the forearm vein is palpable, its calibre, patency and course can be readily assessed. Arterial examination is limited to palpation of the pulse and measurement of the blood pressure (32, 78). The pulse may appear strong due to raised blood pressure, common among renal patients. By contrast, ultrasound gives relatively more information about the artery than the vein, as we have discussed above. Wong et al (32) compared the accuracy of predicting AVF failure for pre-operative evaluation of the forearm cephalic vein: Positive predictive value was better for ultrasound than for physical vein palpation (1.0 versus 0.5), indicating that AVF failure occurred for all veins which were abnormal on the ultrasound scan (defined by a diameter < 1.6 mm or a stenosis) (32).

Clinical examination alone is insufficient in a considerable proportion (approximately 25-50%) of patients (78, 104). Parmley et al (105) reported good AVF outcomes (98% AVF of all access formation, 94% functional primary patency at 1 year) for ultrasound use in a selected group of 47 patients with insufficient clinical findings. Two prospective studies compared the findings of clinical and ultrasound examinations directly. The assessments were performed by different examiners unaware of each others' findings. Robbin et al (106) followed 52 patients and reported that ultrasound information changed the clinical plan in 32%: Among those, half could receive a fistula rather than a graft while 19% did not undergo unnecessary wrist exploration. Wells et al (104) followed 145 patients and found that clinical examination was insufficient in 27%; ultrasound made a relevant contribution for half of

those. However, clinical examination appeared sufficient in 73%; in this group ultrasound changed the surgical plan in < 1%. This shows that clinical assessment correctly identifies those patients who benefit from further imaging. Furthermore, Nursal et al (107) studied 70 selected patients who had adequate vessels on physical examination. and randomised them to have AVF formation on the basis of physical or ultrasound examination alone. There was no significant difference in terms of immediate or simple primary patency at one year, but the sample size was too small for a true negative study. These studies provide evidence that selected patients with insufficient clinical examination benefit from ultrasound, while ultrasound is not needed when adequate vessels are defined by clinical examination.

Routine use of ultrasound was compared to exclusive physical examination in two randomised trials. Mihmanli et al (108) reported that immediate patency among 124 patients was significantly greater in the ultrasound than in the clinical group (95% versus 75%). Zhang et al (109) reported that among 68 patients, simple primary patency at 6 months was higher in the ultrasound than in the clinical group (90% vs 80%). These studies show that a strategy of routine ultrasound, compared to one of exclusive physical examination without imaging, can reduce early AVF failure. However, as exclusive clinical assessment without an option for imaging is not usual clinical practice, the benefit of routine ultrasound may appear greater than it would be, if ultrasound had been used selectively in the clinical groups.

### *3.2.4. Conclusion:*

More haemodialysis patients nowadays are older and have diabetes or cardiovascular disease. These vascular risk factors are associated with increased arterial disease and an increased risk of AVF failure. Pre-existing arterial disease can be assessed by ultrasound assessment which is particularly important for the radial artery.

Furthermore, clinical assessment may be inconclusive in a considerable proportion of patients, for instance when veins are not apparent in the obese. Pre-operative ultrasound assessment predicts AVF patency and maturation for dialysis. Ultrasound is of particular benefit when physical examination is insufficient, but has little added value when physical examination is satisfactory.

Therefore, physical examination should be used initially for all patients to evaluate a suitable site for AVF surgery. Patients who are likely to benefit from pre-operative ultrasound evaluation are those with:

- insufficient clinical examination (obese, absent pulses, multiple previous access surgery)
- possible arterial disease (older age, diabetes, cardiovascular disease)
- possible venous disease (previous cannulation)

In summary, there is good evidence to recommend pre-operative ultrasound in selected patients to improve AVF outcome. By contrast, there is limited research on routine pre-operative ultrasound for all patients. There is an argument that routine ultrasound may offer further benefits given the widespread problems of vascular disease, older age and venous



cannulation among HD patients. Therefore, routine pre-operative ultrasound is a central part of research in this thesis.

### **3.3. Post-operative AVF screening**

#### ***3.3.1. Clinical relevance***

Despite efforts at improving AVF outcome with pre-operative screening and good surgery, a considerable proportion of AVF still fail, particularly among women (30, 40). However, because further surgery or endovascular intervention can rescue a good proportion of failed AVF, appropriate screening is important.

AVF failure most commonly is due to thrombosis or a flow-limiting stenosis, which in turn can trigger thrombosis. In more than half of new AVF, stenoses develop within 12 weeks after surgery (110). Such stenoses do not always cause sufficient flow-limitation to prevent AVF use for haemodialysis (111). They are probably due to de novo myo-intimal hyperplasia (26). At a clinical level, thrombosis is usually obvious, because the AVF outflow vein lacks the signs of a patent AVF of high blood flow (thrill on palpation, bruit on auscultation). However, it can be challenging to judge whether a patent AVF develops (matures) adequately to be usable for haemodialysis later. In particular, the distinction between a slowly maturing AVF and one which fails to mature is difficult. Often, nephrologists wait for further maturation, but in an AVF with maturation failure due to an underlying stenosis, waiting longer results in delay of the necessary intervention, loss of the AVF through clot, or use of a CVC for haemodialysis (19).

The best method and timing of post-operative screening has not been established; published screening methods include intra-operative blood flow measurement, physical examination and duplex ultrasound, which will be discussed below.

### ***3.3.2. Intra-operative blood flow***

Intra-operative blood flow, measured with a transit-time ultrasonic flowmeter, was significantly lower (98 versus 230 ml/min) in AVF which then thrombosed or failed to mature within a month after surgery (23). This approach is not widely used and may be affected by intra-operative spasm of the artery (32).

### **3.3.3. Physical AVF examination**

Physical examination of the AVF after surgery has been described particularly for forearm (radiocephalic) AVF (112, 113). In brief, this is carried out by inspection and palpation of the AVF from the anastomosis along the course of the vein to the point of drainage into the deep venous system. A mature, functioning AVF has a good calibre vein along its course which has a palpable thrill and audible bruit continuous over the cardiac cycle because of high blood flow and resultant vessel wall vibration; thrill and bruit decrease in intensity with increasing distance from the anastomosis; arm elevation above the head empties the outflow vein because the blood pressure drops at and beyond the anastomosis. A flow-limiting stenosis changes the haemodynamics and hence alters the physical findings, depending on its site: A weak thrill and poor vein calibre suggest stenosis in the feeding artery or at the anastomosis. A pulsatile, aneurysmal venous segment in the vein beyond the anastomosis, which fails to empty on arm elevation, suggests a stenosis in the vein a short distance beyond the anastomosis, which often is palpable as a focal venous calibre

narrowing. An engorged, pulsatile outflow vein with dilated venous collaterals, which fails to empty on arm elevation, suggests a venous stenosis further downstream; the stenosis itself may be manifest as a palpable narrowing, or a sudden increase in pitch and loudness of the bruit along the course of the vein, because of further increase in blood velocity and turbulence at the site of stenosis (112). Recently, physical AVF examination of dysfunctional AVF was compared to angiography findings and good agreement was demonstrated for most stenoses (114). Conversely, adequate AVF maturation can be reliably determined by experienced haemodialysis nurses by 4 months after surgery (106). Physical examination can be carried out at the bedside, rapidly without need for any equipment, but a detailed examination as described above is required by an experienced examiner (113, 114). Although a rudimentary physical assessment is common practice, the nephrology community currently does not have adequate skills for such detailed examination. Staff training as well as development of a standardised physical examination protocol would be needed before screening with physical examination can be implemented. Also further research is needed to determine whether physical examination predicts AVF failure early, within 4 weeks after surgery.

#### ***3.3.4. Ultrasound AVF examination***

Duplex ultrasound allows detailed, non-invasive vascular imaging and portable machines nowadays allow bedside assessment, although the examination is time-consuming and also requires an experienced examiner (74). Many studies have used post-operative ultrasound screening and a recent meta-analysis concluded that post-operative AVF ultrasound screening was a useful predictor (relative risk 4.3, confidence interval 3.4-5.5) of subsequent

AVF failure (115). The examination technique with colour duplex ultrasound is well described (74). In brief, the vasculature is followed from the brachial artery to the anastomosis, then along the venous system to the level of the clavicle; the central veins cannot be directly assessed by ultrasound. Grey-scale (B-mode) ultrasound can orientate about the anatomy and vascular morphology initially, but colour duplex ultrasound is used to detect significant stenoses along the course of the vasculature. Colour duplex ultrasound is based on detecting the direction and the speed of red blood cells travelling along a vessel and therefore is sensitive to changes in blood haemodynamics: minor vessel wall irregularity can show as focal reversal of blood flow, whilst significant stenoses show as turbulent flow. Further evaluation with spectral duplex ultrasound is needed to determine the increase in peak systolic velocity (116). A stenosis is usually considered as haemodynamically significant if there is at least doubling of velocity; the anastomosis is an area with a lot of turbulence resulting from the vessel angle as well as the blood pressure drop across the anastomosis, and therefore an at least three-fold increase in velocity is considered significant (110). Because poor AVF blood flow is associated with AVF failure and non-maturation, blood flow has been estimated from ultrasound measurements of diameter and time-averaged velocity (117). As blood flow is proportional to the product of diameter and velocity, any measurement error may distort the blood flow estimate considerably; therefore, measuring blood flow in the brachial artery (a straight uniform vessel) may be more reliable than in the fistula vein (15). The exact cut-off for inadequate blood flow is not clearly established and time-dependent, because AVF blood flow increases within the first month after surgery (15). Further research is needed to establish accurate weekly brachial artery blood flow limits during the first month, which distinguish maturing AVF from those which fail. Also it is

unknown whether an early strategy of pre-emptive screening and intervention in AVF with low blood flow would improve AVF outcome.

#### **4. Interventions to manage AVF failure: focus on non-maturation**

The traditional approach to a dysfunctional AVF has been to assess it with imaging by ultrasound or angiography, identify the underlying abnormality, and salvage the AVF by appropriate surgical or endovascular repair, if possible. More recently, preventative drug treatment has been considered. We will present both approaches in the following.

##### **4.1. Surgical and endovascular salvage of dysfunctional AVF**

Stenoses and other abnormalities develop in over half of new AVF within the first 3 months after surgery and can be detected with ultrasound (110). However, stenoses may be found in 2/3 of well-functioning AVF used for haemodialysis (111). Therefore, any intervention should be targeted to a lesion in an AVF which is not working well for dialysis: both surgical and endovascular salvage can be used (118). A recent meta-analysis of treatment of non-matured AVF concluded that various endovascular interventions, including angioplasty, were effective at improving AVF usability for dialysis from a 51% primary to a 76% secondary patency rate at 1 year; on average there was a 47% increased chance of AVF maturation with intervention (115). Because stenosis recurs after initial angioplasty, then requiring further AVF salvage, there are early reports on the use of drug eluting stents (25). Surgical revision by creating a more proximal anastomosis is the preferred option for forearm (radiocephalic) AVF with anastomotic or peri-anastomotic stenosis; this salvage had an AVF patency of 64 % at 1 year (119). The treatment of thrombosed AVF is more challenging but successful treatment has been reported with endovascular thromb-aspiration followed by angioplasty to the

underlying stenosis, although a variety of techniques exist for thrombectomy (120). Surgical thrombectomy, usually carried out with a Fogarty catheter, is an alternative approach (118). It is unknown whether surgical or endovascular AVF salvage result in better outcomes.

## **4.2. Drugs and AVF patency**

Given the threat of thrombosis and excessive intimal proliferation in new AVF, the effect of medication has been assessed.

### **4.2.1. Aspirin**

A Cochrane review concluded that patients on Aspirin had less early access thrombosis among AVF and AVG in the first 6 months after surgery (121). A large international observational study of patients starting haemodialysis found that persistent Aspirin use for at least one year was associated with a reduced risk of final, unsalvageable AVF failure (hazard ratio 0.63, confidence interval 0.42-0.95), but without increased risk of gastrointestinal bleeding (122). These data support the hypothesis that Aspirin may be useful for early and later AVF patency; however a randomised controlled trial is required to assess safety and efficacy of the use of Aspirin for AVF patency.

### **4.2.2. Clopidogrel**

In a randomised, double-blind, placebo-controlled trial, a six week treatment with clopidogrel starting the day after AVF formation resulted in significantly reduced early AVF

thrombosis (12% versus 20%) by 6 weeks; primary AVF failure in terms of dialysis use was equivalent between groups (123).

#### *4.2.3. Angiotensin-converting enzyme inhibitors*

A large international observational study reported that treatment with angiotensin-converting enzyme inhibitors was associated with significantly better (relative failure risk 0.56;  $p=0.010$ ) secondary AVF patency (124). This observation is consistent with the presumed role of angiotensin II in terms of promoting vascular smooth muscle cell proliferation and the development of neo-intimal hyperplasia (25). These data are hypothesis-generating and the efficacy of angiotensin-converting inhibitors should be tested with a randomised controlled trial.



## 5. Purpose of thesis

In summary, AVF are the preferred form of vascular access for haemodialysis treatment in endstage renal disease, but have a considerable failure rate, in particular forearm AVF which depend on smaller calibre vessels. Primary failure of AVF includes all AVF never develop (mature) to become usable for haemodialysis and occurs in a considerable proportion of AVF usually within the first three months after surgery. Three aspects of primary failure are important – recognition of risk factors, screening, and intervention.

We saw that the risk of primary AVF failure is higher in some patient groups, including the elderly, women, diabetics and those with cardiovascular disease; the significance of ethnicity is unclear. We considered the risk of pre-existing vascular disease and saw that intimal hyperplasia of the feeding artery was associated with increased risk of early AVF failure; whilst venous changes with intimal hyperplasia and medial fibrosis have been described in renal patients, their relevance to AVF outcome is unknown. We found evidence that the skill of the operating surgeon can affect AVF outcome, but that surgical trainees can produce good results if appropriately supervised.

In terms of screening, physical as well as ultrasound examination are clinically usable, both for vascular mapping prior to AVF formation, as well as for AVF examination after surgery. The aim of pre-operative assessment is to avoid unsuitable vessels and select good vessels for surgery. Ultrasound investigation is useful in selected patients such as those with suspected vascular disease but it is unknown whether routine pre-operative ultrasound for vascular mapping has any benefit. Early post-operative AVF examination may allow earlier diagnosis and treatment of immature AVF, but exact protocols are not established.

Interventions with endovascular or surgical technique are well established to salvage failed AVF and result in longer AVF survival (= primary assisted or secondary patency). Medications may also be of benefit; Clopidogrel has been shown to reduce early AVF thrombosis in a randomised controlled trial.

Current knowledge is limited in the following areas, which we used as hypotheses for this thesis:

1. AVF outcomes may be different among ethnic groups, given a higher rate of diabetes among African and Indo-Asian minorities.
2. Structural pre-existing venous wall changes may be related to AVF outcome and patient characteristics.
3. Routine pre-operative vascular ultrasound imaging may produce better AVF outcomes than clinical assessment, supported by selective imaging.
4. Early post-operative AVF assessment at 4 weeks may identify AVF failing to mature.

## **B) Thesis Methods**

For the work in this thesis, three populations of patients with endstage renal disease who underwent AVF surgery were studied at a single large urban hospital (Heart of England Hospital, Birmingham, United Kingdom). Details of the study methodologies are given in the individual chapters.

### **1. Ethnicity study**

For this retrospective analysis, all patients who had vascular access (AVF or AVG) surgery between 01.04.1998 and 31.03.2004 were identified through the clinical coding system.

Electronic patient records were searched for details of access surgery, access failure, access salvage, haemodialysis use, as well as patient characteristics including ethnicity and patient comorbidity. Patients with first-time permanent access formation (n=453) were included in the analysis, whereas patients with previous access formation were excluded.

### **2. Pre-operative ultrasound mapping study**

In a prospective randomised study we randomised 218 patients to routine ultrasound (experimental) versus physical (control) vascular examination, prior to AVF formation. All patients referred for formation of first- or second time permanent vascular access between 01.09.2004 and 30.09.2006 were eligible to take part in the study; patients for AVF salvage operations, or those with more than one previous AVF were excluded.

### **3. Vein histology study**

All patients who underwent AVF formation between 16.12.2005 and 10.01.2007 were eligible for participation in the study and n=70 were recruited; we excluded those for AVF salvage or AVG formation. At surgery, a short (< 1 cm) segment of the most distal part of the vein was resected, placed in formalin and transferred for histological preparation and staining; n=60 specimens were available for histological analysis. Follow-up ended on 08.02.2008.

### **4. Post-operative AVF examination study**

Patients who were recruited to the pre-operative ultrasound mapping study above had also consented to be examined after AVF formation. A proportion (n=133) for whom re-attendance was practical, attended a follow-up some 4 weeks after surgery. Those who preferred not to attend, and those whose AVF had thrombosed by 4 weeks, were not examined.

## **C) Thesis Results**

### **1. The effect of ethnicity and other patient characteristics on outcomes of arteriovenous fistulae for haemodialysis**

Presented as poster (SU110: Primary vascular access in patients of different ethnic origin) at the 5<sup>th</sup> International Congress of the Vascular Access Society at Nice (France) from 11-13.06.2007

#### **1.1. Abstract**

**OBJECTIVES:** Ethnic minority populations in the United Kingdom have an increased incidence of renal disease and of diabetes. We studied whether primary vascular access outcome was different between Caucasian and non-Caucasian ethnic groups.

**METHODS:** Data were collected retrospectively for primary vascular access from all patients who had surgery between 1998 and 2004 at a single centre. Ethnic origin was defined as Caucasian and non-Caucasian (Indo-Asian, African-Caribbean and other). We analysed ethnicity in terms of early AVF outcomes (immediate and primary AVF failure) and longer term AVF outcomes (primary and assisted primary AVF patency), and corrected for other patient variables.

RESULTS: Of 453 vascular access formations in 453 patients, 449 were AVF. Patients were mainly Caucasian (76%), most non-Caucasians were from the Indian Subcontinent (Indo-Asian = 18%, African = 5%, other = 1%). Most AVF were in the forearm and this was not different among ethnic groups (Caucasian = 88%, non-Caucasian = 91%,  $p=0.425$ ). Early AVF outcome was not different among Caucasian and non-Caucasian ethnic groups: There was similar immediate AVF failure (5% versus 7%, respectively,  $p=0.505$ ) and primary failure (44% versus 47%, respectively;  $p=0.616$ ). Longer term AVF outcomes were also not affected by ethnicity: There was similar 1-year primary AVF patency (Caucasians = 53%, non-Caucasian 47%;  $p = 0.371$ ) and 1-year assisted primary AVF patency (Caucasians = 58%, non-Caucasian 58%;  $p = 0.708$ ). Non-Caucasian ethnicity was significantly associated with Diabetes (odds ratio 3.018,  $p<0.001$ ).

CONCLUSION: Early and longer term AVF outcomes are equivalent for Caucasian and non-Caucasian patients with endstage renal disease in the United Kingdom, despite a higher rate of Diabetes. AVF formation should therefore be attempted regardless of the patient's ethnic origin.

## 1.2. Background

Over the last 3 decades, there has been an increasing demand for haemodialysis, particularly among older patients (1). Diabetes and cardiovascular disease are now common among haemodialysis patients (2). Some authors have ascribed an increased risk of primary AVF failure older age, cardiovascular disease, or diabetes (37-39).

Ethnic minorities in the United Kingdom most commonly are of Indo-Asian or African background have a higher risk of renal disease, and may form up to 40% of the renal patient population in some parts of the country (1, 125). Differences in vascular access outcomes might be expected among ethnic minorities because diabetes is more common.

Therefore, we examined the effect of ethnicity on AVF outcome, alongside other patient demographics and comorbidities.

## 1.3. Methods

### *1.3.1. Patients and Data acquisition*

This project was approved and registered as an audit of vascular access outcome at the clinical governance department of Heart of England Hospital, Birmingham.

All patients who had permanent vascular access (AVF or AVG) surgery between 01.04.1998 and 31.03.2004 at Birmingham Heartlands Hospital were identified via the hospital coding system. We included all new formations of the first permanent vascular access (AVF or AVG) and all first salvage interventions in our analysis; this included both patients already

dependent on haemodialysis and those expected to start in the near future. We excluded all vascular access surgery beyond the first access.

Prior to access formation, all patients underwent physical vascular examination; additional imaging (ultrasound or angiography) was carried out if deemed necessary by the surgeon.

Surgery (access formation and salvage) was performed by 6 consultant vascular surgeons and trainees operating under their supervision. It was not possible to retrieve the names or grades of the surgeons from the electronic patient record. Non-functional but patent access was usually assessed by angiography. Endovascular or surgical salvage was carried out as appropriate.

Data were acquired from the electronic case-note record and the dialysis sessions record:

- Patient demographics:
  - age, gender, ethnicity
  - dependency on haemodialysis at time of access surgery,
  - Diabetes (type1 and type2),
  - cardiac disease (angina, past myocardial infarction, coronary artery bypass graft or cardiac transplant),
  - cerebrovascular disease (transient ischaemic attack or ischaemic vascular event),
  - peripheral vascular disease (claudication, past angioplasty or bypass graft in lower limb arteries).
- vascular access type



- haemodialysis use

### *1.3.2. AVF outcomes definitions*

The primary outcome was early AVF outcome (= immediate and primary failure). We also examined longer term AVF outcome in terms of time from surgery to AVF failure.

We defined vascular access outcome as “patent” when used for dialysis, “failed” when not usable for haemodialysis. Failure meant that the access was unusable for dialysis, so that a new AVF, salvage of the existing AVF, or insertion of CVC was required.

We defined short-term access outcomes as “immediate failure” (= all access failures within 24 hours of surgery) and “primary failure” (= all access which was never adequate for haemodialysis following initial surgical formation) (30).

We defined longer term access outcomes by access survival. “Primary patency” referred to all access used for haemodialysis, from date of surgical formation to date of access failure. “Assisted primary patency” referred to all access used for haemodialysis, from date of surgical formation to date of access failure, including extra patency time gained by interventions to salvage patent access (33). Data were censored at the time of patient death, change to kidney transplant or peritoneal dialysis, or end of follow-up period on 19 Jan 2005.

Access outcomes were classified as “unknown” if the patient had not started dialysis by the end of the follow-up period, or died within 12 weeks after surgery without using the access. We excluded these unknown outcomes from the analysis because, although patent, we did not know their usability for haemodialysis.

### *1.3.3. Statistics*

Univariate analysis of access failure and clinical variables was made using Chi-square test with Yates' continuity correction for categorical data. Age as the only continuous variable was skewed and was analysed by independent sample t-test after square transformation.

Short-term access outcomes: Immediate failure was analysed in all patients. Primary failure outcomes were analysed for the patients with known outcome.

For multivariate analysis we used logistic regression with forward stepwise exclusion of non-contributing variables based on the likelihood ratio. One statistical model was to explain ethnicity by patient variables. A further model was to predict primary AVF failure by patient variables including ethnicity. Among patient variables, age was corrected for skew by =  $(\text{age}^2)/1000$ .

Longer term access outcome: access patency (primary and assisted primary patency) was assessed by life table analysis and significance was tested with log-rank test. Cox regression model was used to examine patient variables as possible predictors for longer term AVF outcome as the dependent variable. Predictors were included by a forward stepwise approach based on the likelihood ratio.

SPSS version 16 was used for analysis and a p-value of  $\leq 0.05$  was considered significant.

## 1.4. Results

### 1.4.1. *Characteristics of vascular access and patients*

There were 453 first permanent access operations (in 453 patients): 449 (99%) were AVF, 4 were AVG. Therefore, further analysis was restricted to AVF. Most AVF were in the forearm and this was not different among ethnic groups (Caucasian = 88%, non-Caucasian = 90.9%,  $p=0.425$ ).

Patients were elderly (median 68.5 years, interquartile range 57.9 to 76 years), 63% were male, 76% were Caucasian (non-Caucasian: 18% Indo-Asian, 5% African, 1% other). At the time of access formation, 54% of patients had not yet started haemodialysis, 46% were on dependent on renal support (43% haemodialysis, 2.6% failing peritoneal dialysis, 0.4% failing transplant). Thirty percent had diabetes, 33% had cardiac disease, 9% had peripheral vascular disease, and 11% had cerebrovascular disease.

Patient variables, including ethnicity, revealed several associations on univariate comparison: Non-caucasian ethnicity was significantly associated with younger age (mean 63.4 versus 70.1 years,  $p<0.001$ ), more diabetes (46.7% versus 24.3%,  $p<0.001$ ), and less peripheral vascular disease (96.2% versus 89.5%,  $p=0.046$ ). Older age was significantly associated with cardiac ( $p<0.001$ ), peripheral vascular ( $p=0.008$ ) and cerebrovascular disease ( $p=0.004$ ). Diabetes was associated with cardiac ( $p=0.001$ ), peripheral vascular ( $p=0.001$ ), cerebrovascular disease ( $p=0.046$ ).

We carried out a multivariate analysis with ethnicity (non-Caucasian versus Caucasian) as the dependent variable, and the other patient variables as covariates. Diabetes, younger age and

the absence of peripheral vascular disease were the only independent “predictors” of non-Caucasian ethnicity (table 9). Thus, diabetes was three times more likely among non-Caucasians, but peripheral vascular disease had only a one in five chance to occur in non-Caucasians, and older age was less likely among non-Caucasians.

Table 9: Logistic regression - ethnicity (non-Caucasian versus Caucasian ethnicity) as dependent variable, predicted by patient characteristics as covariates

	p-value	OR	95% C.I. for OR	
			Lower	Upper
PVD	0.010	0.199	0.058	0.681
Diabetes	<0.001	3.018	1.870	4.869
Age	0.001	0.793	0.695	0.904

Notes: 95% C.I. = 95% confidence interval; OR = odds ratio; PVD = peripheral vascular disease; age was corrected for skew = (age<sup>2</sup>)/1000. n=433/449; incomplete data n=16

#### ***1.4.2. Early AVF outcomes predicted by patient variables***

Early AVF failure was not associated with ethnicity (table 10). Immediate failure had no significant association, although immediate failure rates tended to be higher among women compared to men (8.3% versus 3.9%, p=0.084). Primary failure was significantly associated with diabetes (55.9% versus 39.5%, p=0.004) and tended to be worse among women (50.7% versus 40.7%, p=0.065).

Table 10: AVF failure predicted by patient variables

		Immediate failure			Primary failure		
Ethnicity	Caucasian	n=337	5%	p = 0.505	n=294	43.5%	p = 0.616
	Non-caucasian	n=109	7.3%		n=102	47.1%	
Gender	Female	n=169	8.3%	p = 0.084	n=152	50.7%	p = 0.065
	Male	n=279	3.9%		n=246	40.7%	
Diabetes	no Diabetes	n=312	4.8%	p = 0.332	n=276	39.5%	p = 0.004
	Diabetes	n=130	7.7%		n=118	55.9%	
Cardiac	no cardiac disease	n=298	5.4%	p = 0.876	n=273	42.1%	p = 0.206
	cardiac disease	n=144	6.2%		n=121	49.6%	
PVD	no PVD	n=404	5.9%	p = 0.633	n=361	43.2%	p = 0.160
	PVD	n=38	2.6%		n=33	57.6%	
CVD	no CVD	n=393	6.1%	p = 0.404	n=354	44.4%	p = 1.000
	CVD	n=49	2%		n=40	45%	
Dialysis	pre-dialysis	n=258	6.2%	p = 0.646	n=222	46.4%	p = 0.444
	on dialysis	n=190	4.7%		n=176	42%	
AVF site	Forearm	n=391	6.4%	p = 0.135	n=347	44.7%	p = 0.755
	upper arm	n=49	0%		n=44	40.9%	
Age		n=448		p = 0.764	n=448		p = 0.776

Notes: Univariate comparisons, of total n=449; data available for analysis are shown as n; n for primary failure was restricted to those with known AVF outcome in terms of dialysis use versus non-use. Age was corrected by square transformation and then tested by t-test. AVF site = forearm versus upper arm AVF. CVD = cerebrovascular disease. DM = diabetes mellitus. Dialysis = patients established on dialysis versus those who had not started at time of AVF formation. PVD = peripheral vascular disease.

For multivariate analysis, we examined primary failure predicted by patient variables.

Diabetes doubled the risk of primary AVF failure, whilst women had a 1.7 higher risk than men (table 11). The other patient variables, including ethnicity, did not explain primary failure.

Table 11: Logistic regression with primary AVF failure as dependent variable, predicted by gender and Diabetes

	p-value	OR	95% C.I. for OR	
			Lower	Upper
Female gender	.015	1.684	1.107	2.564
Diabetes	.002	1.983	1.272	3.090

Notes: 95% C.I. = 95% confidence interval; OR = odds ratio. Complete outcomes available for analysis available in n=389 of n=398 with known primary failure as dialysis use / non-use

Further analysis of primary AVF failure by main clinical presentations revealed that thrombosis and stenosis were the most common causes, and these were similar between ethnic groups, as shown in table 12 (p=0.220).

Table 12: Main presentations of primary failure by ethnicity

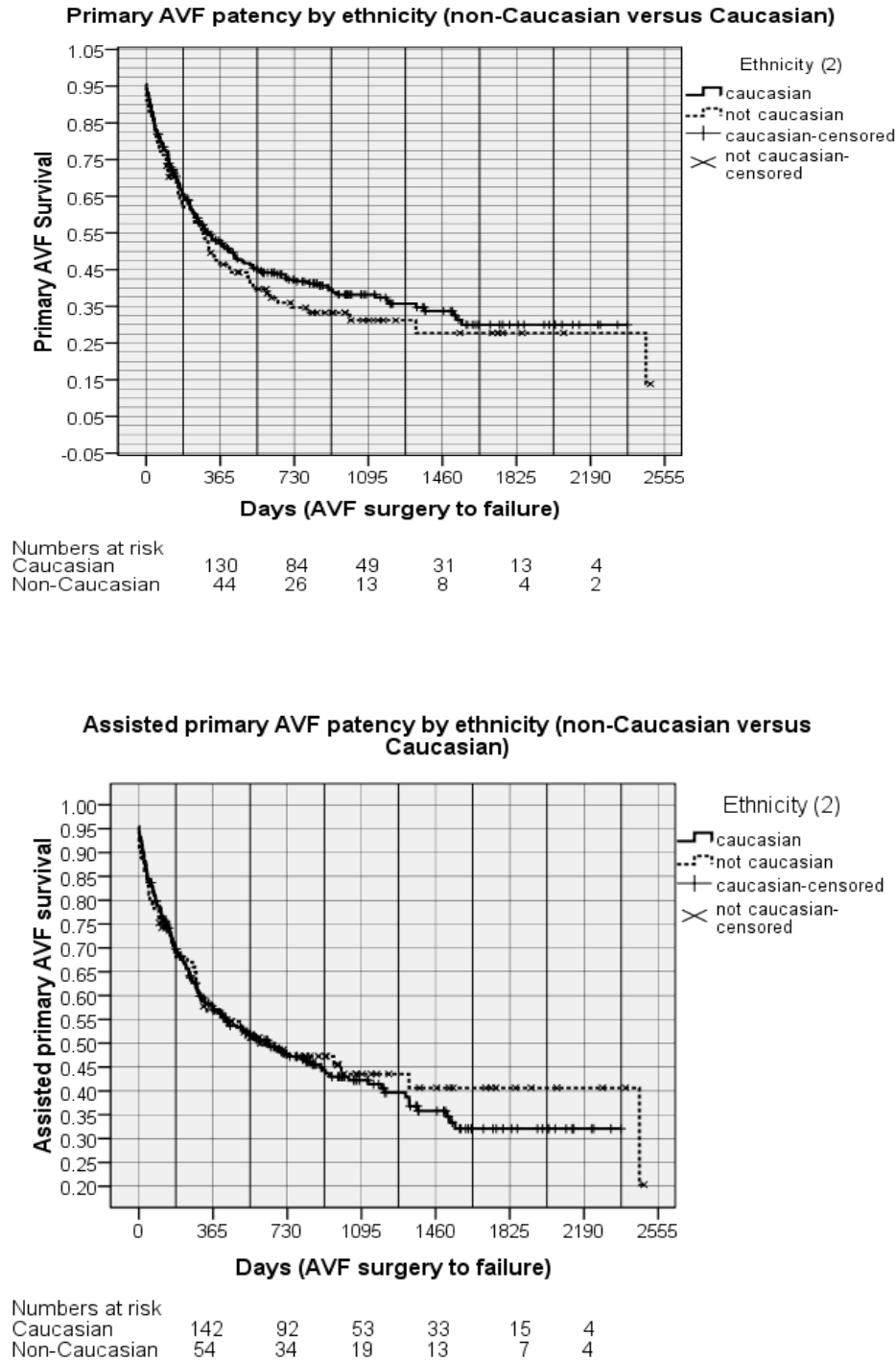
		main presentations of primary failure			
		clot	stenosis	other failure	Total
Ethnicity	caucasian	93	68	13	174
		53%	39%	8%	100%
	not caucasian	28	31	8	67
		42%	46%	12%	100%
Total		121	99	21	241
		50%	41%	9%	100%

#### *1.4.3. Longer term AVF outcomes predicted by patient variables*

Primary and assisted primary AVF patency rates were not significantly different between Caucasian and non-Caucasian ethnic groups (figure 5). Specifically, primary AVF patency rates at 1-year and 5-years were 53% and 30% for Caucasians, respectively, and 47% and 28% for non-Caucasians, respectively (log rank Chi-Square=0.801, df=1, p=0.371). Assisted primary AVF patency rates at 1-year and 5-years were 58% and 40% for Caucasians,

respectively, and 58% and 32% for non-Caucasians, respectively (log rank Chi-Square=0.140, df=1, p=0.708).

Figure 5: a) Primary and b) assisted primary AVF patencies by ethnicity



Using Cox-regression we examined the effect of patient variables including ethnicity on AVF survival (primary and assisted primary patency, respectively) as the dependent variable.

Ethnicity did not predict AVF survival, but both female gender and diabetes were associated with worse AVF survival (table 13). More specifically, women had 50% more risk of longer term AVF failure, and diabetics had at least 40% more risk. Also, cardiac disease had worse primary patency and peripheral vascular disease worse assisted primary patency (table 13).

Table 13: Cox regression for primary and assisted primary patency as dependent variables, respectively; patient characteristics as predictors (significant predictors shown)

		P-value	HR	95% CI for RR	
				Lower	Upper
Primary patency	Female gender	.001	1.539	1.186	1.997
	Cardiac disease	.032	1.355	1.026	1.789
	Diabetes	.008	1.450	1.101	1.911
Assisted primary patency	Female gender	.005	1.475	1.122	1.939
	PVD	.029	1.640	1.052	2.558
	Diabetes	.028	1.391	1.035	1.868

Notes: 95% CI = 95% confidence interval; PVD = peripheral vascular disease; HR = hazard ratio. Of n=449, n=388 had complete data for primary patency, and n=338 had complete data for assisted primary patency



## 1.5. Discussion

In summary, nearly a quarter of our patients were of non-Caucasian origin and the majority of those were from the Indian Subcontinent. Non-Caucasian patients had a significantly higher rate of diabetes; they were also younger and had less peripheral vascular disease. Both early and longer term AVF outcomes were not affected by ethnicity, even though they were all consistently worse among diabetics. Other patient variables associated with worse AVF outcomes included female gender, cardiac and peripheral vascular disease.

Vascular health and adaptation to AVF formation may be affected by diverse genetic predispositions among populations from different ethnic origin. Thus our analysis can be criticised because we combined different ethnic minorities together as “non-Caucasians”. This was done for pragmatic reasons because the African and other groups would have been too small to allow statistical analysis; three quarters of “non-Caucasians” were of Indo-Asian origin.

There are only few studies on AVF outcomes in different ethnic groups. Among African Americans, primary AVF failure and primary patency were comparable to Caucasians in two smaller size studies (28, 48). Compared to Caucasians, Indo-Asian patients in the United Kingdom had similar immediate and primary AVF failure in a smaller size study presented in abstract form, which is consistent with our findings (49). In contrast to our results, Lok et al report in a study equally as large as ours, that Canadian Caucasians had significantly better (hazard ratio 0.63, 95% confidence interval 0.44-0.91) cumulative AVF survival (a measure equivalent to ours of assisted primary patency) than non-Caucasians (37). There is no immediate explanation for this discrepancy between our studies, but we suspect a possible

difference in the ethnic populations, or in the choice of vascular access. Caucasians in Canada and Caucasians in the United Kingdom may not be the same; moreover, the non-Caucasian proportions are probably different between our studies: In the study by Lok et al, one third of patients were of non-Caucasian ethnicity, which were more evenly mixed between South-Asians, Southeast-Asians, and Africans whereas our non-Caucasian patients were predominantly Indo-Asian (37). Furthermore, neither Lok et al nor we performed a comprehensive analysis of all permanent haemodialysis access (=AVF, AVG, long-term CVC) by ethnic group, rather both our studies focussed on AVF. In theory, we could have missed a real difference (in terms of worse AVF outcomes among ethnic minorities), if all non-Caucasians with difficult access were dialysed via a CVC (AVG were used in less than 1% and hence not important). Our overall CVC rate was < 20% (not shown). Lok et al do not show the data but in general Canadian practice involves a higher proportion of AVG and CVC than in the United Kingdom (37, 44). In summary, the diverse outcomes in the studies might be due to different patient populations or use of different types of vascular access.

Diabetes has been associated with more primary AVF failure, which is consistent with our results (28, 38). Konner et al report a similar 1-year primary and assisted primary patency for diabetic and non-diabetic patients; however, this is achieved by a considerably smaller proportion of forearm AVF among diabetics. We found worse AVF survival among diabetics, but the default for our surgeons was to form a forearm AVF in nearly all patients. Poorer AVF outcome among diabetics may result from cardiovascular disease.

Cardiovascular disease itself may adversely affect AVF outcomes, presumably by restricting AVF blood flow, because cardiac disease may limit an increase in cardiac output whilst distal

arterial disease, particularly of the small calibre radial artery, may restrict arterial dilation (8, 37, 38, 126). Although there would be a rationale to combine coronary, peripheral and cerebral artery disease in the analysis as markers of vascular disease, we examined cardiac disease, peripheral and cerebrovascular disease separately to allow comparison with the literature where these are often reported as separate entities (34, 36-38).

In our study, diabetes was three times more likely among non-Caucasians, but surprisingly AVF outcomes were consistently not affected by ethnicity. Younger age and less peripheral vascular disease was more common among non-Caucasians, suggesting that the smaller calibre distal radial artery might have been less affected by pre-existing vascular disease than in Caucasians. Indeed, considerably less lower limb peripheral arterial disease was found in Indo-Asian compared to Caucasian patients in the United Kingdom, despite the presence of more cardiovascular risk factors (127).

Potential strengths of this study are its large sample size and analysis of congruous data (first-time vascular access only). Potential limitations are the retrospective design of a single-centre study including data incompleteness, and lack of detail concerning the operating surgeons and long-term CVC use.

In conclusion, we report equivalent early and longer term AVF outcomes in terms of haemodialysis use among Caucasian and non-Caucasian patients with endstage renal disease at a single centre in the United Kingdom. Hence, ethnic minorities, specifically Indo-Asians, should be offered AVF in the same way as Caucasians.

## **2. Routine pre-operative vascular ultrasound improves patency and use of arteriovenous fistulae for haemodialysis: a randomised trial.**

Published in: Ferring M, Claridge M, Smith SA, Wilmink T. Routine Preoperative Vascular Ultrasound Improves Patency and Use of Arteriovenous Fistulas for Hemodialysis: A Randomized Trial. Clin J Am Soc Nephrol. 2010 September 9, 2010;5: 2236-44 (128); reproduced with permission (Copyright Clearance Center licence n.: 3145781313444)

(National Research Register, UK, Trial number N0046131432)

### **2.1. Abstract**

**AIM:** Arteriovenous fistulae (AVF) are the preferred vascular access for haemodialysis but have a considerable failure rate. We investigated whether routine pre-operative vascular ultrasound results in better AVF outcome than physical examination.

**METHODS:** Patients with endstage kidney disease referred for permanent access formation were assessed by independent examiners using physical examination and ultrasound.

After random allocation, the ultrasound report was disclosed to the surgeon for patients in the ultrasound group but not for the clinical group. Endpoints were AVF failure and survival rates, analysed by intention to treat and by use for haemodialysis.

**RESULTS:** AVF were made in 208 of 218 randomised patients. Clinical and ultrasound groups were similar in terms of patient characteristics, allocation to individual surgeons and proportion of forearm AVF.

The ultrasound group had a significantly lower rate of immediate failure (4% versus 11%,  $p = 0.028$ ), and, among failed AVF, less thrombosis (38% versus 67%,  $p = 0.029$ ). Primary AVF

survival at 1 year was not statistically different (ultrasound = 65%, clinical = 56%,  $p = 0.081$ ).

Assisted primary AVF survival at 1 year was significantly better for the ultrasound group (80% versus 65%,  $p = 0.012$ ). The number of patients requiring pre-operative ultrasound to prevent one AVF failure was 12.

**CONCLUSION:** Routine pre-operative vascular ultrasound in addition to clinical assessment improves AVF outcomes in terms of patency and use for dialysis.

## 2.2. Introduction

The arteriovenous fistula (AVF) is the preferred vascular access for haemodialysis (10, 11). However, AVF failure is a common problem, particularly for radiocephalic AVF with a modest 2/3 survival by one year (31). AVF failure is often attributed to vascular comorbidity as an increasing number of elderly patients with diabetes and cardiovascular disease are accepted for haemodialysis treatment (2, 8, 28, 35, 37, 38).

Physical examination has traditionally been used to identify a suitable artery and vein for AVF formation (50). Pre-operative vascular mapping with ultrasound has recently been shown to predict AVF outcome (29, 50, 67, 78). The KDOQI guidelines recommend the use of routine ultrasound mapping for all patients but noted the lack of level one evidence to support this recommendation (10).

We conducted a randomised trial to evaluate the outcome of routine pre-operative assessment with ultrasound, in terms of patency and use of AVF for haemodialysis.

## **2.3. Methods**

### ***2.3.1. Patients***

The study was approved by the Local Ethics Committee, East Birmingham, UK, and registered with the UK National Research Register, Trial number N0046131432. Declaration of Helsinki was adhered to.

All patients with endstage kidney disease at Heart of England Hospital (Birmingham, UK) who were referred for formation of AVF were invited to take part in the study. We included patients with either none or one previous AVF. Patients who had already participated in the study, who had more than one previous AVF, or a previous upper arm arteriovenous graft were excluded. Patients who gave informed written consent were recruited between 31.08.2004 and 30.09.2006, and followed for up to 40 months. Clinical data were obtained from electronic patient and haemodialysis session records.

### ***2.3.2. Pre-operative assessment***

All patients were evaluated with physical and ultrasound examination, by independent assessors blinded to each other. Physical examination was carried out by one of 4 vascular surgeons (1 consultant, 3 trainees) with experience in AVF formation. Following a standardized protocol, the pulses at elbows and wrists, and the superficial veins in the forearm and upper arm (with tourniquet) were assessed. Vessels were considered suitable if the artery had a good pulse and the vein was patent and of good calibre. The most distal possible site was chosen for AVF formation, so that, where possible, a wrist or forearm AVF

was proposed in favour of an upper arm AVF, and a brachiocephalic in favour of a brachiobasilic AVF.

The ultrasound examination was carried out by a nephrology trainee (myself, n=216 scans) and initially by a vascular access nurse specialist (n=36 scans); both had received vascular ultrasound training by a consultant radiologist. Patients were assessed with a portable ultrasound scanner (SonoSite 180 plus, SonoSite Inc., Bothell, USA) with a 5-10 MHz linear probe. A standardized scan protocol was used based on a technique described in the thesis (section A, Chapter 3.2.); again the most distal possible site for AVF formation was recommended.

Briefly, the arterial scan followed the vasculature from brachial artery in mid-upper arm to radial artery at the wrist in B-mode. Internal diameters were measured of the radial artery at the wrist and in the distal forearm, of the brachial artery at the elbow and in the mid-upper arm. The average of two diameter measurements at each site was the diameter reported. Minimum arterial diameters suitable for AVF formation were 2 mm in the forearm, 3 mm in the upper arm (50, 129). Colour power and spectral duplex ultrasound were recorded in the brachial artery just above the elbow, in the radial artery just above the wrist. The spectral Doppler waveform was considered adequate if triphasic or biphasic in antegrade direction, but inadequate if retrograde, damped or absent (130). The ulnar artery was assessed in the distal forearm with B-mode and duplex ultrasound. Complete or near-occlusion of the ulnar artery was considered a contraindication to access formation in the same arm, because of the increased risk of hand ischaemia.

After application of a tourniquet, the superficial veins were followed in cross-section with B-mode from wrist to mid-upper arm, with intermittent vein compression. The cephalic vein was scanned from wrist to mid-upper arm. The basilic vein was scanned from the elbow level to its drainage into the deep brachial veins, and also in the forearm if the cephalic vein was unsuitable (79). Internal diameters and vein depth were measured at the wrist, in the distal forearm, in the proximal forearm, at the level of the elbow and in the upper arm. The average of two diameter measurements at each site was the diameter reported. Suitable veins had to be fully compressible and morphologically normal, with minimum diameters of 2 mm in the forearm and 3 mm in the upper arm (50). Suitable veins also had to be fully compressible and morphologically normal.

### *2.3.3. Random allocation*

An independent trial coordinator, unaware of the results of the assessments, stratified patients by age (below 65 years versus older) and dialysis status (pre-haemodialysis versus on haemodialysis) into four strata, and randomised to clinical and ultrasound groups using four sets of consecutively numbered sealed envelopes containing the assessment allocation, based on a predefined computer-generated random sequence in blocks of eight. The allocation was revealed to the main researcher after consent, recruitment and pre-operative assessments were complete.

For the clinical group, the ultrasound findings were not disclosed to the surgeon. For the ultrasound group, the ultrasound findings were disclosed to the surgeon; in case of discrepancy between the clinical and ultrasound assessments the surgeon could decide but usually followed the ultrasound recommendation. In the either group, the surgeon had the



option to request further imaging (ultrasound, angiography) if deemed necessary. Patients were unaware of the random allocation.

#### *2.3.4. Surgery*

A team of 6 consultant vascular surgeons and 13 vascular surgical trainees shared the workload of AVF formation. Trainees generally operated with the consultant under direct supervision, but trainees with sufficient experience (n=5) operated under indirect supervision.

#### *2.3.5. AVF use for haemodialysis*

After surgery, all AVF were used by clinical need as defined by the attending nephrologist, and by AVF maturity as assessed by an experienced haemodialysis nurse. When necessary, AVF salvage was undertaken by endovascular or surgical intervention for failed AVF.

#### *2.3.6. Study objectives and outcome definitions*

We hypothesized that routine pre-operative ultrasound use would result in better short-term and possibly longer term AVF outcomes.

Primary endpoint was primary AVF failure: all AVF which were never adequate for HD following initial surgical formation, including immediate failure on the day of surgery, early thrombosis and failure to mature (8, 30).

Secondary endpoints:

1. Immediate AVF failure: AVF thrombosis on the day of surgery, defined as absence of a thrill, or inadequate vein found at surgery (30, 108)
2. Primary AVF survival: patency of all AVF , defined by patency and usability for dialysis, from date of surgical formation to date of first access failure (33).
3. Assisted primary AVF survival: patency of all AVF , defined by patency and usability for dialysis, from date of surgical formation until the access was thrombosed, including time gained by successful salvage procedures (33).

AVF failure meant that AVF were unusable for dialysis, requiring either a salvage intervention, new access formation or insertion of a haemodialysis catheter (40). AVF were considered usable for dialysis if they were used for at least 6 consecutive 4 hour dialysis sessions by two needle cannulation without assistance from a catheter, with a minimum blood pump rate of 200 ml/min after the third session.

Censoring occurred at the time of patient death, kidney transplantation, change to peritoneal dialysis, patient transfer to another unit, or the end of the follow-up period.

#### *2.3.7. Statistics*

Sample size calculation: We decided that AVF outcome describing the usability of AVF for dialysis was an endpoint of key clinical interest to define the usefulness of pre-operative vascular ultrasound. Primary failure is the reverse outcome, meaning all AVF which cannot be used after initial surgery, but the rate is variable in the literature between 20-50% (8). In a pilot analysis (unpublished) of 103 AVF formations at our hospital in 95 patients based on clinical pre-operative assessment we found a primary patency rate of 59%. For the purpose

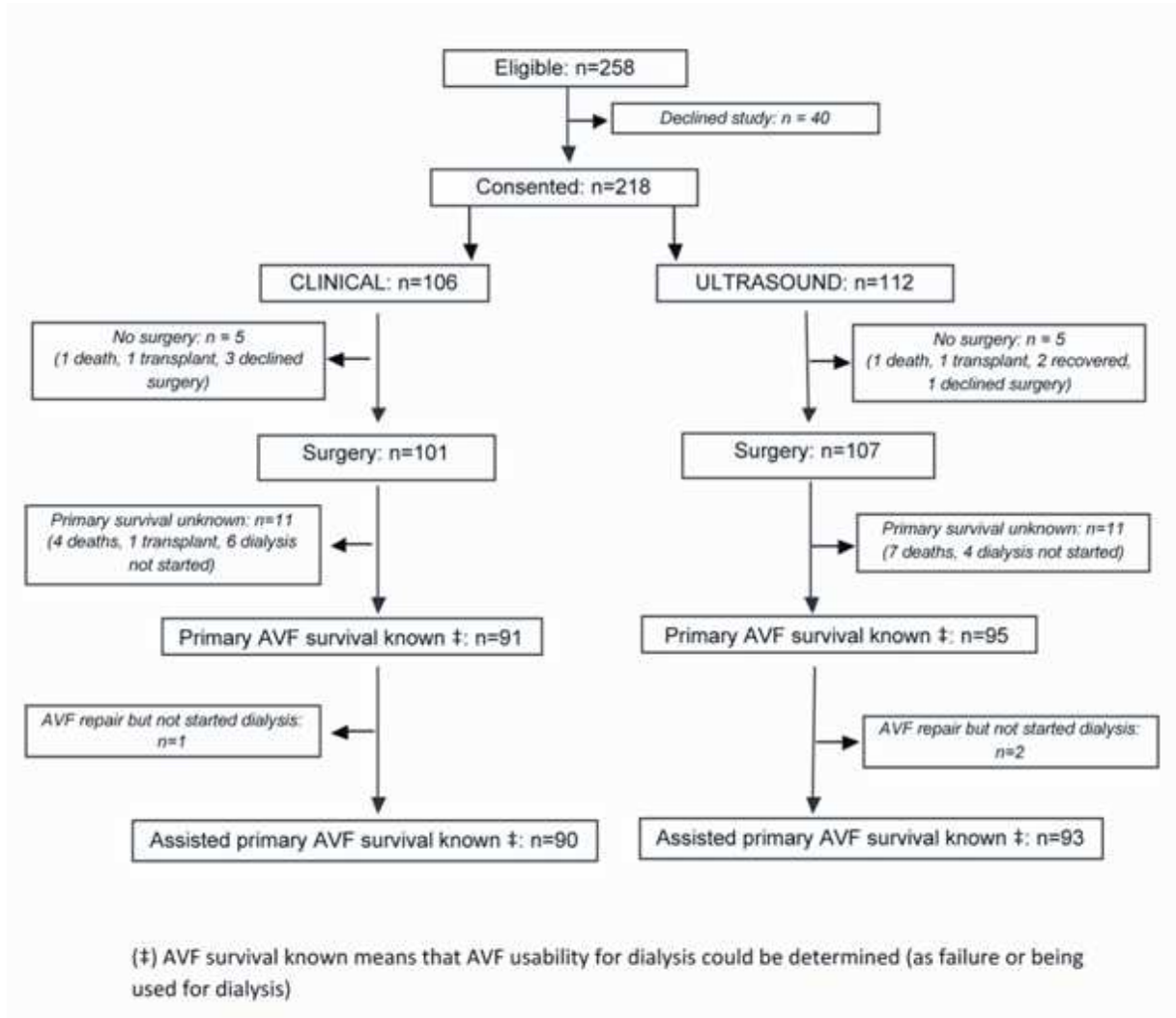
of this study, we set the primary AVF failure rate expected with clinical pre-operative examination conservatively to 35% because we considered possibly some improvement in outcomes in the setting of a research study with a structured clinical assessment. We then considered pre-operative ultrasound a useful tool if it resulted in a reduced primary failure rate by at least 15%, or specifically that ultrasound examination would result in a primary AVF failure rate of 20% or less (8). At a significance level of 0.05 and a power level of 0.8, a minimum sample size of 280 patients (140 in each group) was required.

Univariate analysis of categorical variables was made with CHI square test. After cube transformation, age was tested by independent t-test (131). Number needed to treat (NNT) was calculated as  $1/ARR$  (absolute risk reduction). ARR was calculated as the difference, relative risk (RR) as the ratio of failure risk in the clinical versus that in the ultrasound group (132). Primary and assisted primary AVF survival was examined by life-table analysis. Multivariate analysis of AVF survival was examined by Cox regression. Predictor variables were excluded by a backward stepwise approach based on the likelihood ratio (131). All analyses were by intention-to-treat, and also under the condition that AVF usability for haemodialysis was known. We used SPSS version 16 for statistical calculations. A p-value of 0.05 or less was considered significant.

## 2.4. Results

### 2.4.1. Patients

Figure 6: Consort Diagram



Of 258 eligible patients, 218 consented to the study (Figure 6). All 208 patients undergoing surgery (clinical n=101, ultrasound n=107) received an AVF for which immediate outcome was known. AVF usability for haemodialysis was known in 186 for primary survival, and in 183 for assisted primary survival. Primary survival was unknown in 22 patients due to death or not having started dialysis by the end of follow-up. Assisted primary survival was

unknown in a further 3 patients who had undergone AVF salvage but not started dialysis (Figure 6). Thus the number required by our power calculation was not reached.

#### ***2.4.2. Baseline characteristics of randomised groups***

Clinical and ultrasound groups were similar in terms of baseline patient characteristics, difficulty of access or surgeon's experience (table 14). Upper arm or right arm AVF location and the rate of vein transpositions were not significantly different, although the latter was more common in the ultrasound group (table 15).

Table 14: Baseline characteristics

	Clinical group n=106	Ultrasound group n=112	p-value
Age : median [range] 25 percentile 75 percentile	67 [20-89] 55 76	69 [26-88] 60 75	0.760
Gender: Male Female	70 (66.0%) 36 (34%)	69 (61.6%) 43 (38.4%)	0.496
Ethnicity: Caucasian Indo-Asian African Other	71 (67%) 21 (19.8%) 11 (10.4%) 3 (2.8%)	80 (71.4%) 25 (22.3%) 4 (3.6%) 3 (2.7%)	0.477
Body mass index #	26.9 ± 6.2	27.1 ± 6.31	0.471
Hypertension	79 (75.2%)	87 (78.4%)	0.584
Diabetes	36 (34.3%)	48 (43.2%)	0.177
Cardiac disease £	38 (36.2%)	33 (29.7%)	0.312
Vascular disease &	23 (21.9%)	16 (14.4%)	0.153
Antiplatelet agent §	55 (52.9%)	47 (42.3%)	0.122
Haemodialysis Not on haemodialysis ‡ On haemodialysis	82 (77.4%) 24 (22.6%)	87 (77.7%) 25 (22.3%)	0.955
Difficult access ¶	12 (11.3%)	12 (10.7%)	0.886
Surgical experience Surgical consultant alone Surgical consultant + trainee Surgical trainees alone	39 (39.8%) 41 (41.8%) 18 (18.4%)	47 (46.5%) 35 (34.7%) 19 (18.8%)	0.549

(#): Body mass index data only available for approximately 62% of patients (clinical 57%, ultrasound 67%). (£): Cardiac disease was defined by a clinical history of previous angina, acute coronary syndrome, coronary artery bypass graft or cardiac transplant. (&): Vascular disease was defined by a clinical history of claudication, critical leg ischaemia, and endovascular or surgical intervention for peripheral arterial disease. (§): antiplatelet agent = Aspirin or clopidogrel or both. (‡): not on haemodialysis included patients with advanced chronic kidney disease (majority), failing peritoneal dialysis or renal transplant with plan to transfer to haemodialysis, and patients having started haemodialysis for less than 90 days. (¶): Difficult access was defined as patients in whom no suitable site for AVF formation could be identified by clinical examination alone

Table 15: Site of AVF (in all patients who underwent surgery n=208)

Site of AVF	Clinical: n=101	Ultrasound: n=107
ALL FOREARM	64 (63%)	63 (59%)
Left radiocephalic	47 (47%)	42 (39%)
Right radiocephalic	16 (16%)	20 (19%)
Other forearm	1 (1%)	1 (1%)
ALL UPPER ARM	37 (37%)	44 (41%)
Left braciocephalic	21 (21%)	26 (24%)
Right brachiocephalic	12 (12%)	10 (9%)
Left brachio basilic	2 (2%)	2 (2%)
Right brachio basilic	1 (1%)	5 (5%)
Other upper arm	1 (1%)	1 (1%)
All AVF with superficialisation / transposition	5 (5%)	11 (10%)

Other forearm AVF: left radiobasilic (ultrasound); left ulnobasilic (clinical). Other upper arm AVF: right upper arm ulnocephalic (clinical); left upper arm ulnocephalic (ultrasound). Forearm versus upper arm AVF: CHI square 0.266; p=0.606. Superficialisation / transposition of vein versus none: Chi square 2.079; p=0.149.

#### *2.4.3. Early AVF outcomes (including Primary Failure as 1<sup>o</sup> endpoint)*

Immediate failure was significantly worse in the clinical group (Table 16). Of all 16 immediate failures, 2 were due to an inadequate vein found at surgery (clinical = 2, ultrasound = 0).

Primary failure occurred less in the ultrasound group but this was not statistically significant (Table 16). Primary failure after excluding immediate failure was similar (clinical n = 21, ultrasound n = 20). On univariate comparison, primary failure was significantly worse in forearm compared to upper arm AVF (39.7% versus 15.7%; p = 0.001). Further analysis of primary failure by thrombosis versus other causes showed significantly more early thrombosis in the clinical group (clinical n=22 of 33 (67%); ultrasound n=9 of 24 (38%); p = 0.029). The number of ultrasound scans needed to avoid one immediate failure event was low (Table 16).

Table 16: Early AVF outcomes (failure rates)

	Clini- cal group	Ultra- sound group	RR [95% CI] ‡	p- value	ARR §	NNT [95% CI] ¶
<b>IMMEDIATE FAILURE</b>						
all AVF: n failures	12	4			8.1%	12 [6; 132]
ITT #: n=218	11.3%	3.6%	0.315 [0.105; 0.944]	0.028		
Surgery £: n=208	11.9%	3.7%	0.315 [0.105; 0.944]	0.028		
forearm AVF: n failures	11	4			11.1%	9
ITT: n=131	16.7%	6.2%	0.369 [0.124; 1.100]	0.054		
Surgery: n=126	17.5%	6.3%	0.364 [0.122; 1.081]	0.054		
<b>PRIMARY FAILURE</b>						
all AVF: n failures	33	24			11.0%	9
ITT: n=218	31.1%	21.4%	0.688 [0.437; 1.084]	0.103		
Dialysis use Δ: n=186	36.3%	25.3%	0.697 [0.449; 1.082]	0.104		
forearm AVF: n failures	27	19			12.4%	8
ITT: n= 131	40.9%	29.2%	0.715 [0.444; 1.151]	0.139		
Dialysis use: n=116	45.8%	33.3%	0.728 [0.460; 1.154]	0.171		
<b>NON-MATURATION §</b>						
all AVF: n failures	21	20			4.6%	22
ITT: n=218	19.8%	17.9%	0.901 [0.519; 1.565]	0.712		
Dialysis use: n=170	26.6%	22.0%	0.827 [0.485; 1.409]	0.484		
forearm AVF: n failures	16	15			5.0%	20
ITT: n= 126	25.4%	23.8%	0.938 [0.509; 1.729]	0.836		
Dialysis use: n=101	33.3%	28.3%	0.849 [0.472; 1.526]	0.584		

n failures means absolute number of AVF failures; % failures means percentage of AVF failures to total number AVF. (‡): RR [95% CI]: relative risk of AVF failure with 95% confidence interval [lower and upper limits]. (§): ARR: absolute risk reduction of AVF failure (¶) NNT [95% CI]: number needed to treat, meaning the number of ultrasound scans needed to avoid one AVF failure; the 95% confidence interval is shown for immediate failure, but not for primary failure or the forearm subgroups as these are not statistically significant. (#): ITT = analysis by intention to treat, ie for all patients randomised. (£): analysis restricted to patients who had surgery. (Δ): analysis restricted to AVF for which the outcome (dialysis use or failure) was known. (§): non-maturation = primary failure excluding immediate failure



#### *2.4.4. Longer term AVF outcomes*

Primary AVF survival at one year was 56% for the clinical group and 65% for the ultrasound group but this was not statistically significant ( $p=0.081$ ; Figure 7). The assisted primary survival at one year was significantly better for ultrasound (80% versus 65%,  $p = 0.012$ ; Figure 8).

With Cox regression we tested which baseline clinical variables were independent predictors of AVF survival. First, we included all variables, that means ultrasound, age, gender, ethnicity, diabetes, cardiac disease, vascular disease, antiplatelet medication, dialysis status, AVF site or surgeon's experience (Table 17). In the final analysis, after backward exclusion of non-significant variables, pre-dialysis status, upper arm AVF site and male gender remained independent predictors of better primary survival; pre-dialysis status, upper arm AVF site and ultrasound were independent predictors of better assisted primary survival (annotation to Table 17).

Figure 7: Primary AVF survival (time to failure) of clinical and ultrasound groups

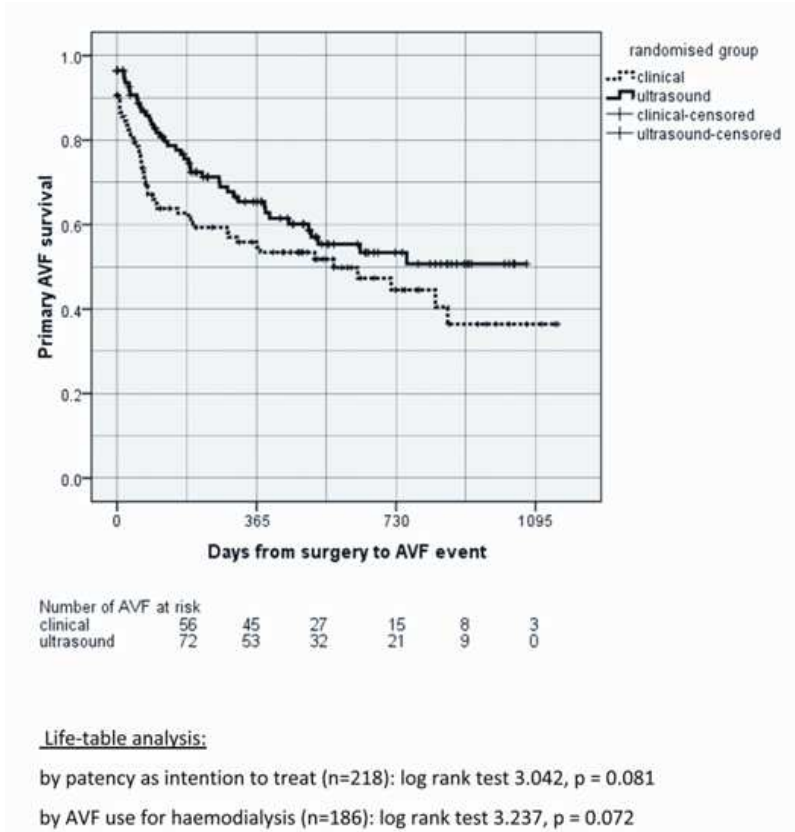


Figure 8: Assisted primary AVF survival (time to failure) of clinical and ultrasound groups

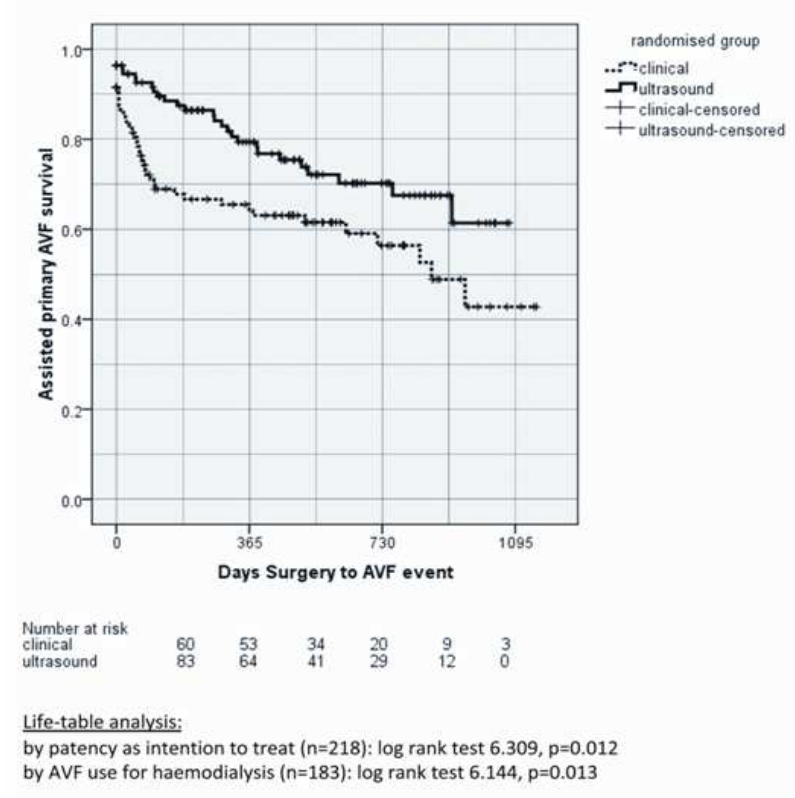


Table 17: Longer term AVF outcomes - Cox regression for primary and assisted primary AVF survival (time to failure)

<i>Predictor variables</i>	<b>Primary AVF survival (time to failure)</b>		<b>Assisted primary AVF survival (time to failure)</b>	
	Hazard ratio (HR)	95% confidence interval (CI): lower; upper	Hazard ratio (HR)	95% confidence interval (CI): lower; upper
<i>Randomisation to ultrasound</i>	0.669	0.436; 1.027	0.573	0.348; 0.945
<i>Age ‡</i>	1.001	0.986; 1.015	0.999	0.983; 1.016
<i>Gender §</i>	0.672	0.426; 1.060	0.857	0.504; 1.458
<i>Ethnicity ¶</i>	0.951	0.579; 1.564	1.364	0.782; 2.380
<i>Diabetes</i>	1.659	1.002; 2.747	1.648	0.922; 2.945
<i>Cardiac disease</i>	1.033	0.597; 1.788	1.037	0.555; 1.936
<i>Vascular disease</i>	1.105	0.605; 2.018	1.434	0.735; 2.794
<i>Antiplatelet drug</i>	0.733	0.422; 1.273	0.609	0.324; 1.145
<i>Dialysis status ∆</i>	2.060	1.217; 3.487	2.668	1.451; 4.907
<i>Surgical experience #</i>				
<i>Trainee alone</i>	0.912	0.487; 1.708	1.419	0.731; 2.756
<i>Consultant + trainee</i>	0.945	0.580; 1.540	0.751	0.418; 1.349
<i>AVF site £</i>	0.449	0.273; 0.741	0.460	0.257; 0.823

Cox regression for AVF survival by intention to treat (for primary and assisted primary patency, respectively) with AVF survival as the dependent variable; the initial regression model with all predictor variables is shown in the table above with hazard ratios (HR) for AVF failure and 95% confidence intervals. Age ‡: Age was transformed to  $[(age^3)/10000]$  to correct for skew. Gender §: female gender as baseline hazard. Ethnicity ¶: Caucasian as baseline hazard, versus non-caucasian. Dialysis status ∆: “not yet on haemodialysis” as baseline hazard, versus “on haemodialysis”. Surgical experience #: consultant operating alone as baseline hazard, compared to trainee alone and to consultant with trainee operating. AVF site £: forearm AVF as baseline hazard, versus upper arm AVF.

**Primary AVF survival:** For the initial model with all predictor variables shown in the table above, CHI square was 25.312, 12 degrees of freedom,  $p = 0.013$ . The final model after backward stepwise exclusion of non-significant variables included only dialysis status (HR 2.196 [95% CI: 1.323; 3.644], AVF site (HR 0.448 [95% CI: 0.277; 0.724]), gender (HR 0.637 [95% CI 0.412; 0.985]), randomisation to ultrasound (HR 0.691 [95% CI 0.453; 1.054]), and diabetes (HR 1.504 [95% CI: 0.965; 2.344]). Final model CHI square was 23.263, 5 degrees of freedom,  $p < 0.001$ .

**Assisted primary AVF survival:** For the initial model with all predictor variables shown in the table above, CHI square was 26.759, 12 degrees of freedom,  $p = 0.008$ . The final model after backward stepwise exclusion of non-significant variables included only dialysis status (HR 2.601 [95% CI: 1.473; 4.594]), AVF site (HR 0.491 [95% CI: 0.284; 0.849]), randomisation to ultrasound (HR 0.594 [95% CI: 0.364; 0.969]), and diabetes (HR 1.657 [95% CI: 0.989; 2.777]). Final model CHI square was 19.613, 4 degrees of freedom,  $p = 0.001$ .

## 2.5. Discussion

Our randomised trial shows that routine pre-operative ultrasound in addition to physical examination improves AVF patency and dialysis use in a patient population without complex access problems. Specifically, ultrasound results in less immediate failure, less early AVF thrombosis and better assisted primary AVF survival. Ultrasound is effective with a small number of ultrasound scans needed to prevent failure.

Our finding that immediate failure is significantly reduced by pre-operative ultrasound is consistent with another randomised trial, which showed significantly less failure (6% versus 25%) on the day of surgery in the ultrasound compared to the clinical group (108).

Inadequate vessels can be identified by ultrasound, which reduces the rate of immediate failure (29, 78, 133).

Our data show no statistically significant difference between clinical and ultrasound groups for primary failure as the primary endpoint. Particularly, we saw no difference when primary failure is counted without immediate failure. This could indicate that ultrasound improves AVF patency but not AVF maturation, in agreement with published experience that maturation failure remains an important problem, even when pre-operative ultrasound is used (110, 134, 135). However, our study is underpowered and cannot give a definitive answer.

Our study shows a significantly better assisted primary AVF survival, ie survival time gained by AVF salvage, in the ultrasound group. The clinical group had a significantly greater early AVF loss due to a higher rate of early thrombosis, and thus had poorer potential for salvage.

We found that factors other than pre-operative assessment affect AVF outcome, such as gender, diabetes and AVF site. This is in keeping with the literature (28, 35, 41, 135). As noted by others, prior haemodialysis strongly predicted poorer outcome (41). Our stratified randomisation produced equal allocation of patients with prior haemodialysis.

Our study supports the recommendation by KDOQI and European guidelines for routine pre-operative ultrasound (10, 11). However, a recent trial, which preselected only patients with normal pre-operative physical findings and then randomised some to additional ultrasound mapping, found no advantage for ultrasound in terms of immediate patency or early AVF survival (107). In comparison, our patients were considerably older and not preselected, and our data show that such patients generally benefit from ultrasound. In our experience, ultrasound is unlikely to add information in the small subgroup of young non-diabetic men with normal physical findings.

Potential strengths of our study are its randomised design, the strict endpoint definition for functional AVF patency by use on dialysis, a real-life setting including the option of selective imaging for the clinical group and a low drop-out rate in terms of surgery not being done (n=10). Potential weaknesses include the lack of blinding of the operating surgeons to the randomisation group, because the surgeons needed to know the details of the pre-operative assessment which led to the decision for AVF formation. Furthermore, the study is underpowered and it is therefore unclear whether pre-operative ultrasound can or cannot reduce primary AVF failure, the primary endpoint. Finally, the pre-operative assessments are likely due to inter- and intra-observer variation, which may affect the measurements made and possibly the decision-making to propose or dismiss a particular site for AVF formation.

Although we considered the importance and practicalities of studying inter- and intra-observer variability, particularly for ultrasound, there were significant organisational and funding limitations in terms of bringing renal patients back for a second assessment under identical circumstances to study intra-observer variation, and for having two experienced sonographers available for scanning at the same time. We note the findings by others who describe minimal interobserver variation but considerable day-to-day variation of native venous diameters, measured with ultrasound in renal patients (85).

In conclusion, our randomised clinical trial demonstrates benefit of routine pre-operative ultrasound on AVF outcomes with a small number needed to treat to save one fistula. While non-maturation may remain an important problem, pre-operative ultrasound combined with appropriate AVF salvage leads to better long term AVF use on haemodialysis.

### **3. The quality of histological and ultrasound measurements of native veins, prior to arteriovenous fistula formation, in patients with endstage renal disease**

#### **3.1. Abstract**

INTRODUCTION: Pre-existing structural changes have been described in native veins prior to formation of arteriovenous fistulae (AVF) for haemodialysis. Whilst others studied histological width in vein cross-sections, we considered a new approach: We measured histological area of lumen, intima and media because this allows more quantitative detail, particularly of medial fibrosis. The validity and reproducibility of such histological measurements is unknown.

We investigated whether histological measurements compare to in vivo ultrasound measurements, and whether histological width compares to histological area.

METHODS: Native vein specimens were harvested from patients with endstage renal disease undergoing AVF formation. Histological examination was carried out using a computer-assisted image analysis system. In cross-section, venous lumen, intima and media were studied. In vivo ultrasound measurements of the vein lumen were obtained prior to surgery. Bland Altman plots were used to compare vein lumen measurements obtained from ultrasound, with and without tourniquet; from ultrasound and histological width; from ultrasound and histological area.

**RESULTS:** Sixty vein specimens were available for histological analysis and 48 had been scanned with ultrasound. Ultrasound vein diameter was mildly (on average 13%) larger with tourniquet compared to without. Histological and in vivo ultrasound measurements did not agree; specifically the former substantially underestimated the latter. However, the larger calibre of upper arm veins was equally well discerned by ultrasound and by histological measurements. Medial fibrosis, a characteristic change in native veins in endstage renal disease, was only quantified by histological area measurements.

**CONCLUSION:** Histological vein measurements cannot be directly compared to those made with ultrasound. Histological area may be more useful than histological width as it quantifies medial fibrosis.

### **3.2. Introduction**

Arteriovenous fistulae (AVF) are the preferred access for haemodialysis treatment but early AVF failure is an important problem. One cause may be pre-existing native vein disease: Abnormal native vein morphology or small lumen diameters found on ultrasound have been associated with AVF failure (32, 108). Native vein histology may provide more detail on morphology and thus give further insight into native vein disease. However, it is unknown whether histological findings compare to ultrasound findings in vivo. Besides, there is no established standard for quantitative histological examination.

Two authors reported on native vein histology prior to AVF formation. Wali et al examined cross-sections of native cephalic vein in patients with and without renal disease, and



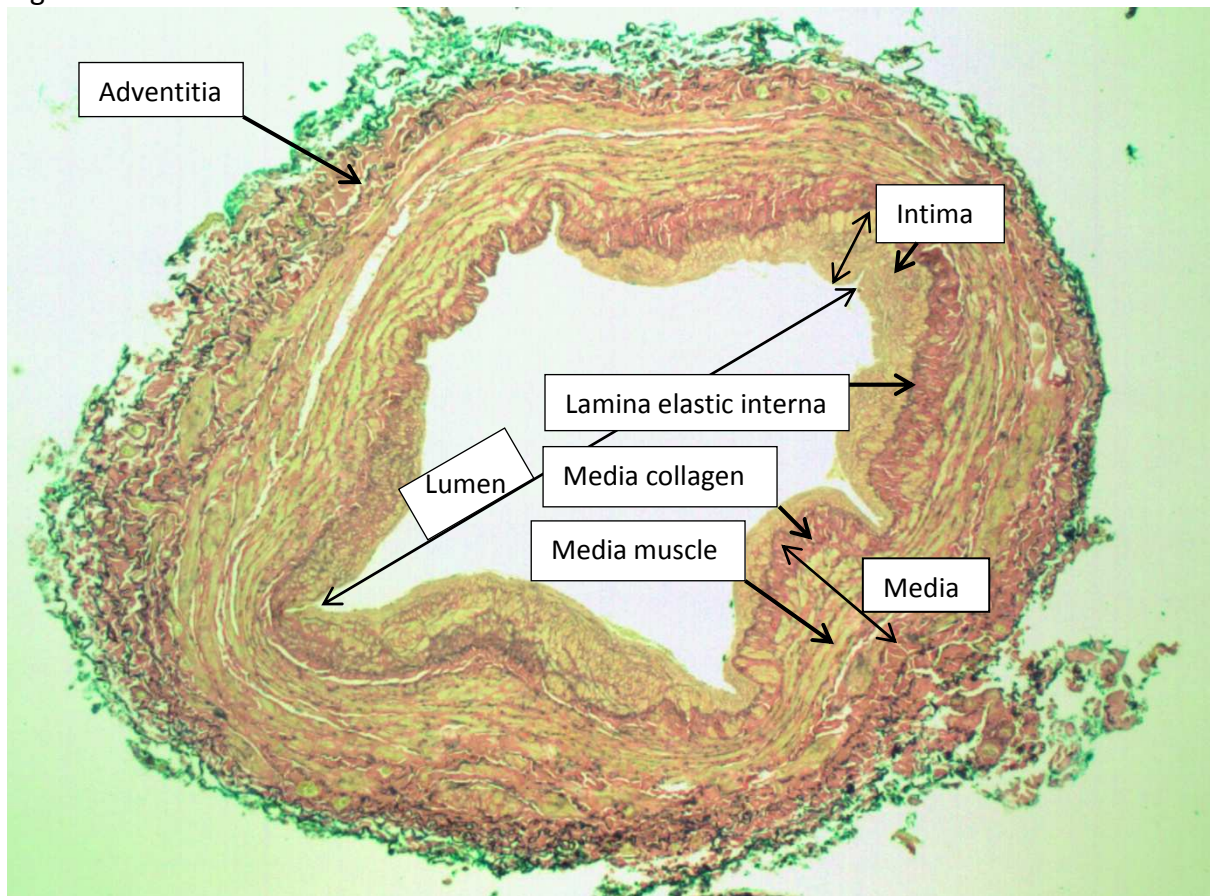
described particular changes in renal disease, including intimal hyperplasia and fibrotic media thinning (60). Wali et al measured the width of intima and media at focal areas of intimal hypertrophy and found an intima to media ratio of 5:1, inverse to the ratio in patients without renal disease (60). Such observations illustrate changes in renal disease but have limitations because they are not fully quantitative. Feinfeld et al measured the average width of intima and media of native brachial veins in cross-section in renal patients (Figure 9); media width was significantly greater in longer term haemodialysis patients compared to recent haemodialysis starters (61). Although quantitative, these measurements do not inform of the degree of medial fibrosis which could be important for vein dilatation after AVF formation.

We considered a new approach by measuring histological area in cross-section, because this provides a single quantitative measurement for each structure (lumen, intima, media), and the degree of media fibrosis can be measured as the percentage collagen to total media content. The validity and reproducibility of such histological measurements is unknown.

Furthermore, we do not know how well histological measurements compare to in vivo measurements with ultrasound. There is also a debate in the literature whether venous diameters should be measured with or without tourniquet (86).

Therefore, we studied native veins in patients with endstage renal disease to compare in vivo ultrasound to histological measurements, and to compare histological width to histological area. We also assessed the measurement variability of vein lumen on ultrasound, with and without tourniquet.

Figure 9: Vein wall structure in cross-section



Elastic van-Gieson stain: the lamina elastic interna stains black and separates intima and media; the smooth muscle of the media is yellow; collagen in media and adventitia is red. Width measurements are shown by double arrows for lumen, intima and media. For area measurements the relevant structure (lumen / intima / media) was outlined and measured using an image analyser.

### 3.3. Methods

Native veins were harvested from patients with endstage renal disease during surgery for new AVF formation.

### *3.3.1. Patients*

The study protocol was approved by the East Birmingham Local Ethics Research Committee. All patients who had surgery for formation of a new AVF were eligible for the study between 01/01/2005 and 31/01/2007. Patients who had already taken part in the study were also included (n=2). Patients with an existing AVF which required surgical repair were excluded. Baseline patient data and comorbidity were obtained from electronic patient records.

The site of AVF surgery was determined pre-operatively by physical examination of blood vessels; some patients also had ultrasound or angiography.

### *3.3.2. Ultrasound*

Just before the operation, internal vein diameters (from near to far wall intima) in cross-section were measured with ultrasound (Sonosite 180 plus) by a trained nephrologist (myself), prior to and after venous congestion with a tourniquet for 30 seconds, with patients seated and the arm supported by a pillow. Two measurements were made each time and the average was used as the ultrasound diameter.

### *3.3.3. Surgery*

Surgery was carried out by vascular surgeons experienced in AVF surgery (5 consultants, 5 trainees). Trainees usually operated under direct supervision by a consultant, but experienced trainees operated under indirect supervision.

AVF surgery was carried out with established technique usually under local anaesthesia. Following skin incision vein and artery were identified. The artery was occluded proximally

and distally using a sloop or a small vascular clamp, before a longitudinal incision was made. The artery was then injected with heparinised saline. The vein was divided by transverse section, and from the distal end an approximately 10 mm long segment was resected and placed in a sterile container with formalin. The proximal vein in situ was flushed with heparin and anastomosed to the artery in an end-to-side fashion.

#### *3.3.4. Specimen transport and preparation*

The vein was submitted in formalin for immediate dissection. When transfer was delayed it was cooled at 5 degrees Celsius overnight until the next day. The specimen was sliced transversely at 3 to 4mm intervals, yielding on average 2 cross sections per vein.

The specimens were fixed in 10% neutral buffered formalin, embedded in paraffin and cut at 4 micron thickness prior to staining with Haematoxylin and Eosin and elastic van-Gieson (EVG). The EVG is a trichrome stain which allows distinction between the elastic which is stained black and the smooth muscle of the media which has a yellow colour. The collagen in the adventitia stains red.

#### *3.3.5. Vein histomorphometry*

Microscopic images were captured using an Olympus BX40 microscope and a Paxcam 2 Megapixel camera. Vein dimensions (width and area) were measured using image analysis software (Twain Image Solutions Software, ISS Group Services Ltd, Withington, UK).

One Consultant Pathologist analysed and measured the vein structure in cross-section after identifying intima, lamina elastic interna, media, lamina elastic externa (Figure 9). Width and

area measurements were made for lumen, intima, media. For width measurements, the maximum and minimum thickness was obtained for each structure. Area measurements were also made for the collagen and muscular parts of the media. The ratio of collagen to total media area, and the ratio of muscle to total media area were calculated and expressed as a percentage; thus, percent collagen = 100% media - percent muscle.

### *3.3.6. Statistics*

SPSS version 16 and Microsoft Excel 2007 were used for data analysis. A p-value of  $\leq 0.05$  was considered significant.

We compared vein lumen measurements made with ultrasound, histological width and histological area, for agreement. More specifically, we made the following comparisons:

Vein lumen diameter comparisons:

- Ultrasound diameter before *versus* after tourniquet
- Ultrasound diameter (before tourniquet) *versus* histological diameter; the latter was calculated as (maximum diameter + minimum diameter)/2

Vein lumen area comparisons:

- Area of ultrasound measurements: before *versus* after tourniquet; this was calculated as  $\pi \times (\text{ultrasound diameter}/2)^2$ , assuming a circle shape

- Area from ultrasound before tourniquet (see calculation above) *versus* area from histological width; the latter was calculated as  $[\pi \times (\text{maximum histological diameter} / 2) \times (\text{minimum histological diameter} / 2)]$ , assuming an ellipse shape
- Area, measured histologically *versus* area, from histological width (see ellipse calculation above)

The agreement between ultrasound, histology width and histology area measurements was examined with Bland Altman plots: We plotted the estimated true value (calculated as the averages of measurements obtained from two methods) against the error between methods (calculated as the differences between measurements obtained from two methods) (136). Because our raw data showed that differences became larger with increasing averages, we also produced Bland Altman plots after logarithmic transformation of the raw data, as described by Bland and Altman (136). The mean difference (calculated as the average of differences) indicates bias; the limits of agreement (calculated as mean difference  $\pm 2 \times$  standard deviation of differences for upper and lower limit, respectively) inform about the scatter of most (95%) differences around the mean difference. As recommended by Bland and Altman, we transformed the logarithmic results of mean difference and limits of agreement back to their antilogs so as to appreciate the magnitude of error at a clinical level (136). The back-transformed data are “dimensionless” and indicate the proportional increase or decrease in error, rather than an absolute error difference (136).

Furthermore, we used Pearson's correlation and Student's t-test to compare histological width [mm] and histological area [mm<sup>2</sup>] measurements of intima and media, respectively; because the same structure was measured with different units of measurement, Bland Altman plots were impossible. The same applied to comparison of vein dimensions in the forearm versus the upper arm. To correct skew we transformed our data appropriately (logarithmic or square).

### 3.4. Results

Seventy patients (n=52 forearm, n=18 upper arm) consented to the study. Of those, 60 vein specimens (n=45 forearm cephalic, n=2 forearm basilic, n=6 upper arm cephalic, n=7 upper arm basilic) were available for histological examination. Of those, 48 had been measured with ultrasound in vivo prior to surgery (n=34 forearm cephalic, n=2 forearm basilic, n=5 upper arm cephalic, n=7 upper arm basilic). Table 18 summarises the measurements in forearm and upper arm veins.

We examined the level of agreement of vein lumen measurements between ultrasound, histology width and histology area using the Bland-Altman method by plotting averages against differences between methods (136). Using raw data, differences generally increased with averages (Figure 10 a, c; Figure 11 a, c, e); therefore, logarithm-transformed data were plotted (Figure 10 b, d and Figure 11 b, d, f) as described by Bland and Altman (136).

The Bland Altman plots show moderate agreement for ultrasound comparison of lumen diameter, with versus without tourniquet (Figures 10b, 11b). There is no agreement

Table 18 a-c: Forearm versus upper arm vein dimensions for lumen, intima and media (a: ultrasound, b: histological width, c: histological area)

<b>a) Ultrasound</b>	<b>forearm veins</b>		<b>upper arm veins</b>		<b>p-value</b>
	median	[interquartile range]	median	[interquartile range]	
lumen diameter [mm], no tourniquet	2.35	[1.75 to 2.85]	3.48	[2.96 to 4.56]	<0.001
lumen diameter [mm], with tourniquet	2.40	[1.75 to 3.20]	4.00	[3.28 to 4.70]	<0.001

<b>b) Histology (width)</b>	<b>forearm veins</b>		<b>upper arm veins</b>		<b>p-value</b>
	median	[interquartile range]	median	[interquartile range]	
maximal lumen diameter [mm]	1.027	[0.645 to 1.562]	1.343	[1.118 to 2.021]	0.026
minimal lumen diameter [mm]	0.343	[0.229 to 0.467]	0.585	[0.354 to 0.745]	0.003
maximal intima width [mm]	0.025	[0.010 to 0.086]	0.170	[0.068 to 0.198]	0.001
minimal intima width [mm]	0.000*	[0.000 to 0.000*]	0.000*	[0.000* to 0.004]	0.450
maximal media width [mm]	0.354	[0.293 to 0.411]	0.455	[0.342 to 0.583]	0.001
minimal media width [mm]	0.138	[0.104 to 0.169]	0.156	[0.118 to 0.198]	0.369

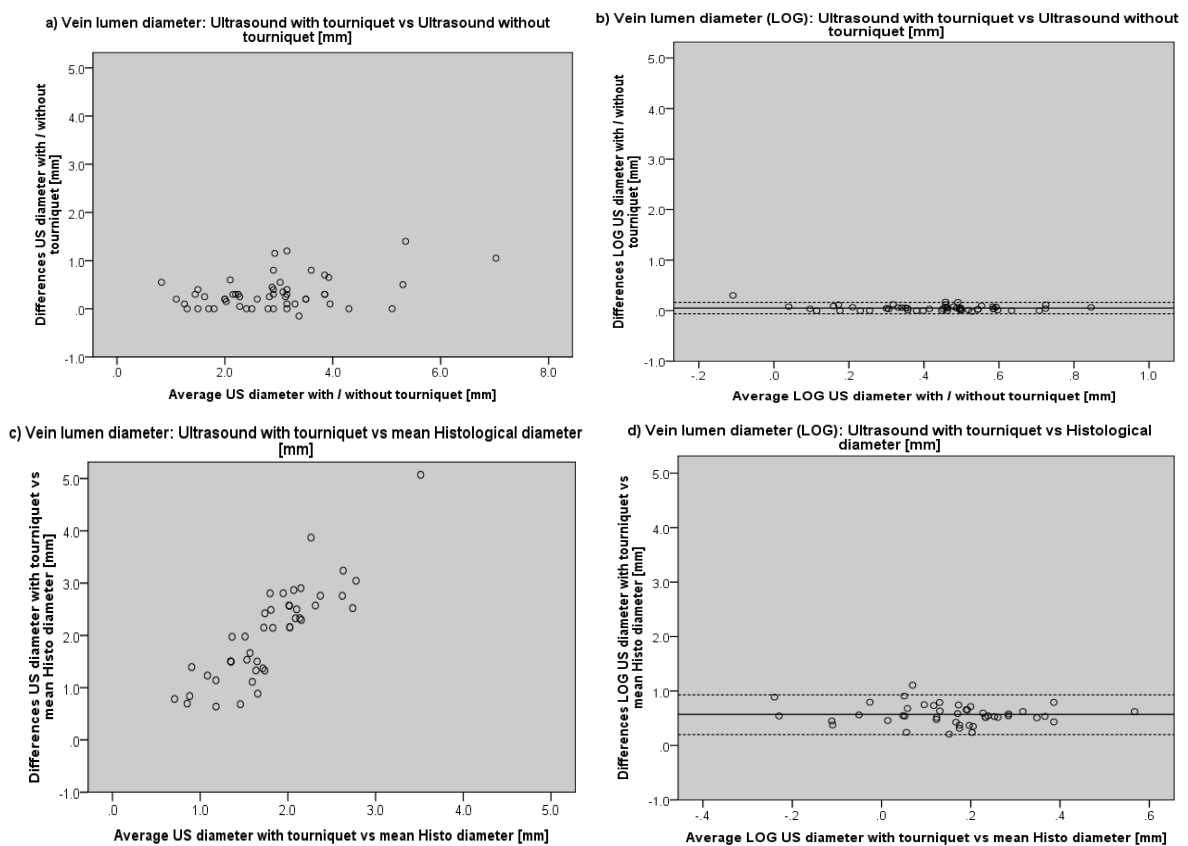
<b>c) Histology (area)</b>	<b>forearm veins</b>		<b>upper arm veins</b>		<b>p-value</b>
	median	[interquartile range]	median	[interquartile range]	
area of lumen [mm <sup>2</sup> ]	0.159	[0.085 to 0.310]	0.327	[0.152 to 0.560]	0.011
area of intima [mm <sup>2</sup> ]	0.041	[0.030 to 0.093]	0.180	[0.090 to 0.420]	<0.001
area of media [mm <sup>2</sup> ]	0.849	[0.646 to 1.029]	1.165	[0.934 to 1.843]	<0.001
media area of collagen [mm <sup>2</sup> ]	0.321	[0.276 to 0.401]	0.602	[0.401 to 0.730]	<0.001
media area of muscle [mm <sup>2</sup> ]	0.496	[0.366 to 0.634]	0.638	[0.513 to 1.105]	0.023
media collagen [%]	39.3	[35.9 to 42.8]	41.7	[38.5 to 46.3]	0.059
media muscle [%]	60.7	[57.2 to 64.1]	58.3	[53.7 to 61.5]	0.059

(\*) Minimal intima width = 0.000; this means that the media was too thin to be measurable on light microscopy at its lowest point in the vein circumference. P-values show significance of t-test comparisons between forearm and upper arm veins after raw data transformation by square root for histological width data (except for minimal width) and by logarithm for ultrasound and histological area data, as well as for minimal intima width



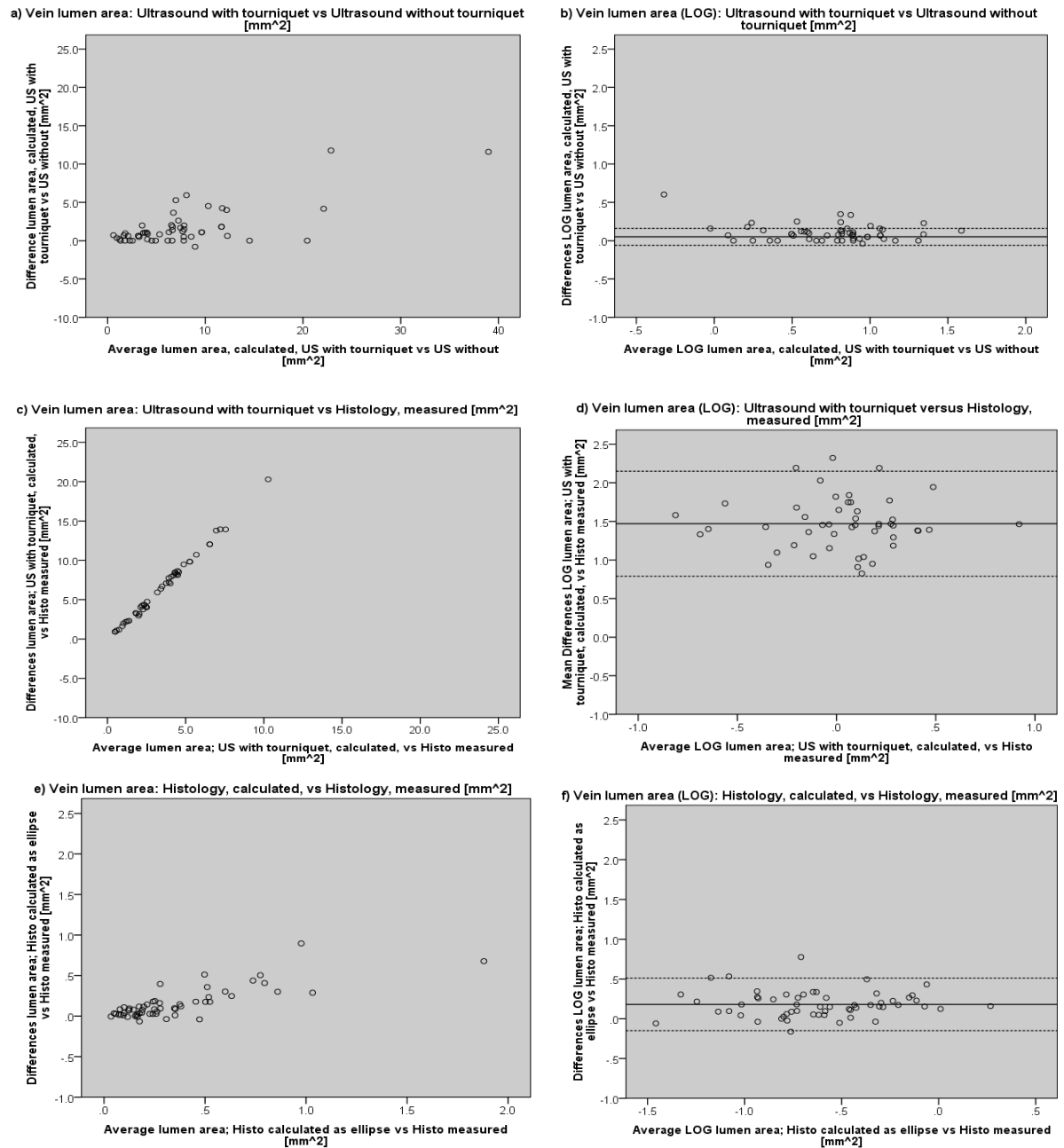
between ultrasound and histology (Figure 10d, 11d). There is some agreement between histology area, measured versus calculated from diameter (Figure 11e, f). Agreement is generally poorer for area than for diameter, and poorer for comparison between ultrasound and histology (Figures 10, 11). Specifically for comparison of ultrasound versus histology, there is considerable bias, shown by mean differences well above zero, and substantial spread of error, shown as wide limits of agreement.

Figure 10: Vein lumen diameter comparisons - Bland Altman plots (raw data left, log-transformed data right “LOG”): Ultrasound, with tourniquet versus without tourniquet; a) raw data, b) log-transformed. Ultrasound versus histology; c) raw data, d) log-transformed.



Log-transformed plots show mean difference (straight line) and limits of agreement (dotted lines)

Figure 11: Vein lumen area comparisons - Bland Altman plots (raw data left, log-transformed data right “LOG”): Ultrasound lumen area (calculated from diameter, with tourniquet versus without tourniquet); a) raw data, b) log-transformed. Ultrasound lumen area (calculated from diameter, with tourniquet) compared to histology area (measured); c) raw data, d) log-transformed. Histology area (calculated) compared to histology area (measured); e) raw data, f) log-transformed.



Log-transformed plots show mean difference (straight line) and limits of agreement (dotted lines). Note that y-axis scale is -10 to +25 for a) and c), but -1 to +2.5 for b), d), e) and f).

Table 19: Bias and spread of error between methods measuring vein lumen dimensions

Methods	Measurement	Bias (mean difference)	Spread of error [lower; upper limit of agreement]
ultrasound (with tourniquet) <i>versus</i> ultrasound (no tourniquet)	diameter [mm]	13% above	[13% below to 45% above]
	area [mm <sup>2</sup> ]	27% above	[24% below to 112% above]
ultrasound (with tourniquet) <i>versus</i> histology (measured)	diameter [mm]	268% above	[59% below to 755% above]
	area [mm <sup>2</sup> ]	2856% above	[522% below to 13954% above]
histology (calculated) <i>versus</i> histology (measured)	area [mm <sup>2</sup> ]	52% above	[29% below to 227% above]

Back-transformation from logarithmic results of mean differences and limits of agreement: The error between measurements is shown as the percentage of error above or percentage error below (136)

Table 19 shows the error illustrated in the Bland-Altman plots in its magnitude at a clinical level. Bias and spread of error are shown as the proportional increase (+) or decrease (-) in error. The error is small between ultrasound diameter measurements: with tourniquet the average diameter measurement increases by 13%. Most diameter measurements with tourniquet vary between 13% below to 45% above the measurements without tourniquet. Thus, application of tourniquet may approximately increase the lumen diameter measurement by up to 50%. In contrast, ultrasound lumen measurements grossly overestimate histological lumen measurements, on average by an extra 268% for diameter and by an extra 2856% for area (Table 19). In comparison, there is less disparity between lumen area based on histological width and histological area measurements (Table 19). Thus, we find reasonable agreement between ultrasound measurement with and without tourniquet, some agreement between histological measurements, but ultrasound lacks agreement with histology and produces substantially larger measurements.

Histological measurements correlated with ultrasound measurements in vivo: maximal and minimal lumen diameters, as well as lumen area, correlated with ultrasound vein diameter ( $r=0.450$ ,  $p=0.002$  and  $r=0.489$ ,  $p=0.001$  and  $r=0.542$ ,  $p<0.001$ , respectively). Histological width correlated with histological area measurements: Specifically, maximal and minimal lumen diameters correlated with lumen area ( $r=0.696$ ,  $p<0.001$  and  $r=0.769$ ,  $p<0.001$ , respectively). Maximal intima width correlated with intima area ( $r=0.871$ ,  $p<0.001$ ). Maximal and minimal media width correlated with media area ( $r=0.742$ ,  $p<0.001$  and  $r=0.405$ ,  $p=0.001$ ).

We also examined whether the larger calibre of upper arm compared to forearm veins could be demonstrated with histological and ultrasound measurements. We found significant differences for most measurements, except for minimal intima and minimal media width, as summarised in Table 19. Media composition (% collagen, % muscle) was not significantly different between forearm and upper arm veins, although there was a tendency towards more collagen in upper arm veins.

### 3.5. Discussion

In summary, our data show that histology measurements of the venous lumen considerably underestimate in vivo ultrasound measurements. Ultrasound vein lumen with tourniquet slightly overestimates vein lumen without. Histological measurements, similar to ultrasound, can discern calibre differences between forearm and upper arm veins. Finally, we describe

that the degree of media fibrosis can be quantified by histological area but not by histological width measurement.

Controversy exists whether venous diameters should be measured with or without tourniquet. Considerable variability of venous lumen diameters measured with ultrasound has been reported associated with patient position, temperature, tourniquet use as well as day-to-day variation (85, 86). However, venous diameters are not dependent on the congestion method – tourniquet or pressure cuff (85). After venous congestion, an increase in venous diameter on ultrasound is found in some patients and associated with better AVF outcome (46, 78). Our ultrasound findings confirm that with tourniquet use the vein diameter increased on average by 13%. In our experience, venous diameters with ultrasound are easier to measure with than without tourniquet, even when appropriate care is taken to avoid venous collapse during the ultrasound scan. Because the average calibre increase is minor, we therefore recommend using a tourniquet for ultrasound measurements of venous diameters.

By contrast, we found no agreement between histological measurements and in vivo ultrasound measurements of vein lumen. More specifically, ultrasound measurements were consistently larger than histological measurements, and the magnitude of scatter was greater for area than for diameter measurements. There may be several reasons for this lack of agreement: Firstly, there are potential errors underlying our vein lumen estimates: A vein in cross-section on ultrasound is often not a perfect circle (86). Thus, calculating the area from the ultrasound diameter squares any error and results in a wider scatter. Similarly, histological area calculated from an ellipse is an imperfect estimate because the vein lumen

outline is more complex, as illustrated by Figure 9 and shown by comparisons of calculated versus measured histology area. Secondly, formalin fixation may lead to shrinkage due to smooth muscle alteration, as observed for other histological specimens (137, 138). We conclude that vein measurements on ultrasound in vivo and on histology are not equivalent as the former considerably overestimate the latter.

If measurements lack agreement, this raises the question whether histological measurements have any relationship to in vivo measurements at all. As one might expect intuitively, histological width and histological area measurements have strong correlation ( $r \geq 0.7$ ) for corresponding structures, whilst the relation between in vivo ultrasound and histology is less strong ( $r \geq 0.5$ ). Furthermore, a larger vein calibre in upper compared to forearm veins is distinguished by ultrasound, histological area and most histological width measures. More specifically, from Table 18 we calculate average increases of 1.7 times and 1.5 times of the ultrasound lumen diameter (with and without tourniquet, respectively), which compares reasonably to average increases of 1.3 and 1.7 times for maximal and minimal histological lumen diameters, respectively. Thus, even though histological vein measurements are not equivalent to ultrasound measurements made in vivo, they have some proportionality.

Finally, vein changes in endstage renal disease such as intimal hyperplasia can be quantitatively assessed by histological width and area measurements. Histological area and width measurements were only very recently reported for native veins used for access formation, although the degree of medial fibrosis was not assessed in this study (62). Our data show that only histological area can quantify the degree of media fibrosis, in terms of

absolute collagen area as well as media collagen content. The clinical relevance of pre-existing native vein disease to arteriovenous fistula (AVF) failure is unknown. Possibly, increased media fibrosis may prevent post-operative vein dilatation, which could impede AVF blood flow particularly in smaller calibre forearm veins.

In conclusion, we show that venous diameter on ultrasound increases on average by only 13% after tourniquet congestion; as measurements with tourniquet are easier, we recommend tourniquet use. We show that ultrasound and histology vein lumen dimensions are not equivalent as the former substantially overestimates the latter. Furthermore, only histological area measurements allow quantification of the degree of vein medial fibrosis.

## **4. Vein histology, patient demographics and outcomes of arteriovenous fistula formation in patients with endstage renal disease**

### **4.1. Abstract**

**BACKGROUND:** Successful arteriovenous fistula (AVF) formation is vital to the success of haemodialysis treatment. Abnormal native vein morphology found on pre-operative ultrasound has been associated with early AVF failure. Histology may provide more detail of native vein disease and characteristic changes have been described in endstage renal disease. We used a new quantitative approach by measuring histological area of lumen, intima and media, and examined whether vein histology could be linked to early AVF outcome.

**METHODS:** Native veins were harvested from patients with endstage renal disease undergoing AVF formation. Vein histology was examined with a camera linked to a computer image analysis program for quantitative area measurements. Medial fibrosis (collagen content of the media) was quantified as the percentage of collagen area in media relative to total media area. Electronic records were searched for patient demographics and early AVF outcomes defined by usability on haemodialysis. Specifically we examined primary AVF failure (= all AVF which never became usable for haemodialysis) and immediate AVF failure (= all AVF which failed on the day of surgery).

**RESULTS:** Of 70 AVF formations, 60 vein specimens were available for histological analysis. Immediate failure occurred in n=3 (all forearm), primary failure occurred in n=16 (forearm: n=14), of which n=10 were due to thrombosis (forearm: n=10).



As all histological dimensions were significantly larger in upper arm (n=13) compared to forearm (n=47) veins, we focussed further analysis on forearm AVF.

Immediate failure was linked significantly to higher vein medial fibrosis (51% versus 31%,  $p=0.002$ ), but not to other histological measurements. A cut-point of 47% medial fibrosis was 98% accurate to predict immediate failure. Primary AVF failure had no relation to histological measurements.

Female gender was the only independent predictor of a smaller vein media area on multiple regression ( $p=0.001$ ). This finding could be interesting because worse AVF outcomes among women are well recognised. Age, Ethnicity, Diabetes were not associated with histology measurements.

**CONCLUSION:** In patients with endstage renal disease, increased medial fibrosis in native veins appears to predispose to immediate AVF failure. Vein media area is significantly smaller in women. These findings need validation in a larger study.

## **4.2. Introduction**

Arteriovenous fistulae (AVF) are preferred to arteriovenous grafts (AVG) or haemodialysis catheters (CVC) because of a lower infection risk and better patient survival (139). For normal development of an AVF, the vein needs to dilate to accommodate high blood flow needed for haemodialysis. Native venous disease may not allow appropriate dilatation and result in AVF failure: Indeed, abnormal vein morphology and distensibility on ultrasound,

found prior to AVF formation was associated with a higher rate of early AVF failure (32, 78, 108).

Altered native vein histology has been described in endstage renal disease, specifically intimal hyperplasia and medial fibrosis; whether these changes affect AVF outcome is unknown (60, 61). Vein structure may also be affected by diabetes mellitus as reported for saphenous veins (56). We used a new approach by measuring vein area (lumen, intima, media) in cross-section, which allowed us to quantify the degree of medial fibrosis, defined by the percentage collagen of total media area.

We hypothesised that pre-existing native vein disease quantified by vein histology may be associated with early AVF outcomes. Therefore, we harvested native veins from patients with endstage renal disease undergoing AVF formation and related venous histology to patient factors and early AVF outcomes.

### **4.3. Methods**

#### **4.3.1. Patients**

The study protocol was approved by the East Birmingham Local Ethics Research Committee. All patients who had surgery for formation of a new AVF were eligible for the study between 01/01/2005 and 31/01/2007. Patients who had already taken part in the study were also included (n=2). Patients with an existing AVF which required surgical repair were excluded. Baseline patient data and comorbidity were obtained from electronic patient records.

The site of AVF surgery was determined pre-operatively by physical examination of blood vessels; some patients also had ultrasound or angiography. Just before the operation, internal vein diameters (from near to far wall intima) in cross-section were measured with ultrasound (Sonosite 180 plus) by a trained nephrologist, prior to and after application of a tourniquet for 30 seconds, with patients seated and the arm supported by a pillow.

#### *4.3.2. Surgery*

Surgery was carried out by vascular surgeons experienced in AVF surgery (5 consultants, 5 trainees). Trainees usually operated under direct supervision by a consultant, but experienced trainees operated under indirect supervision.

AVF surgery was carried out with established technique usually under local anaesthesia. Following skin incision vein and artery were identified. The artery was occluded proximally and distally using a sloop or a small vascular clamp, before a longitudinal incision was made. The artery was then injected with heparinised saline. The vein was divided by transverse section, and from the distal end an approximately 10 mm long segment was resected and placed in a sterile container with formalin. The proximal vein in situ was flushed with heparin and anastomosed to the artery in an end-to-side fashion.

#### *4.3.3. Specimen transport and preparation*

The vein was submitted in formalin for immediate dissection. When transfer was delayed it was cooled at 5 degrees Celsius overnight until the next day. The specimen was sliced transversely at 3 to 4mm intervals, yielding on average 2 cross sections per vein.

The specimens were fixed in 10% neutral buffered formalin, embedded in paraffin and cut at 4 micron thickness prior to staining with Haematoxylin and Eosin and elastic van-Gieson (EVG). The EVG is a trichrome stain which allows distinction between the elastic which is stained black and the smooth muscle of the media which has a yellow colour. The collagen in the adventitia stains red.

#### *4.3.4. Vein histomorphometry*

Microscopic images were captured using an Olympus BX40 microscope and a Paxcam 2 Megapixel camera. Vein diameter and area was measured using image analysis software (Twain Image Solutions Software, ISS Group Services Ltd, Withington, UK).

One Consultant Pathologist analysed and measured the vein structure in cross-section after identifying intima, lamina elastic interna, media, lamina elastic externa (Figure 9). Width and area measurements were made for lumen, intima, media. For width measurements, the maximum and minimum thickness was obtained for each structure. Area measurements were also made for the collagen and muscular parts of the media. The ratio of collagen to total media area, and the ratio of muscle to total media area were calculated and expressed as a percentage; thus, percent collagen = 100% media - percent muscle, which quantified the severity of medial fibrosis.

#### *4.3.5. Definitions of early AVF outcomes*

Immediate AVF failure meant failure on the day of surgery. Primary AVF failure was defined as failure to develop (mature) for dialysis use including immediate failure; as a consequence AVF repair, new AVF formation or dialysis catheter insertion was required. Analysis was restricted to AVF with known outcomes (=used for haemodialysis or failed) by the end of the follow-up period (08/02/2008), whilst AVF with unknown outcomes (=patent AVF but not used for haemodialysis during follow-up period) were excluded. Dialysis use of the AVF was defined by at least 6 consecutive full dialysis sessions with two needles and without catheter assistance. Outcomes were unknown if a patent AVF had not been used for dialysis by the end of the follow-up period (due to patient death, transfer to other treatment modality, or patient had not required to start dialysis).

#### *4.3.6. Statistics*

Histological area measurements were skewed and this was corrected with logarithmic transformation. We used Pearson's correlation, Student's t-test, Chi-square tests with Yates' continuity correction as appropriate for univariate comparisons of AVF failure, vein histology, surgical experience and patient characteristics. The latter included age, gender, ethnicity, diabetes, dialysis status (on haemodialysis versus not yet started), vein diameter on ultrasound and antiplatelet use. Surgical experience distinguished between consultant vascular surgeons operating alone, experienced trainees operating alone, and junior trainees operating with vascular surgeons. The diagnostic value of medial fibrosis was evaluated using a receiver operating ROC curve. Logistic regression, using a stepwise backward approach based on the likelihood ratio, was attempted to analyse AVF failure as the

dependent variable, against vein histology, ultrasound vein diameter, and surgical experience. Multiple regression, using a stepwise backward approach, was used to test patient factors (gender coded female = 0, male = 1) to predict vein histology as the dependent variable. The diagnostic value of vein media fibrosis as a predictor of immediate AVF failure was calculated with sensitivity, specificity, accuracy, and a receiver operating curve was plotted. We used SPSS version 15 and Microsoft Excel 2007 for data analysis. A p-value of < 0.05 was considered significant.

#### ***4.3.7. Study and follow-up period***

Study period was from 01/12/2005 until 31/12/2006; follow-up was until 08/02/2008.

#### **4.4. Results**

Of 70 AVF formations, 60 vein specimens (n=47 forearm, n=13 upper arm) were available for histological examination, 48 of those had been scanned just before surgery with ultrasound (n=36 forearm, n=12 upper arm). Immediate failure occurred in n=3 (all forearm), primary failure occurred in n=16 (forearm: n=14), of which n=10 were due to thrombosis (forearm: n=10).

Of the 70 operations, in 10 none or inadequate specimens were obtained at surgery (n=5 forearm cephalic, n=5 upper arm cephalic). Twenty-three patients had declined to take part in the study.

Upper arm veins (n=13) were significantly larger than forearm (n=47) veins for all histological area measurements, including area of lumen (mean 0.61 versus 0.45 mm<sup>2</sup>, p=0.011); intima (mean 0.48 versus 0.28 mm<sup>2</sup>, p<0.001); media (1.12 versus 0.91 mm<sup>2</sup>, p<0.001); collagen part of media (0.76 versus 0.61 mm<sup>2</sup>, p<0.001). Therefore, we focussed further analysis on the larger subgroup of forearm veins with available histology (n=47).

#### *4.4.1. Early AVF failure and histology of forearm veins*

Primary AVF failure had no relation to histological area measurements (table 21). Immediate failure was linked to histological media composition (table 21): The two AVF with immediate failure and available histology had significantly more medial fibrosis, defined by the collagen content of the media (51% versus 39%, p=0.002). Other histological measurements (lumen, intima, media, collagen media areas) were not associated with immediate failure. Vein diameters on ultrasound appeared smaller in AVF with immediate failure, but the difference was not statistically significant (median failed: 2.0 mm, patent: 2.5 mm; p=0.306). Both AVF with immediate failure and available histology were constructed by the same consultant surgeon team, but surgical experience (p=0.106) was not associated with immediate failure (Figure 12). Patient demographics were not associated with immediate or primary failure (table 21).

We attempted logistic regression to examine immediate AVF failure as the dependent variable predicted by histological media collagen content (medial fibrosis), vein diameter on ultrasound, and surgical experience. Because there were only 2 failure events, it was not possible to reach statistical significance: medial fibrosis was the only predictor of immediate failure (odds ratio for failure = 1.813, p=0.083, 95% confidence interval 0.925 to 3.551).

Table 20: Early AVF failure (= primary failure and immediate failure) in forearm AVF with available histology. Univariate comparisons with (a) histological area measurements and with (b) patient demographics and surgical experience

Histological area		Primary AVF failure			Immediate AVF failure		
		Patent n=28	Failed n=13	p-value	Patent n=45	Failed n=2	p-value
Area lumen [mm <sup>2</sup> ]	median	0.16	0.16	0.815	0.16	0.16	0.952
Area intima [mm <sup>2</sup> ]	median	0.04	0.04	0.924	0.04	0.04	0.691
Area media [mm <sup>2</sup> ]	median	0.83	0.90	0.677	0.85	0.84	0.947
Collagen area media [mm <sup>2</sup> ]	median	0.32	0.35	0.509	0.32	0.43	0.372
Medial fibrosis (media collagen content %)		38%	39%	0.345	39%	51%	0.002

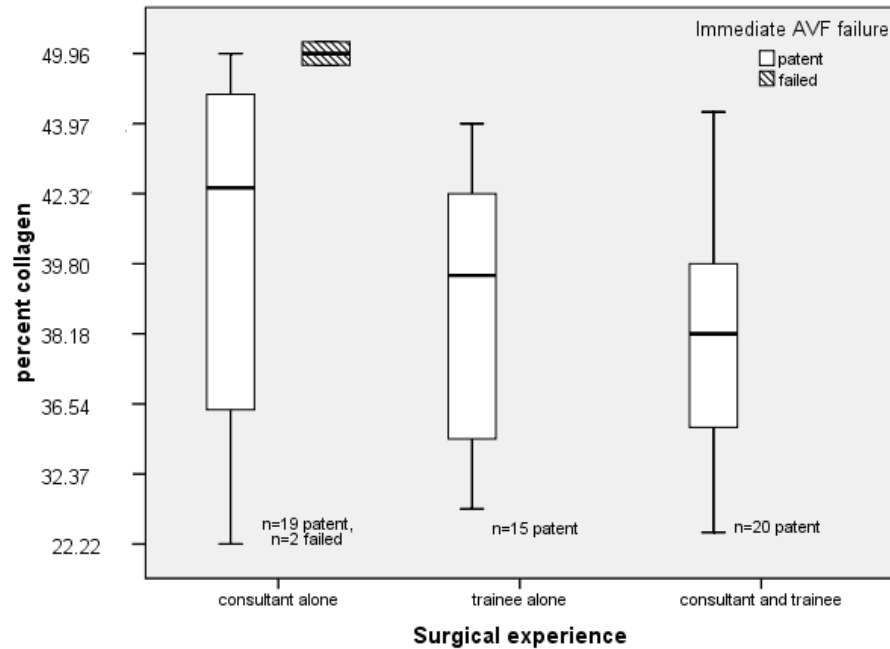
Patient demographics		Primary AVF failure			Immediate AVF failure		
		Patent n=28	Failed n=13	p-value	Patent n=45	Failed n=2	p-value
Age [years]	median	66.0	68.6	0.268	67.7	68.6	0.928
Gender	female	63%	37%	0.749	91%	9%	0.451
	male	73%	27%		100%	0%	
Ethnicity	Caucasian	67%	33%	0.975	97%	3%	0.830
	other	75%	25%		89%	11%	
Diabetes	not diabetic	76%	24.0%	0.307	96%	4%	1.000
	diabetes	56%	44%		95%	5%	
Dialysis	pre-dialysis	64%	36%	0.724	100%	0%	0.093
	on dialysis	74%	26%		90%	10%	
Antiplatelet drug use	yes	72%	28%	0.919	92%	8%	0.571
	no	62%	38%		100%	0%	
Vein diameter US [mm]	median	2.7	2.5	0.523	2.5	2.0	0.306
Surgical experience	Consultant	73%	27%	0.251	86%	14%	0.106
	Trainee	58%	42%		100%	0%	
	Consultant with trainee	87%	13%		100%	0%	

Note: Only primary failure with known outcome (n=41) was analysed; n=6 AVF patent but not used for dialysis.



Figure 12: Immediate failure and medial fibrosis (media collagen content) in forearm AVF grouped by surgical experience

**Medial fibrosis (% collagen content media) grouped by immediate AVF failure and surgical experience**



We investigated how well medial fibrosis predicts immediate AVF failure with sensitivity, specificity and ROC curve (Figure 13). The ROC curve shows that medial fibrosis has reasonable diagnostic value to predict immediate AVF failure. We found that a cut-point of 47% medial fibrosis had the best prediction for failure (Table 22).

Figure 13: Sensitivity, specificity and ROC curve of medial fibrosis (% media collagen)

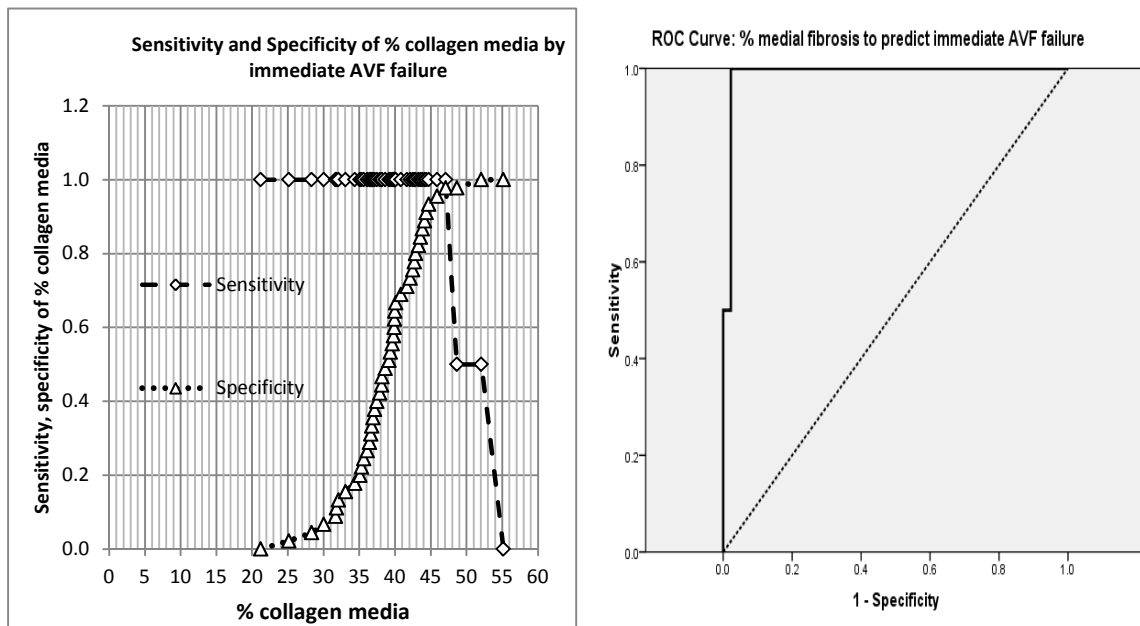


Table 21: Diagnostic value of increased media collagen for immediate failure in forearm AVF

Test / outcome	Failed	Used for dialysis	Total
Collagen > 47%	2	1	3
Collagen ≤ 47%	0	44	44
Total	2	45	47

Sensitivity = 2/2 (100%)

Specificity = 44/45 (98%)

Accuracy = 46/47 (98%)

Positive predictive value = 2/3 (67%)

Negative predictive value = 44/44 (100%)

#### 4.4.2. Patient factors and histology of forearm veins

We found histological differences associated with gender and dialysis status (figure 14).

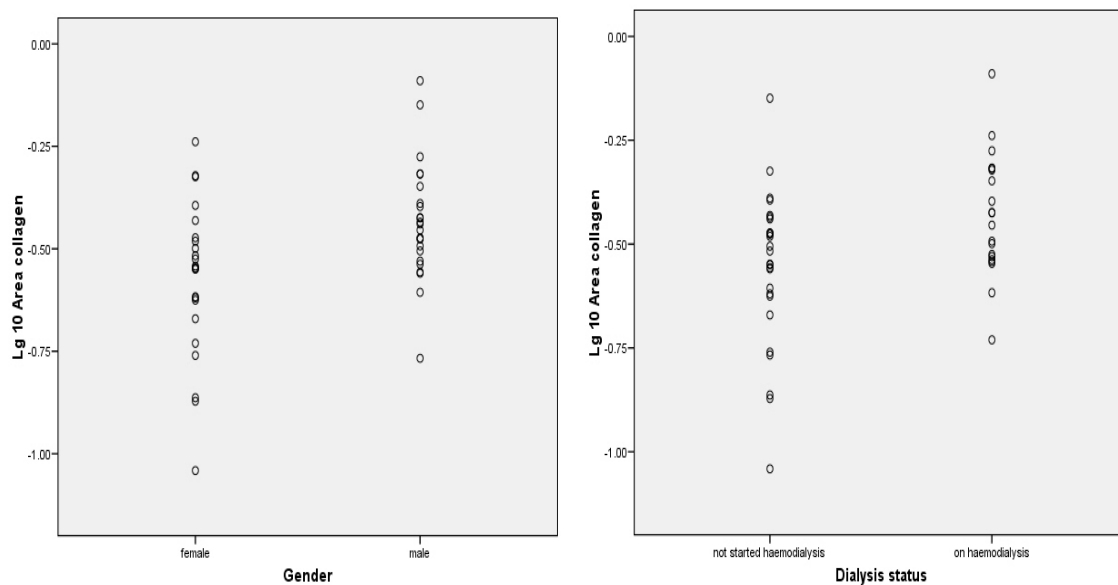
Women had significantly smaller media area and collagen area of media than men ( $p=0.002$

and  $p=0.007$ , respectively); however, medial fibrosis (media collagen content) was not

different for gender ( $p=0.922$ ). Patients not yet on haemodialysis had significantly smaller

media area, as well as smaller collagen area of media, than those already on dialysis ( $p=0.031$  and  $p=0.017$ , respectively). Other patient variables (age, ethnicity, Diabetes) were not associated with histology measurements (area lumen, area intima, area media, area collagen of media, media collagen content). Multiple regression analysis showed that only female gender (but not dependency on haemodialysis) was significantly associated with a smaller total vein media area ( $p=0.001$ ), and a smaller collagen media area ( $p=0.004$ ).

Figure 14: Forearm vein collagen area of media, compared with gender and dependence on dialysis (pre- versus on dialysis)



#### 4.5. Discussion

In summary, we found that increased medial fibrosis as measured by media collagen content was the only histological measurement predictive of immediate AVF failure in forearm AVF of patients with endstage renal disease. No histological measurement predicted primary failure. We also found that arm vein media dimensions were smaller in women. Other histological measurements were not related to patient demographics.

We found that immediate AVF failure was associated with increased medial fibrosis in the form of increased media collagen content); to our knowledge this has not yet been described. Our data can be criticised because only two AVF were affected which were made by the same consultant surgeon team. However, surgical experience was not significant on multivariate analysis. The box-plot figure illustrates that there was increased medial fibrosis in the two AVF that failed immediately, whilst the patent AVF made by the same consultant team show considerably less medial fibrosis, at a degree comparable to those of the other surgeons. Therefore, in our view medial fibrosis is the determinant of immediate failure, and the particular team were unfortunate enough to have the two veins with severe medial fibrosis. Our data suggest that a cut-point of 47% medial fibrosis or above predicts immediate AVF failure, but because of small numbers, this finding is of hypothesis-generating value only. Our result would be consistent with the hypothesis that severe medial fibrosis may result in increased blood flow resistance after AVF formation because of reduced venous distensibility, which may be a particular problem in the smaller calibre forearm veins. This is suggested by ultrasound studies of native veins that have shown reduced venous distensibility related to AVF failure (46, 78).

We presumed that pre-existing native vein disease found on histology could be associated with early AVF failure (60). However, our data showed no association between vein histology and primary AVF failure. We considered the following: Possibly, significant venous disease could have been excluded already through pre-operative assessment. Perhaps, primary AVF failure depends on other factors, such as myo-intimal hyperplasia that develops after AVF formation and may progress to stenosis, or native arterial disease which has been associated

with early AVF failure (25, 66). Finally, our study may be underpowered to show a difference. But an alternative interpretation of our findings could be that native vein disease, specifically medial fibrosis, might impair vein dilatation at surgery causing immediate AVF failure, whilst inappropriate arterial development or neo-intimal hyperplasia primarily affect primary AVF failure. More research is needed in this area.

To our knowledge, our data are the first to show that women have significantly smaller total and collagen media area than men, but equivalent degrees of medial fibrosis between the genders. This could simply mean that smaller calibre veins in women reflect an on average smaller body size. However, the data are interesting because poorer AVF outcomes in women have been consistently reported and the reasons for this are unknown (30, 36, 37). Ultrasound-based studies show that women have smaller vessel lumen diameters than men (30, 45). Miller et al therefore used pre-operative vascular ultrasound to select only vessels with an appropriate minimum diameter for AVF surgery: Even so, significantly more early AVF failure was found among women (30). Whilst our data are suggestive of poorer outcome in women compared to men (immediate AVF failure: 9% versus 0%, primary AVF failure: 37% versus 27%), this is not significant in our small sample size. Although the clinical significance of smaller vein media dimensions is not clear from our data, this finding requires further research given the well-known poorer AVF outcomes among women.

We also found larger media dimensions in patients dependent on haemodialysis compared to those waiting to start haemodialysis, although this was not significant on multivariate

analysis. This finding is consistent with observations by Feinfeld et al, who found that long term haemodialysis patients had a greater medial width compared to recent haemodialysis starters (61).

In conclusion, we found that immediate AVF failure was associated with increased medial fibrosis, which might impair the ability of the vein to dilate after AVF formation.

Furthermore, smaller vein media dimensions were associated with female gender, which is interesting as women are generally known to have worse AVF outcomes. These findings need validation in a larger study.

## 5. Does assessment of arteriovenous fistulae early after surgery predict their usability on haemodialysis?

### 5.1. Abstract

**INTRODUCTION:** Early AVF assessment after surgery is desirable for timely detection of early AVF failure, as well as to determine AVF maturity. This would help to avoid haemodialysis catheter use. The clinical challenge with patent AVF is to distinguish those that are still maturing versus those which cannot mature due to an underlying problem. We therefore examined patients with newly formed AVF at 4 weeks to determine whether clinical or ultrasound assessments were useful in terms of predicting AVF failure.

**METHODS:** Patients with endstage renal disease who had first time or second time AVF formation were included in the study. Clinical examination was carried out by experienced haemodialysis nurses. Ultrasound examination was carried out by a trained nephrologist using a portable machine and included measuring vascular lumen diameters as well as blood velocities. Both examiners were not aware of each others' findings. Primary failure defined by haemodialysis use was the outcome measure.

**RESULTS:** Of 208 AVF formations, 133 patients returned for follow-up with a clinically patent AVF, and AVF outcome was known in 119. Physical examination by experienced dialysis nurses was 79% accurate: the physical finding of a pulse or the absence of a palpable thrill predicted failure, and a thrill predicted AVF usability. For ultrasound we established cut-points predicting primary AVF failure: These were arterial velocity less than 110 cm/s and venous diameter less than 5 mm (accuracy 66% and 80%, respectively). Ultrasound

measurements remained the only independent predictors of less primary failure on multivariate analysis (larger vein diameter of  $\geq 5$  mm: Odds ratio (OR) = 0.104, 95%CI [0.036 to 0.297,  $p < 0.001$ ] and higher end-diastolic arterial velocity of  $\geq 110$  cm/s: OR = 0.274, 95% CI [0.095 to 0.785,  $p = 0.016$ ]). Whilst vein diameter was distinctive to predict failure from early on, arterial velocity became increasingly useful from 2 weeks after surgery.

**CONCLUSION:** Post-operative assessment of patent AVF as early as 4 weeks after surgery is useful to predict maturation failure.

## 5.2. Introduction

Guidelines on vascular access for long-term haemodialysis agree that central venous catheters (CVC) should be avoided because of well recognised risks including infection and central vein stenosis; instead, arteriovenous fistulae (AVF) should be used (10, 11). Despite these guidelines, there is still considerable use of CVC world-wide as reported by the dialysis outcomes and practice patterns study group; one factor associated with more CVC use may be a practice of delayed AVF cannulation in some countries (44). In terms of the proportion of AVF cannulated by 8 weeks after surgery, this occurs in 14% in the United States, 45% in the United Kingdom, and in over 90% in mainland European countries (44). Provided the AVF is mature, cannulation as early as 2 weeks after surgery was associated with equivalent AVF survival as later cannulation (17). Because early failure may occur in about 20-50% of new AVF, clinicians often face the task of assessing AVF in terms of their future usability for haemodialysis (8).



If AVF failure is due to thrombosis, this is usually easy to diagnose clinically because the physical findings of thrill and bruit over the AVF outflow vein are absent. However, an equally common form of failure is in patent AVF which have inadequate blood flow to allow haemodialysis; often there is an underlying cause which can be treated, and subsequently the AVF can be used for haemodialysis (19). Not infrequently, it is a clinical challenge to decide whether a patent AVF will be usable for haemodialysis or not, particularly if this is done very early after surgery and in a patient not yet dependent on haemodialysis. In the first month after surgery, blood flow through the AVF increases during normal maturation; low blood flow, as may be found in the early phases of AVF maturation, also occurs in AVF with a flow-limiting stenosis (15, 140).

Four months after surgery, assessments predict AVF maturity well: Robbin et al studied whether haemodialysis adequacy of patent AVF could be predicted by physical examination or ultrasound scanning at 2-4 months after surgery (106): In terms of predicting AVF maturity, physical examination was 80% accurate whilst ultrasound had an up to 95% positive predictive value for combined measurement of vein diameter and blood flow.

An early assessment after surgery would be clinically more useful, but it is unknown whether such an assessment would be accurate. We therefore assessed patent AVF at four weeks after surgery with physical and ultrasound examination, so as to see whether this predicted AVF failure to mature.

### 5.3. Methods

#### 5.3.1. Patients

Patients belonged to a cohort recruited for the purpose of a randomised trial conducted at a single centre and described in detail earlier (section B, Chapter 2). In brief, patients with endstage renal disease requiring vascular access were randomly allocated to pre-operative assessment with physical examination (control) or routine ultrasound (experimental); however, routine contrast venography was performed in all patients with a central venous catheter in place for 3 months or longer. In the ultrasound group, the surgeon was made aware of the ultrasound findings, but not in the clinical group. Early AVF failure was the primary endpoint. Patients were included if they required first or second time formation of a new AVF, whereas new AVF beyond the second time and AVF salvage surgery were excluded.

The post-operative assessment of this patient group was observational and carried out using physical and ultrasound examination: All patients initially recruited were invited to return at 4 weeks after surgery for post-operative assessment of the AVF. Patients were not further assessed if the AVF was thrombosed, or if patients chose not to attend (free transport was offered).

#### 5.3.2. Physical AVF examination

Experienced haemodialysis nurses with known good AVF cannulation skills were asked to perform a physical examination of the AVF. This included the flow character (thrill, pulse or bruit without a thrill), the vein calibre, the straightness and depth of the vein. Finally they

were asked to conclude about the usability of the AVF for haemodialysis, whether the AVF was mature at this point, whether they expected the AVF to become mature, whether they felt it was not maturing, or whether the outcome was uncertain. The nurses were unaware of the ultrasound findings.

### *5.3.3. Ultrasound scan of AVF*

A trained nephrologist (myself) scanned the AVF with a portable ultrasound (Sonosite 180 plus, 5-10 MHz linear transducer), using a protocol based on a technique described earlier (section B, Chapter 3.3.). In brief, patients were seated in a chair with the AVF arm supported by a pillow at 60 degrees. With greyscale ultrasound, an initial overview of the feeding artery, AVF anastomosis and draining vein was obtained. Radiocephalic (forearm) AVF were followed from the radial artery some 5 cm proximal to the anastomosis, and then along the course of the vein up to the antecubital fossa. Brachiocephalic or brachio basilic (upper arm) AVF were followed from some 5 cm proximal to the anastomosis, and then along the course of the vein up to the shoulder. The entry of the cephalic vein into the subclavian vein, the subclavian vein, and the brachial veins were not studied. Representative cross-sectional vascular lumen diameters were obtained at the following sites: Brachial, radial and ulnar arteries (the AVF feeding artery was scanned within 3 cm distance proximal and distal to the anastomosis); AVF anastomosis (diameter in 2 planes, parallel and perpendicular to feeding artery); curved vein beyond anastomosis; straight vein (=future cannulation site). Cross-sectional vascular diameters were measured provided that the vessel was on a plane parallel to the skin surface, from the near to the far wall interface between vessel wall and lumen. The average of two diameters was used. To avoid venous

compression, a layer of at least 3 mm of ultrasound gel was placed between skin surface and transducer, whilst the transducer was stabilised by resting part of it on skin surface area to the side of the vein. At the same standard sites for diameter measurements, blood velocities were recorded with spectral duplex (peak systolic and end-diastolic velocities). The velocities were obtained with spectral duplex, with the vessel in longitudinal view at maximum diameter, the Doppler range gate encompassing the entire lumen and with the angle of insonation set at 60 degrees or less. Pulse repetition gain was adjusted to avoid aliasing. The average of two measurements was used. Velocities in the venous parts of the AVF were often too high to be exactly quantified, but arterial measurements could all be obtained. Our Sonosite 180 plus machine did not provide directional colour Doppler and hence this was not used when following the vasculature. However, focal vessel narrowing on greyscale by  $> 1/3$  was noted in terms of diameter and anatomical site, and further interrogated with spectral duplex; a two-fold increase of peak-systolic velocity was considered as haemodynamically significant, but for the anastomosis it was a three-fold increase (110).

#### *5.3.4. Statistics*

AVF outcome was described by primary AVF failure which included any cause of failure preventing haemodialysis use after initial AVF formation; the effects of AVF salvage was not taken into account (30). Primary failure was defined by use for regular haemodialysis (at least 6 consecutive sessions with 2 needle AVF cannulation and blood flow rate  $> 200$  ml/min) or failure; patients without known AVF outcome (usually because of not yet having started haemodialysis) were excluded from the analysis.

Continuous variables were explored and transformed if necessary to obtain an approximately normal distribution (age transformed by square/1000, time periods between surgery, AVF assessment and AVF haemodialysis use by logarithm, arterial diameter and velocities by square root, venous diameters by logarithm\*1000). Initial analysis was performed with CHI square tests and student t-tests; multivariate analysis was carried out using logistic regression. Primary AVF failure was the dependent variable, predicted by patient demographics, timing of assessment, and assessment findings (clinical and ultrasound). Significant variables from the logistic regression were further examined in terms of sensitivity, specificity and receiver operating (ROC) curves, to decide on appropriate cut-off points (141). Confidence intervals (95%) for sensitivity, specificity, accuracy and predictive values were calculated using an online calculator (142). Empirical normal ranges were calculated as 2.5<sup>th</sup> to 97.5<sup>th</sup> centile around median for skewed data (143). Analyses were carried out with SPSS version 16 and Microsoft Excel 2007. A p-value of < 0.05 was considered significant.

#### **5.4. Results**

In the pre-operative study described earlier (section B, Chapter 2), 208 patients had an AVF formed, of whom 57 had primary failure and 151 had a patent AVF (of those, 129 used their AVF for dialysis whilst in 22 the AVF outcome was unknown). Of the 151 patients with a patent AVF, 133 presented for follow-up examination and these formed the cohort examined in this study. Of those 133, 14 patients were excluded from the analysis because AVF outcome was unknown (they had not started haemodialysis during the follow-up

period). Among the remaining 119 patients, physical examination was carried out in 109 and ultrasound in 116.

Primary failure occurred in 22% (n=26 of 119), largely due to thrombosis (n=12) or stenosis (n=9) of the AVF.

#### 5.4.1. Parameters that predict primary AVF failure

Patient demographics and timing of assessments are shown by primary failure in table 23.

Table 22: Primary AVF failure (known in n=119): univariate comparisons of patient demographics and timing of assessments

		AVF used for dialysis	Primary AVF failure	p-value
Age	Median [IQR]	68 years [61 to 74]	69 years [66 to 72]	0.148
Gender	Female	71%	29%	0.281
	Male	82%	18%	
Diabetes	No	83%	17%	0.173
	Yes	71%	29%	
Randomisation (in pre-operative trial)	Clinical	77%	23%	0.906
	Ultrasound	79%	21%	
Site AVF	Forearm	70%	30%	0.011
	Upper arm	91%	9%	
T Surgery-Assess	Median [IQR]	28 days [21 to 34]	28 days [21 to 33]	0.852
T Assess-AVF event	Median [IQR]	43 days [19 to 152]	32 days [12 to 76]	0.056

Notes: IQR=interquartile range; T Surgery-Assess = time from surgery to assessment; T Assess-AVF event = time from assessment to haemodialysis use or failure of AVF

Among patient demographics, only forearm AVF site significantly predicted primary AVF failure (forearm: n=22/73, upper arm n=4/46; p=0.011). Randomisation group was not significantly different for primary failure (p=0.906), nor was time from surgery to assessment (p=0.852), but time from assessment to AVF haemodialysis use / failure tended to be shorter in those with primary failure (p=0.056).

At 2-6 weeks after surgery, the nurses' opinion on whether the AVF would be usable for haemodialysis or not, did not predict primary failure ( $p=0.235$ ). For physical examination, the only significant predictor for primary AVF failure was flow evaluation as shown in table 24 ( $p=0.018$ ). Specifically, a pulse or absence of a thrill was very specific (96%) for AVF failure, but not very sensitive (21%). Overall this test was 79% accurate.

Table 23: Physical examination for AVF flow (no thrill versus thrill) at 4 weeks and its diagnostic value to predict primary AVF failure (data available for analysis  $n=106$ )

Test / outcome	Failed	Used for dialysis	Total
No thrill	5	3	8
Thrill	19	79	98
Total	24	82	106

Diagnostics	Result	95% confidence interval [lower and upper limit]
Sensitivity	5/24 (21%)	0.090 to 0.298
Specificity	79/82 (96%)	0.929 to 0.990
Accuracy	84/106 (79%)	0.715 to 0.870
Positive predictive value	5/8 (63%)	0.269 to 0.895
Negative predictive value	79/98 (81%)	0.777 to 0.828

The ultrasound parameters for all AVF ( $n=119$ ) are summarised in table 25.

Table 24: Ultrasound measurements grouped by primary AVF failure

	all AVF (forearm and upper arm)								
	used for dialysis				primary failure				t-test p- value
	n	median	IQR		n	median	IQR		
Diameter proximal artery [mm]	90	4.0	3.1;	4.9	26	3.1	2.6;	3.6	0.001
Diameter anastom. right angle [mm]	89	3.1	2.5;	3.9	25	2.7	2.0;	3.4	0.060
Diameter anastom. parallel [mm]	68	3.6	2.7;	4.4	15	3.0	2.4;	3.6	0.258
Diameter vein curve [mm]	80	4.7	3.9;	5.8	24	4.2	3.1;	4.8	0.001
Diameter vein straight [mm]	89	5.8	5.1;	6.6	25	4.5	3.5;	5.5	<0.001
PSV proximal artery [cm/s]	87	236.2	187.0;	299.0	26	176.9	94.6;	213.8	<0.001
EDV proximal artery [cm/s]	87	134.3	106.4;	169.0	26	98.5	52.6;	117.1	<0.001
RI proximal artery [%]	86	41%	36%;	48%	26	46%	36%;	56%	0.003

Notes: Diameter anastom. right angle = diameter of the anastomosis at right angle to feeding artery; Diameter anastom. Parallel = diameter of the anastomosis parallel to feeding artery; diameter=internal lumen diameter of vessel; EDV=end-diastolic velocity; n=number of AVF scanned; n = number of measurements available for analysis; PSV=peak systolic velocity; RI=resistive index, shown as percentage ( $\frac{PSV-EDV}{PSV} \times 100\%$ ). Because the data were skewed, median and interquartile range of the raw data are shown, but transformed data were used for the t-tests.

#### 5.4.2. Ultrasound predictions of failure to mature

We investigated the diagnostic value of ultrasound parameters (arterial end-diastolic velocity and vein diameter) to predict primary failure. We first calculated the empirical normal ranges around the median for vein diameter and for arterial velocity for all AVF (table 26). There was considerable overlap between primary failure and vein diameter or arterial velocity.



Table 25: Empirical normal ranges (shown for all AVF)

Ultrasound measurements	Vein diameter [mm]	Arterial EDV [cm/s]
failure	4.5 [2.0 to 6.7]	99 [14 to 166]
dialysis use	5.8 [3.7 to 9.6]	134 [32 to 326]

We calculated sensitivity, specificity and plotted ROC curves to determine the best cut-off values for vein diameter and arterial velocity, respectively, to predict primary AVF failure (table 27 and 28; figure 15). This showed that a vein diameter below 5 mm and an end-diastolic velocity below 110 cm/s in the feeding artery were the best cut-points to predict failure. The cut-points were the same for forearm as for all AVF; upper arm AVF had slightly larger cut-points but using those did not change the diagnostic value of ultrasound predicting AVF failure (data not shown).

Table 26: Diagnostic value of AVF vein diameter on ultrasound to predict primary failure (all AVF)

Test / outcome	Failed	Used for dialysis	Total
Below 5 mm	17	15	32
At least 5 mm	8	74	82
Total	25	89	114

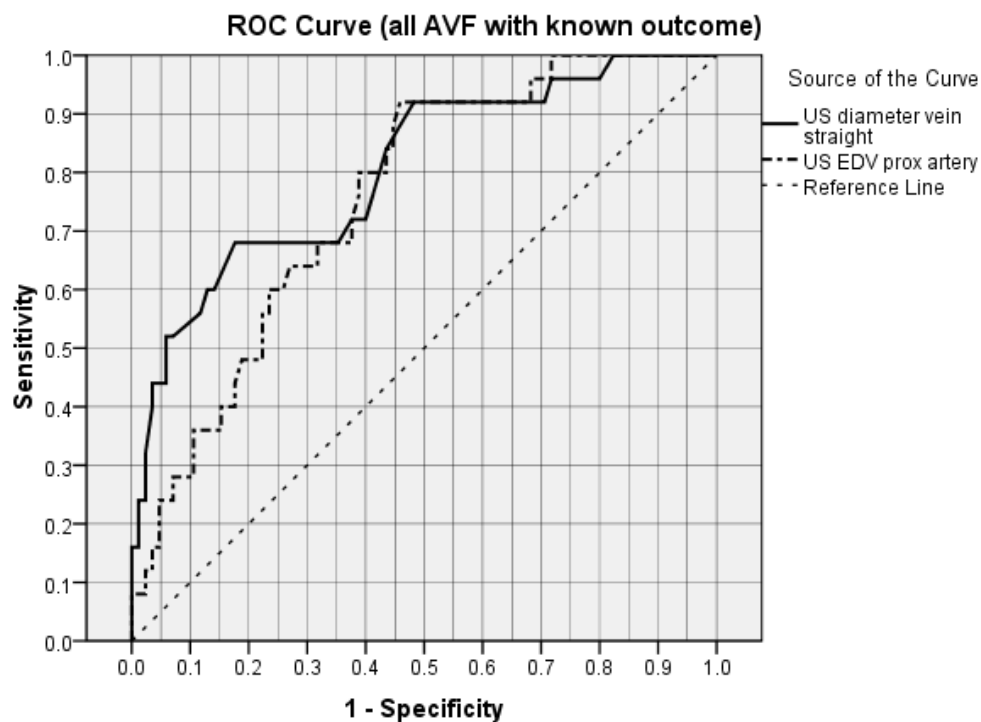
Diagnostics	Result	95% confidence interval [lower and upper limit]
Sensitivity	17/25 (68%)	0.495 to 0.827
Specificity	74/89 (83%)	0.779 to 0.873
Accuracy	91/114 (80%)	0.725 to 0.874
Positive predictive value	5/8 17/32 (53%)	0.387 to 0.646
Negative predictive value	74/82 (90%)	0.846 to 0.947

Table 27: Diagnostic value of arterial end-diastolic velocity on ultrasound to predict primary failure (all AVF)

Test / outcome	Failed	Used for dialysis	Total
Below 110 cm/s	17	29	46
At least 110 cm/s	9	58	67
Total	26	87	113

Diagnostics	Result	95% confidence interval [lower and upper limit]
Sensitivity	17/26 (65%)	0.467 to 0.809
Specificity	58/87 (67%)	0.611 to 0.713
Accuracy	75/113 (66%)	0.577 to 0.751
Positive predictive value	17/46 (37%)	0.264 to 0.458
Negative predictive value	58/67 (87%)	0.793 to 0.926

Figure 15: ROC curve for ultrasound vein diameter and arterial velocity to predict primary AVF failure (all AVF)



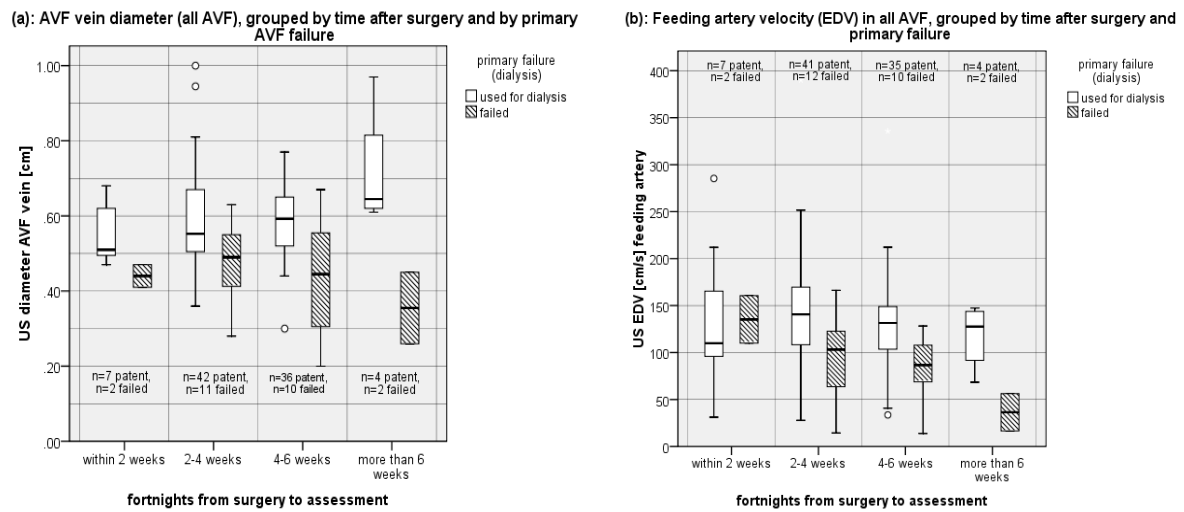
The ROC curve plots the cumulative values of true positive against false positive test results for every ultrasound measurement: In the case of perfect discrimination for primary failure the curve would be close to the upper left corner, but lack of discrimination would show as

overlap with the diagonal (12). Both vein diameter and for arterial velocity are significant predictors of primary AVF failure, as demonstrated by the area under the curve (Vein diameter: AUC=0.806, 95% confidence interval 0.704 to 0.906,  $p<0.001$ . Arterial end-diastolic velocity: AUC=0.756, 95% confidence interval 0.659 to 0.854,  $p<0.001$ ).

With logistic regression we examined primary failure as the dependent variable, predicted by patient characteristics, randomisation to routine pre-operative ultrasound, AVF site, timing of assessment, clinical vein flow and post-operative AVF ultrasound parameters. Post-operative ultrasound assessment of the AVF (diameter of the straight AVF vein (below 5 mm versus at least 5 mm) and end-diastolic velocity in the feeding artery (below 110 cm/s versus at least 110 cm/s) were the only independent predictors of primary AVF failure: There was less failure with a larger vein diameter (Odds ratio = 0.104; 95% confidence interval 0.036 to 0.297;  $p<0.001$ ) and a greater arterial velocity (Odds ratio = 0.274; 95% confidence interval 0.095 to 0.785;  $p=0.016$ ). Physical AVF examination was not an independent predictor.

We considered that blood flow in new AVF continues to increase during the first month, and this could affect ultrasound parameters within the first 4 weeks (140). Therefore we plotted ultrasound measurements in AVF grouped by time after surgery and primary AVF failure (figure 16): These show that vein diameter was discriminative early on, whilst arterial velocity became increasingly useful beyond 2 weeks after surgery.

Figure 16: Ultrasound measurements to predict primary failure, grouped by time after surgery. (a) vein diameter; (b): arterial (end-diastolic) velocity



## 5.5. Discussion

In summary, we investigated whether early post-operative assessment of patent (excluding thrombosed) AVF is useful in terms of predicting failure of AVF to be used for haemodialysis (primary failure). Physical examination by experienced dialysis nurses was 79% accurate in as far as the physical finding of a no thrill predicted failure, and a thrill predicted AVF usability. Bedside ultrasound measurements of vein diameter and arterial velocity were the only independent predictors of primary failure on multivariate analysis: We determined cut-points predicting primary AVF failure for arterial end-diastolic velocity below 110 cm/s and for venous diameter below 5 mm.

Few studies examined early AVF assessment after surgery. Blood flow was considered as an important marker of AVF functionality; this can be calculated from the product of time-averaged velocity and vessel diameter, both of which can be measured with ultrasound (74). Reproducibility of blood flow measurements may be affected by changes between cardiac cycles and by measurement errors (which are then multiplied). There is controversy in the literature where to measure blood flow: Some use the draining vein but measurements can be affected by turbulences and diameter irregularities (106, 117). Others proposed the brachial artery which is a straight, regular vessel, but blood flow estimates there include a minor proportion of non-AVF blood flow directed to the upper extremity (15). Blood flow measurements increase during AVF maturation in the first month: Lomonte et al measured blood flow (brachial artery) repeatedly at different times after AVF formation and showed that a 50% rise occurs by the day after surgery, followed by a 20% increase at week one; by one month, approximately 2/3 of the final blood flow are achieved (15). Between 2 to 4 months after surgery, AVF blood flow did no longer appear to change (106).

Observational studies with ultrasound have followed AVF development and outcomes early after surgery. Kim et al reported that the average blood flow (AVF vein) at 1 week after surgery was significantly lower in AVF that subsequently failed, and suggested a cut-point of 350 ml/min (117). Wong et al scanned AVF fortnightly after surgery for 6 weeks; they reported consistently lower average blood flow (AVF vein) in those AVF that later failed; at 12 weeks, a blood flow < 150 ml/min predicted failure (32). Robbin et al established a cut-point for blood flow (AVF vein) of 500 ml/min at 2-4 months after surgery, to predict AVF maturity (106). Thus, although blood flow may characterise AVF functionality, the exact cut-

points are not fully established; particularly during the first month or two, different cut-points may be needed so as to take normal AVF development into account.

Blood velocity has been used as a surrogate marker for blood flow. We focussed on the end-diastolic velocity because this is usually absent in the native artery at rest and increases dramatically after AVF formation; on the other hand, it may considerably reduce in AVF with a very high-grade stenosis. One could regard it as the ultrasound analogue to a continuous thrill found on normal physical AVF examination. Wong et al reported that the peak systolic blood velocity measured in the AVF vein on the day after surgery was significantly lower (on average: 180 versus 530 cm/s) in AVF with subsequent failure compared to those patent (32). Our data are in keeping with these findings, although not directly comparable. We found that the end-diastolic velocity measured in the feeding artery for patent AVF scanned between 2 and 6 weeks after surgery was an independent predictor of primary failure. We found that a cut-point of 110 cm/s was better at predicting AVF maturity (NPV for failure 87%) than AVF failure (PPV for failure 37%) in our population. This may be because we excluded AVF which had clinically failed. Consistent with the fact that blood flow increases considerably in the first month after surgery, we found that arterial velocity measurements did not distinguish AVF outcomes within 2 weeks of surgery, but were more useful beyond 2 weeks.

Vein diameter increases are also part of AVF maturation. Kim et al found a significantly smaller vein diameter (average 3.5 versus 4.1 mm) at 1 week after surgery in AVF that subsequently failed, compared to those patent (117). Wong et al report that at 12 weeks after surgery, AVF with a vein diameter of below 3 mm failed (32). Robbin et al chose a cut-

point of 4 mm for vein diameter at 2 to 4 months after surgery to predict AVF maturity (106). Our data also show that a smaller venous diameter is an independent predictor of primary failure of forearm AVF; for scans of patent AVF between 2-6 weeks, but we arrived at a cut-point of 5 mm. The difference may have arisen because we focussed on AVF failure rather than AVF maturity, or because of a considerable variability in AVF vein diameter. Therefore we conclude that vein diameter and blood flow or blood velocity, measured by ultrasound, are useful to screen for AVF failure early after surgery.

Only Robbin et al investigated the value of ultrasound measurements as a diagnostic test; they analysed for AVF maturity, which is the reverse of our analysis for failure (106). Their data have comparable sensitivity, specificity, accuracy and predictive values for the vein diameter, but their blood flow measurements tend to be better than our velocity measurements. We suspect this is because blood flow in the first months rises, and our data show that beyond 2 weeks blood velocity does become more discriminative for AVF outcomes. Ives et al reported on post-operative ultrasound scanning, mostly within 8 weeks of surgery; they found a very high predictive value at 94% for AVF maturity with a normal scan, but they included thrombosed AVF in their study (144), which we excluded, and as a result differences in measurements would have been more subtle in our study.

The value of physical examination was only investigated by Robbin who showed that experienced dialysis nurses correctly identified maturity in 80% of AVF by 2 to 4 months (106). By contrast, at 4 weeks our nurses were not able to confirm AVF maturity, perhaps because many AVF would still have been in development at that point. However, the absence of abnormal flow on palpation of the AVF (=pulse, absence of thrill) makes AVF

failure unlikely (96% specific), although it is not a sensitive sign (21%). Although physical examination had only modest value in our study, one should consider the possibility to enhance it by following a more systematic examination protocol (112). In AVF in which dysfunction is known, physical examination was shown to compare well with angiography (114). Hence systematic physical examination may have value to screen all AVF post-operatively.

Our data have limitations because we did not measure AVF blood flow. As our data are cross-sectional rather than longitudinal with repeat scans of the same AVF, we cannot produce cut-points for different time intervals after surgery.

In conclusion we show that early post-operative examination AVF can predict primary failure already at 4 weeks. We propose that all new AVF should be screened with ultrasound to measure AVF vein diameter and arterial velocity (or blood flow). We propose that vein dilatation less than 5 mm or end-diastolic velocity in the feeding artery below 110 cm/s are associated with a greater likelihood of AVF failure. Such AVF should be closely monitored and appropriately investigated.



## **D) Thesis Discussion**

### **1. Summary of main research findings**

The research performed in this thesis has made the following new findings: AVF outcomes were equal among Caucasians and ethnic minorities, despite a higher rate of diabetes. However, overall diabetes, female gender and also cardiovascular disease were associated with a higher risk of AVF failure, as previously described in the literature. We also showed in a randomised trial that pre-operative ultrasound performed routinely in all patients prior to AVF formation resulted in better AVF outcomes than standard care based on physical examination with selective imaging; complete AVF outcomes in terms of early and longer term dialysis have not been described by other randomised trials. Furthermore we investigated native vein histology. We found that vein lumen dimensions measured with histology were considerably smaller than those measured with ultrasound in vivo, possibly due to a shrinkage effect with formalin fixation. Moreover, we found a way to quantify medial fibrosis through area measurement; this has not yet been described. Vein histology with a higher degree of medial fibrosis was associated with immediate AVF failure and we found a cut-point which discriminated well for failure; this has not yet been reported. Finally, we describe that early post-operative assessment of patent AVF is useful to predict subsequent AVF failure: Although clinical examination has some use, ultrasound evaluation of vein diameter and blood velocity, a surrogate marker for flow, were independent predictors of AVF failure and had moderate diagnostic value. The diagnostic value of such early measurements has not yet been investigated in detail.

## **2. Implications from the research in this thesis**

### **2.1. Patient demographics and ethnicity**

The optimal timing of AVF formation in patients with advanced chronic kidney disease is controversial. On a practical level, one may conclude from our data (as well as the literature) that AVF formation should be considered earlier in certain patient groups who are at greater risk of AVF failure, such as those with diabetes, vascular disease or female gender, because it may take more time to establish an access functional for haemodialysis. More frequent AVF monitoring or surveillance may also be needed in such risk groups. On the other hand, our data suggest that ethnic minorities such as Indo-Asian and perhaps also African patients can expect similar AVF outcomes as Caucasians in the UK.

### **2.2. Pre-operative vascular ultrasound mapping**

Traditionally, forearm (radiocephalic) AVF were attempted in nearly all patients, with modest AVF outcomes. Increasingly the site for AVF surgery is now selected by the quality of the vessels; there is still a preference for forearm AVF, provided that vessels are suitable. Ultrasound mapping is proven to be useful in selected patients. Our randomised trial has shown that routine pre-operative ultrasound is effective in all patients and should therefore be the standard of care for all patients who require AVF formation.

### **2.3. Native vessel histology**

Area measurement of vein histology as described by us has the advantage that the degree of media fibrosis of the vein can be quantified. Our pilot study which shows that media fibrosis was associated with immediate failure generates the hypothesis that a greater degree of media fibrosis results in greater resistance and impaired vein dilation after arteriovenous anastomosis.

### **2.4. Early post-operative AVF examination**

Our data show that routine early post-operative assessment of patent AVF is worthwhile because it can identify AVF at greater risk of subsequent failure. Based on our data, as well as the work by others (32, 106, 117), we propose routine post-operative screening of AVF with ultrasound to identify AVF which need further investigation and possibly salvage intervention.

## **3. Implications from recent literature publications on AVF patency and maturation**

### **3.1. Endothelial function, matrix metalloproteinases and AVF maturation**

In recent years there has been increasing recognition that the vascular endothelium is an active tissue, producing mediators which work through intracellular signalling cascades to regulate vascular tone and blood flow, vascular permeability and inflammation, and to inhibit or favour vascular thrombosis (145). Healthy endothelium maintains blood flow and

prevents thrombosis or inflammation through nitric oxide and other mediators; specifically, increased blood flow exerts a tangential force known as shear stress on the endothelium which releases further nitric oxide, resulting in flow-mediated arterial vasodilatation (146). But in disease which manifests as endothelial dysfunction or structural injury, the bio-availability of nitric oxide is reduced and the balance shifts towards vasoconstrictive, pro-inflammatory, pro-thrombotic and pro-proliferative mediators which can progress to vessel wall thickening and luminal stenosis (145). Endothelial dysfunction is recognised in older age, diabetes and hypertension, as well as in advanced renal failure (68, 147).

Endothelial function is central to AVF maturation, which is characterised by increased blood flow and dilatation of the muscular artery and superficial vein in the upper extremity. At surgery, there is immediate arterial dilatation due to increased shear stress on the endothelium (148). From experimental AVF models we know that subsequent outward arterial expansion requires remodelling of the arterial wall with fragmentation of the internal elastic lamina, a process which is mediated by matrix metalloproteinases (MMP) and these depend on a functional endothelium releasing nitric oxide (149, 150). MMP are a family of zinc-dependent proteinases capable of degrading extra-cellular matrix and their activity is under tight control: they are released as inactive precursors requiring activation, and their action is terminated by tissue inhibitors of metalloproteinases (TIMP) (151). Specifically, the gelatinases MMP-2 and MMP-9 (inhibited by TIMP-2 and TIMP-4, respectively) have been implicated in arterial dilatation in AVF (148, 152). In a clinical study, AVF maturation was associated with increased ratios of MMP-2:TIMP-2 and possibly MMP-9:TIMP-4 in the serum collected at the time of AVF surgery (153).

Dilatation of the compliant thin-walled vein has traditionally been viewed as passive in response to increased flow; however, a clinical study on venous maturation in men observed “eccentric hypertrophy” during maturation (lumen expansion with stable wall thickness), which was associated with increased flow and thus may be related to endothelial function (154). The role for MMP in AVF maturation is less clear for veins than arteries; a recent clinical study examined vein specimens harvested at AVF surgery and found increased tissue levels of total MMP-2, MMP-2 activator and the inhibitor TIMP-2 among AVF that matured (148, 152).

### **3.2. Non-maturation of AVF: the role of endothelial dysfunction and inflammation**

Research into causes of non-maturation of AVF has been hindered by several problems: There is no consensus about the definition of AVF maturity, particularly in patients not yet on dialysis, causes of AVF failure are heterogeneous, and there is a proportion of slowly maturing AVF that are counted as failure in short-term studies (8, 148). However, the main mechanisms for AVF non-maturation appear to be mediated by endothelial dysfunction or injury, possibly due to the uraemic inflammatory state, as well as trauma and bleeding at surgery, abnormal haemodynamics, and later AVF cannulation for dialysis or endovascular intervention (147). Inflammatory stimulation appears to be worse in AVG than AVF, possibly due to the bio-incompatibility of the synthetic graft and tendency to infection (155).

Histologically, neo-intimal hyperplasia (NIH) that causes luminal stenosis was found to be the substrate of early AVF failure in surgically resected specimens, but more recently it was also described in a proportion of native veins harvested at surgery for AVF formation (26, 62, 156). Moreover, experimental data show increased AVF failure in uraemic versus non-

uraemic mice (147). These findings suggest that the uraemic inflammatory state itself induces endothelial dysfunction in AVF.

Endothelial dysfunction and injury is considered central to the pathogenesis of AVF non-maturation because it produces a downstream signalling cascade that promotes inflammation and oxidative stress, resulting in tissue injury and development of NIH (147). Haemoxygenase-1 is a protein induced in vascular injury to reduce oxidative stress and vascular smooth muscle cell proliferation. Its anti-inflammatory effects may be important for AVF patency: the genetic polymorphism of haemoxygenase-1 may predispose patients with less haemoxygenase-1 to more AVF failure as shown in experimental and clinical studies (147).

Furthermore, the common location of stenotic NIH at the anastomosis or just beyond suggests that factors other than uraemia may be responsible for AVF failure: Whilst surgical trauma has been implicated for juxta-anastomotic stenoses, haemodynamic factors, particularly shear stress that is oscillatory or excessively high, appear relevant for the development of anastomotic stenoses (148).

Clinical studies have also observed an association between increased inflammation and vascular access dysfunction: Increased serum markers of inflammation (high sensitivity C-reactive protein, tumour necrosis factor alpha, interleukin 6, monocyte chemoattractant protein 1) were found in patients with dysfunctional AVF compared to those with well functioning or newly formed AVF (157). Another study compared histological vein specimens of dysfunctional AVF to native veins in renal patients and found increased expression of

growth factors (transforming growth factor beta 1, insulin-like growth factor 1) associated with NIH and inflammatory cells (macrophages and lymphocytes) (156). Histological examination of thrombosed AVF compared to stenotic AVF showed increased tissue inflammation with inflammatory cells (macrophages and lymphocytes) and cytokines (interleukin 6, tumour necrosis factor alpha) in thrombosed AVF (158). Taken together, these studies suggest that inflammatory cytokines and cells activate pro-proliferative and pro-fibrotic pathways that further promote the development AVF stenosis or thrombosis.

Above we described the beneficial effect of MMP on arterial remodelling in AVF maturation; however, MMP are also induced by oxidative stress and may paradoxically promote NIH as they facilitate migration of vascular smooth muscle cells (147, 148). Furthermore, Chan et al studied tissue expression of MMP-2 and MMP-9 in vein specimens resected from thrombosed or stenosed AVF; they found MMP-9 levels located with macrophages near the luminal surface of NIH of thrombosed AVF and hypothesised that intimal disruption by MMP-9 could have contributed to thrombus formation (158).

Although stenosis due to focal excess of NIH appears to be a major cause of AVF non-maturation, a milder degree of diffuse NIH associated with outward vessel expansion ("eccentric hypertrophy") appears necessary for successful AVF maturation; by contrast, lack of both dilatation and vessel wall thickening is a less common but well described cause of early AVF failure (148, 159). More research of the basic science is needed to distinguish appropriate from inappropriate vascular remodelling in AVF and to develop appropriate therapeutic targets (159).

### 3.3. Intra-operative interventions for improving AVF outcomes in inadequate vessels

Surgical dedication, skill and expertise are important for successful AVF outcomes and certain care measures are recommended for all AVF (68, 160). These include avoiding trauma or kinks to vessels to preserve the vascular endothelium, achieve meticulous haemostasis to avoid pro-inflammatory stimuli, and use local drugs to overcome vasospasm (68, 160). Interrupted clips rather than continuous suturing of the anastomosis were associated with better AVF patency in an observational study but lack of randomisation and blinding could have introduced bias (161).

Pre-operative vascular diameter on ultrasound is a well established criterion for future AVF success (89). Clinical studies have shown an increased risk of AVF failure (45% immediate failure and 100% early failure, respectively) when small arteries  $< 1.6$  mm were used (29, 32). By contrast, paediatric surgeons have developed a microsurgical technique to establish AVF from tiny vessels: this technique uses a brachial plexus block which in itself promotes vasodilatation and removes the need for haemostatic clamps; meticulous haemostasis and precise intima-to-intima apposition at the anastomosis may be other advantages (162).

Pirozzi et al showed remarkable success in AVF outcomes (0% immediate failure, and 69% primary patency at 1 year) when using this microsurgical technique in adult renal patients with a small radial artery diameter  $< 1.6$  mm and a majority of women (163). These results of a single surgeon suggest a great benefit from microsurgery and brachial plexus anaesthesia but need to be more widely studied.



Furthermore the dedicated surgeon will review the AVF for patency in the recovery room and re-explore the operation site if there are doubts about the AVF flow (68). Finally, one small randomised study showed increased AVF blood flow in response to a transdermal glyceryl-trinitrate patch placed near the anastomosis 24 hours after surgery although the impact of this intervention on early AVF failure was not reported; glyceryl-trinitrate could be beneficial in uraemic patients to overcome the relative lack of endogenous nitric oxide and maybe useful earlier on (164).

Current research in AVF surgery is testing various adjuncts delivered locally during AVF surgery with the aim to reduce anastomotic and juxta-anastomotic hyperplasia (147): A drug-eluting wrap containing paclitaxel, a drug which interferes with microtubules and interrupts cell proliferation, was successful in animal studies; however, a randomised trial in renal patients had to be abandoned because of a significant increase in peri-operative infections (165). A recent pilot study on rapamycin-eluting wraps for AVF surgery has shown promising outcomes but these findings need to be confirmed in a larger study (165). A trial is underway with the Optiflow device, a small synthetic tube covering the inside of the brachiocephalic AVF anastomosis to serve as a haemodynamic bridge between the artery and vein (165).

### **3. 4. Methods of vein mapping**

Different modalities have been employed for pre-operative vein mapping. As described in this thesis (section A, Chapter 3.2.), ultrasound is preferred because it is non-invasive, but has obvious limitations: the central veins cannot be directly assessed, the examination is time-consuming and it does not produce an anatomic map available for later inspection

(166). Conventional contrast angiography is useful as it allows visualisation of the central veins and of an anatomic map; however, it is invasive, does not inform about the depth of the vein and carries a small risk of contrast-induced nephropathy (166). Until recently, gadolinium enhanced magnetic resonance imaging was considered as it offers clear visualisation of the entire (arterial and venous) vascular tree. In terms of accuracy, native vessel diameters measured by magnetic resonance were equally or more accurate than ultrasound when compared to diameters measured at surgery (167). However, concerns about a link between gadolinium and nephrogenic systemic fibrosis have abandoned the use of contrast-enhanced magnetic resonance in renal patients. Two observational studies investigated a strategy of routine pre-operative ultrasound and contrast angiography on AVF formation and outcome: Huber et al found some additional benefit in invasive arterial and venous contrast imaging over ultrasound, whereas Lampropoulos et al only studied venous contrast imaging and found benefit restricted to patients with central vein stenosis (94, 168). Given the potential risk of invasive imaging, invasive contrast imaging should be reserved to patients with a strong suspicion of central vein stenosis (166).

Non-invasive assessment of functional properties such as venous distension may raise the predictive value of pre-operative native vein assessment (50). A new enhanced ultrasound technique may help: Recently, the feasibility of obtaining in vivo vein compliance measurements during venous distension was reported in a volunteer, using a high resolution ultrasound scan enhanced by phase-sensitive speckle tracking; whether this technique could be useful for pre-operative vein mapping needs further investigation (169).

## **4. Topics for further research**

### **4.1. Patient demographics and ethnicity**

Poorer AVF outcome with diabetes or cardiovascular disease is well recognised but might be improved with appropriate drug treatment. This could be investigated in a controlled trial, stratified by diabetes and cardiovascular disease, which randomly allocates patients to trial medication (for instance Clopidogrel, or Angiotensin-converting enzyme inhibitor, or a statin, or a combination of those drugs) versus placebo. Primary endpoints would be early AVF failure, secondary outcome longer term AVF survival, including the effects of AVF salvage. It is possible that endovascular angioplasty for instance has better results when supported by the administration of antiplatelet agents and a statin.

Poorer outcome among women is also well known but so far poorly understood. Differences in vessel size have been observed but their relevance is unknown. Greater detail on gender-specific difference in patency and maturation of AVF would be of interest. This could be studied in a prospective cohort study in which women are matched to men by body mass index, age, diabetes and cardiovascular disease. Blood viscosity (haematocrit), thrombostasis and clotting should be studied because of the higher thrombosis rate reported. Pre-operative ultrasound assessment, intra-operative blood flow measurement, and post-operative ultrasound follow-up scans during the maturation period would be useful. Main AVF outcomes would be immediate failure and maturation failure (broken down into early thrombosis, stenosis, and a patent but deep vein requiring superficialisation).

Although we found no difference in AVF outcomes among different ethnic groups, our data did not control for long-term haemodialysis catheter use. Therefore it is unknown whether the distribution of permanent vascular access (AVF, AVG, long-term CVC) is different among ethnic groups. Furthermore, there may be international differences between the American and European continent. This may be an appropriate investigation for the Dialysis Outcome and Practice Pattern Study Group (an ongoing international observational study of dialysis patients), because their data would also allow comparison of practice patterns.

#### **4.2. Pre-operative vascular ultrasound mapping**

Our randomised trial showed that routine pre-operative vascular mapping with ultrasound was effective in terms of reducing immediate failure, early thrombosis as well as long-term AVF survival when supported by AVF salvage. However, we found no difference for maturation failure which affected a larger proportion of patients. It is unclear whether maturation failure is mainly caused by post-operative events, or whether our study was underpowered to show a difference for pre-operative ultrasound. A larger randomised controlled trial that randomly allocated patients to routine versus selective ultrasound could clarify the question; a power calculation can be based on the 10% difference observed in our study for primary failure. A multi-centre design would allow better generalisability.

#### **4.3. Native vessel histology**

Native vein histology with area measurements acquired through an image analyser may be useful for further research; however, their reproducibility is unknown and should be compared to that of histological width measurements. Furthermore, measurement variation

between adjacent segments of the same vein should be studied, because intimal hyperplasia and medial fibrosis may be focal.

The hypothesis that arose from our pilot study, specifically that greater medial fibrosis prevents adequate vein dilation after AVF formation, should be tested in a larger prospective study. Veins should be tested with high-resolution ultrasound study that may give better detail of the venous morphology. For best evaluation of venous diameters a strict protocol should be followed including tourniquet use, a defined patient position (eg supine) and regulated room temperature. Venous distensibility may be studied before and after immersion into a warm water bath. If medial fibrosis was confirmed as a predictor of AVF failure, and if ultrasound parameters could predict medial fibrosis, then another important element for pre-operative ultrasound assessment could be described.

Further study of arterial histology, harvested at surgery, may also be useful and could be carried out alongside a venous study. A South-East Asian study described intimal hyperplasia in the radial artery as a predictor of early failure in radiocephalic AVF (66). By contrast, a study from the United States with a large proportion of African Americans found no intimal hyperplasia but found medial calcification particularly in diabetic patients, although this was not associated with AVF non-maturation; it is possible that pre-operative ultrasound evaluation already excluded significantly diseased arteries, or distal (radial) arterial disease may have been underrepresented because 2/3 of patients had an upper arm AVF (63). Again high-resolution ultrasound of the artery should be correlated with the histological findings.

#### 4.4. Early post-operative AVF examination

Physical AVF examination is convenient but non-standardised physical examination in our study had modest diagnostic value. In AVF with known dysfunction, physical examination has been shown to be accurate (114). The diagnostic value of systematic physical AVF examination in unselected patients following a standardised protocol as described by Pourchez is unknown (112). If systematic physical AVF examination was effective early after surgery, then a low-cost, time-efficient, clinically useful screening tool would have been found.

Our data propose that ultrasound is useful as a screening tool for possible AVF dysfunction early after surgery. However, further research would need to study unselected patients (ie not limited to those with known dysfunction) to address the following issues. The reproducibility of different AVF blood flow measurements should be compared (velocity and blood flow in brachial artery and AVF vein, respectively). Furthermore, cut-points for vein diameter and blood flow (and/or velocity) predicting early AVF failure should be defined at weekly intervals in the first four weeks and fortnightly in month two in a large prospective study. Then it may be appropriate to consider a controlled trial that enrolls patients with AVF at risk of failure as defined by ultrasound assessment, and randomising those to immediate AVF salvage versus standard care; this would clarify whether a strategy of systematic post-operative follow-up, as we propose, would be effective.

## **E) Thesis Conclusions**

This thesis has shown that some patient groups have an increased risk of AVF failure and may benefit from earlier AVF formation; however, the benefits of AVF apply similarly to African, Indo-Asian and Caucasian patients. Pre-operative ultrasound should be routinely offered to all patients requiring AVF formation. Medial fibrosis of native veins may result in resistance to vein dilatation after AVF formation but this finding requires more research. Early post-operative AVF examination is useful to identify those at risk of subsequent maturation failure.

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