Patent foramen ovale, cerebral microembolisation and cognitive function in dialysis

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Abstract

Background

Dialysis in patients with kidney disease is associated with cognitive decline. The reasons for this are multifactorial but in haemodialysis (HD) patients it is postulated that paradoxical embolisation of material from the extracorporeal circuit may occur across a right-to-left shunt, such as a patent foramen ovale (PFO), and cause cerebral damage.

Aims

We aimed to identify the prevalence of PFO in dialysis patients and to assess for evidence of cerebral microembolisation during HD and continuous veno-venous haemofiltration (CVVH). We also wished to correlate the presence of PFO with rates of cognitive decline.

Methods

We carried out transthoracic echocardiography to identify PFO and transcranial Doppler to assess for cerebral microembolisation. Cognitive function testing was carried out at baseline and at 1 year follow-up in dialysis patients.

Results and conclusions

The prevalence of PFO in patients undergoing renal replacement therapy is consistent with that in the general population. We confirmed that microemboli are created but found no evidence that they enter the cerebral circulation during HD or CVVH. Although there was significant deterioration in cognitive function in dialysis patients, there was no evidence that this was linked to the presence or absence of a PFO.

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Acknowledgements

Throughout my thesis I refer to the group's contribution to the work, to avoid giving the misleading impression that I was working without supervisory guidance or assistance from others. This also acknowledges the fact that publications resulting from this work are, and will be in the future, co-authored. Nevertheless I have led this research and take primary responsibility for the experimental planning, data acquisition and interpretation.

The following people have been critical to the successful undertaking of this work.

Firstly, my supervisors, Doctors Hildick-Smith, Holt and Medford, and Professor Critchley for their help, support and patience throughout my research period. Their vision made this project what it is, and I am extraordinarily grateful for their faith in me. Secondly, the staff of the Brighton and Sussex University Hospital renal and cardiology departments as well as the Clinical Investigations and Research Unit, for generously sharing space and equipment. Most importantly, I would like to thank the patients who, by their willing and good-natured participation, made this study possible.

Finally I would also like to thank my wife Lindsay.

Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not previously been submitted to this or any other university for a degree, and does not incorporate any material already incorporated for a degree.

Signed:

Dated:

List of Abbreviations

APD	automated peritoneal dialysis
AV	arteriovenous
CAPD	continuous ambulatory peritoneal dialysis
CAVH	continuous arteriovenous haemofiltration
CKD	chronic kidney disease
CRRT	continuous renal replacement therapy
CVVH	continuous venovenous haemofiltration
ERP	event related potential
EEG	electroencephalography
FFT	fast Fourier transform
GFR	glomerular filtration rate
HD	haemodialysis
LA	left atrium
MCA	middle cerebral artery
MMSE	mini-mental state examination
MRI	magnetic resonance imaging
ONS	Office for National Statistics
PD	peritoneal dialysis
PFO	patent foramen ovale
POS	platypnoea-orthodeoxia syndrome
RA	right atrium
RT	renal transplant
SD	standard deviation
TCD	transcranial Doppler
TIA	transient ischaemic attack

TOE   transoesophageal echocardiography

TTE   transthoracic echocardiography

Chapter 1 (Introduction)

Cognitive function

The term "cognition" has traditionally been used to refer to the concepts of awareness, thinking and learning, resulting from receiving, understanding, storing, retrieving and using sensory information. Different components of the cognitive process, including working memory, long term memory, knowledge base, sensory attention and executive function, co-operate to facilitate decision-making, problem-solving, communication and behaviour. Unsurprisingly for such a complex series of processes, cognitive function can be influenced by a multitude of factors, including genetic and environmental backgrounds, as well as by a vast number of disease processes.

Chronic kidney disease (CKD) is one of the conditions known to adversely affect cognitive performance. A landmark study by Rozeman *et al* [1] evaluated neurological and cognitive function in patients with chronic renal disease divided into 3 groups: those close to starting renal replacement therapy; those with end stage renal disease established on haemodialysis (HD); and those established on peritoneal dialysis (PD). They used a combination of neurophysiological (electroencephalograms and visually evoked potentials) and neuropsychological tests (trail making test and the neurobehavioural evaluation system [2]) to assess function. They found that all three groups had significant neuropsychological and neurophysiological impairment compared to age and education matched published

reference groups. Comparisons between the groups themselves showed that there were no significant differences in ability. This suggested a link between chronic kidney disease and cognitive impairment. This finding has subsequently been reiterated by other groups including that of Madan *et al* [3], who used neurophysiological testing alone. Event related potentials (ERPs) are electrical signals from the brain in response to a stimulus and can be measured using electroencephalography (EEG). P3, the third positive wave in a standard ERP, is evoked by a test called the "odd ball paradigm", in which an unusual stimulus is hidden in a sequence of more similar ones. The latency of the P3 wave is known to correspond with the speed of cognitive processing [4] and is an early sign of impaired cognition in metabolic encephalopathies. Madan *et al* compared patients with later stages of CKD (but prior to requiring dialysis) against age and sex matched healthy controls. They found that there was a negative correlation between decreasing renal function as measured by glomerular filtration rate (GFR) and P3 latency, such that the stage of CKD was associated with progressive prolongation (see figure 1).
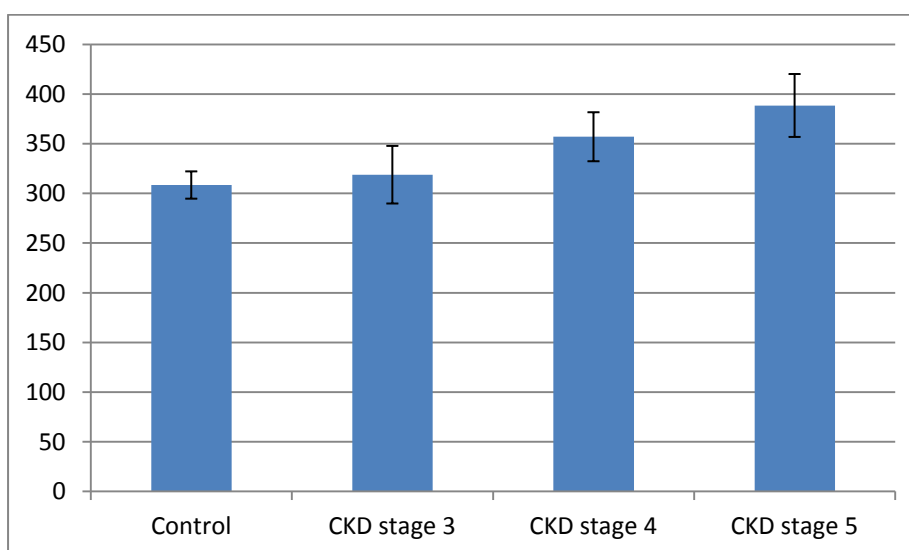
Figure 1 – Graph adapted from *Madan et al* showing P3 latencies according to stage of CKD

This and other similar studies have established the paradigm of cognitive impairment as a predictable and quantifiable consequence of chronic renal insufficiency.

Chronic kidney disease

In a bid to help optimise the care of patients with chronic kidney disease (CKD), the National Kidney Foundation (a United States not-for-profit organisation) set up the Kidney Disease Outcomes Quality Initiative (KDOQI). The role of this multidisciplinary group was to produce a set of clinical practice guidelines on the evaluation, classification and stratification of CKD. These guidelines have become widely accepted internationally and thus CKD can be defined as "a structural or functional abnormality of the kidney for at least three months manifested by kidney damage (with or without an associated fall in glomerular filtration rate) or a decreased glomerular filtration rate without evidence of kidney damage" [5]. CKD is categorised according to severity as summarised in table 1.

Table 1 – Stages of chronic kidney disease

Stage	Description	Glomerular filtration rate (ml/min/1.73m^2)
1	Kidney damage with normal or increased GFR	≥90
2	Kidney damage with mild decrease in GFR	60-89
3	Moderate decrease in GFR	30-59
4	Severe decrease in GFR	15-29
5	Kidney failure	<15 (or dialysis)

Use of a unified set of guidelines and classification system enables early identification and treatment in the early stages of CKD, with the hope that having identified those at risk, intervention strategies may reduce rates of progression to end stage renal disease or other complications. For example, it is known that renal function in patients with hypertension declines faster than a matched normotensive population [6]. It is thus widely accepted that the prescription of antihypertensive medication may slow progression in renal disease [7], and there is some evidence to suggest that use of angiotensin converting enzyme inhibitors are particularly effective in some conditions [8] .

The global prevalence of end stage renal failure has increased significantly in recent years [9]. In 2001, around 1 million people worldwide were receiving dialysis treatment and the dialysis population was growing by 7% a year [10]. By the end of 2004, the total number of people receiving renal replacement therapy (either in the

form of dialysis or renal transplantation) worldwide had climbed to 1.8 million [9].

Demographic changes such as an ageing population and higher rates of

comorbidities such as diabetes mellitus and hypertension are likely to be significant

contributors to the rise [11]. The increasing access to renal replacement therapy in

some parts of the world enables more patients with end stage renal disease to be

treated [9]. More up to date figures are available for the UK population where the

total number of adult patients having renal replacement therapy in 2009 was 49 080

(794 per million population) [12]. The incidence of new onset end stage renal

disease requiring renal replacement therapy in 2009 was 109 per million population

[13] with HD the most commonly used renal replacement modality at 69.1% at 90

days, followed by PD 17.7%, renal transplantation (RT) 6.7% and withdrawal from

renal replacement therapy 6.5%. HD is likely to remain the largest treatment

modality in the near future.

The population of the United Kingdom is getting bigger and getting older. A recent

report by the Office for National Statistics (ONS) has projected that over the 10 years

between 2010 and 2020, the population will increase from 62.3 million to 67.2 million

with the median age increasing from 39.7 years to 39.9 years [14]. As the

prevalence of chronic kidney disease increases with our aging population, the

burden of associated health problems is also likely to rise [15].

Cognitive function and chronic kidney disease

The reason for the relationship between chronic kidney disease and decline in

cognitive function is likely to be multifactorial. Age is a major contributor to cognitive

decline with both structural and neurophysiological changes thought to be important in this process [16]. Separate regions of the brain that work together to provide higher order cognitive function show less coordinated action with ageing, which may be due to a global reduction of integrative function [17]. Age is of course also a key predictor of CKD. One large study showed that up to 11% of the normotensive non-diabetic American population aged over the age of 65 has CKD stage 3 [18]. As well as advancing age, other likely contributing factors are detailed below.

Anaemia

Conventionally, anaemia has been defined by the World Health Organisation as a haemoglobin level at sea level of below 13.0 g/dl in adult males and 12.0 in adult non-pregnant females [19]. Anaemia is common in patients with CKD. A study by Astor *et al* reporting on the results of the Third National Health and Nutrition Examination Survey (1988-1994) found that in North American patients with CKD stage 5 (using a more generous haemoglobin target of 12.0 g/dl in males and 11.0 g/dl in females), 33% of men and 67% of women were anaemic [20]. Anaemia in CKD is often caused by low levels of erythropoietin which is normally released by the healthy kidney in response to hypoxia, although other factors such as haematinic deficiency (iron, folate, and vitamin B12), chronic inflammation, hyperparathyroidism or chronic bleeding can also be responsible. Erythropoietin has been used effectively since the 1980s to treat anaemia in patients with CKD. Grimm *et al* [21] showed some improvement in neurological function (measured both with cognitive and neurophysiological testing) of HD patients when anaemia was treated with erythropoietin. This suggests not only that cerebral dysfunction may be linked to anaemia but also that correcting the anaemia can improve cerebral function. This

picture is complicated by the multiple biological and extra-haematopoietic effects that have been attributed to erythropoietin [22].

Cerebrovascular disease

Another cause for poor cognitive function in patients with chronic kidney disease is cerebrovascular disease. Epidemiological analysis using a population based cohort study by Seliger *et al* found that dialysis patients are at a much higher risk of stroke than the general population [23]. Compared to the general population, Caucasian patients having dialysis had an age-adjusted relative risk of stroke of 6.1 for males and 9.7 for females. The relative risk for African Americans was 4.4 for males and 6.2 for females. There are of course major confounding factors, for example, hypertension and diabetes mellitus being more prevalent in the dialysis population than in the general population. It remains unclear whether cerebrovascular disease is due to the common aetiology of both renal dysfunction and vascular disease [24]. However, population based studies suggest that although the prevalence of these comorbidities is higher in the dialysis population, they are not enough to explain the vastly increased risk of stroke [25][26]. A study by Seliger *et al* looked specifically at risk factors for stroke in patients undergoing PD or HD [27]. They found that markers of malnutrition such as albumin and a subjective judgement of undernourishment by dialysis unit staff were also significantly associated with stroke. Anaemia itself has been associated with a higher risk of stroke in patients with CKD [28], as has correction of anaemia to a high target haemoglobin using high doses of erythropoietin in CKD patients who were not receiving dialysis [29]. In HD patients, especially those with diabetes, reduction in cerebral blood flow during dialysis may also contribute to cerebral vascular disease [30].

Patients who have had a stroke have worse cognitive function than those who have not previously had a stroke [31], but subclinical cerebrovascular disease may also manifest as poorer cognitive function. One recent study has even shown that in the elderly, impaired performance on an assessment of cognitive ability using the Trail Making B test is associated with a higher risk of subsequent stroke [32]. The authors of this paper speculated that even mild cognitive dysfunction may signal unrecognised cerebrovascular injury.

Nutrition

Another possible contributor to cognitive decline is malnutrition, for which there is emerging evidence in a non CKD population [33]. This also appears relevant to patients with CKD. Recent work in HD patients suggests that patients with a poor nutritional status perform poorly on tests of psychomotor function [34]. Malnutrition has been shown to be common in patients with renal impairment and its severity correlates with the severity of the impairment [35]. The decline in nutritional status with CKD may be due to disturbances in protein and energy metabolism, hormonal derangement or a reduction in protein and calorie intake, all of which are seen commonly in this patient population [36]. There are little data on the effect of nutritional intervention on cognitive function in patients with CKD. However one trial performed by Salva *et al* found no improvement in autonomy in a group of patients with dementia following a program of nutritional intervention [37].

Dialysis

The process of dialysis was first described by the Scottish chemist Thomas Graham, who in 1854 managed to separate solutes using a semi-permeable membrane. However, it was not until 1924 that a German physician named Georg Haas used HD to treat a human patient with uraemia for the first time [38]. This first treatment lasted only 15 minutes and did not prolong life, but did show the potential to remove waste products such as urea. As technology has advanced, the effectiveness of HD has increased alongside the number of patients undergoing successful HD treatment [9]. During HD an extracorporeal circulation allows blood removed from a patient to pass over a semi-permeable membrane, on the other side of which is an electrolyte-balanced fluid. By re-circulating blood over the semi-permeable membrane for a period of hours the composition of the plasma comes to resemble that of the dialysate, therefore correcting electrolyte imbalances and removing waste products. Thus HD replaces one of the homeostatic functions of a fully functioning kidney and other important functions have to be addressed separately. For example, recombinant erythropoietins are often necessary to achieve target levels of haemoglobin [39].

In the early days of HD, several neurological impairments known collectively as dialysis encephalopathy were common. This was a condition characterised by dysarthria, apraxia, asterixis, myoclonus, seizures and personality change. It was not until a seminal paper by Alfrey *et al* [40] linked dialysis encephalopathy to abnormally high levels of aluminium when compared both to healthy controls and to dialysis patients without the condition that effective treatments could be found. Use

of aluminium free dialysates and the abandonment of aluminium containing

phosphate binders led to successful prevention of dialysis encephalopathy.

However more subtle neurological impairments, including impaired cognitive

function, have persisted. There is evidence that amongst patients with chronic

kidney disease, those undergoing HD continue to perform least well in cognitive

function testing [41]. There are several possible theories as to how this occurs. In

patients with chronic kidney disease undergoing HD it has been postulated that the

associated fluid shifts and fluctuations in blood pressure may directly cause cerebral

ischaemia leading to a deleterious effect on cognitive function [42]. In support of this

theory, HD patients are known to have changes in the blood flow velocity in the

middle cerebral artery (MCA) during dialysis when measured by using transcranial

Doppler ultrasonography. In a study by Postiglione *et al* [43], blood flow velocity in

the MCA was shown to be significantly lower after HD when compared to flow before

the procedure. Acute change in cognitive function also occurs during HD; Murray *et

al* [44] showed that patients with end stage renal disease performed worst on tests of

cognitive function whilst undergoing the HD treatment, whilst they did better either

just before, or the day after HD.

A further factor in cognitive decline in HD may be due to the presence of a right-to-

left cardiac shunt in the form of a patent foramen ovale (PFO). It is known that the

process of HD produces microemboli which can be detected in the subclavian vein

downstream of an arteriovenous fistula using ultrasound technology [45].

Microemboli are thought to be small platelet aggregates or microbubbles that are too

small to be trapped by the filters used in dialysis machines [45]. Our hypothesis is

that the presence of microemboli, combined with the non-physiological fluid shifts

engendered during HD, predispose to paradoxical cerebral microembolisation where such a potential right-to-left shunt exists. If cerebral microembolisation does occur during HD, then it may lead to clinically silent cerebral ischaemic events, much as it does during cardiac surgery, [46] and lead to a decline in cognitive function.

An alternative form of renal replacement therapy in patients with chronic renal failure is PD. This makes use of the abdominal peritoneum as a semi-permeable membrane across which waste products, water and electrolytes can diffuse between the abdominal cavity and the vasculature of the mesothelium. An indwelling Tenckhoff catheter is inserted into the abdomen and dialysis fluid is introduced. After a period of equilibration, the fluid is drained back out through the catheter and discarded. In continuous ambulatory peritoneal dialysis (CAPD), fluid is exchanged up to four times throughout the day. In automated peritoneal dialysis (APD), a machine is used to exchange dialysis fluid overnight. PD was first used in the United Kingdom by Reid *et al* in 1946 to successfully treat a patient who had become anuric following an ABO incompatible blood transfusion [47]. Over subsequent decades, PD has increasingly been used to treat both acute and chronic renal failure. The main advantages of PD over HD are that it does not require vascular access, there is less restriction of fluid intake and patients are not required to attend the hospital 3 times per week, making them more independent. The main disadvantages are that there is risk of infection (peritonitis), a risk of malnutrition and that the dialysis has to be carried out either by the patient or a carer.

Continuous renal replacement therapy

HD and PD are effective treatments in the management of patients with chronic renal failure. In patients on the intensive care unit, for example following major surgery, a different mode of renal replacement therapy is often used. Continuous renal replacement therapy (CRRT) was first described in 1977 for the treatment of diuretic-unresponsive fluid overload [48]. Originally, this was continuous arteriovenous haemofiltration (CAVH) where the patient's heart acted as the blood pump. More recently, continuous venovenous haemofiltration (CVVH) has become the dominant technology and relies on an artificial peristaltic pump module. This is a slower and more continuous process when compared to HD and uses convection and a pressure gradient rather than a concentration gradient to remove waste products and water. This may result in less haemodynamic instability than HD and may be more useful in critically unwell patients who do not tolerate the rapid fluid shifts of HD. Due to the clinical condition of the patients affected, haemofiltration is almost exclusively carried out in intensive care units.

There have been no previous studies examining whether CVVH also produces microemboli and whether these microemboli could have a clinically significant effect. However, we do know that HD does create microemboli as a result of the use of an extracorporeal circuit that can be detected in the subclavian vein [49]. It is therefore reasonable to speculate that CVVH too causes microembolisation. As the availability and use of CVVH has increased (41), the number of patients at potential risk of microembolisation has also increased.

Aims of the project

The primary aim of this research project was to establish whether of the presence of a PFO influenced cognitive function in patients with renal failure. We therefore posed the questions:

1. Do microemboli created during HD cross a PFO?
2. Are these microemboli identifiable in the cerebral circulation with the use of transcranial Doppler ultrasound scanning?
3. Is cognitive dysfunction associated with having a PFO in patients with an extracorporeal circuit?

On the assumption that cognitive decline in HD patients may partly be mediated by microemboli crossing a PFO during dialysis and causing cerebral pathology, we did not expect the presence or absence of a PFO to have any influence on cognitive function in patients having PD, so these patients served as controls.

A review of the available literature showed that at present the prevalence of PFO in those with renal failure requiring renal replacement therapy is unknown. Thus, a secondary objective of the study was to identify the prevalence of PFO in patients with renal failure undergoing different modalities of dialysis treatment. Finally we carried out a pilot study looking for evidence of cerebral microembolisation in patients who had developed acute renal failure and required CVVH on the intensive care unit.

Ethics

Ethical approval for the studies involving HD and PD patients was obtained from the Kent and Brighton West Research Ethics Committee. The reference number for this project is 10/H1101/14. Final approval was granted on 12/03/2010 and recruitment began on 15/03/2010.

As the study looking for cerebral microembolisation during CVVH involved vulnerable adults and could not be considered at our local ethic committee, we received permission from the London South East Research Ethics Committee. The reference number for this project is 11/LO/0778. Final approval was granted on 08/06/2011 and recruitment began on 04/07/2011.

Chapter 2 (Statistics)

The subsequent chapters in this thesis describe the studies that we carried out in order to try to achieve the aims of the project. In this chapter we describe the statistical methods chosen to analyse the results in those studies. In general, a p value of <0.05 was deemed to be statistically significant. Statistical analyses were carried out using IBM SPSS Statistics, version 20 (New York, USA) and GPower, version 3 (Heinrich Heine University Dusseldorf, Dusseldorf, Germany).

Prevalence of PFO

Where data were normally distributed, mean and standard deviation are used. Where data are not normally distributed, median and inter-quartile ranges are given.

Dialysis and cerebral microembolisation

To analyse whether the data were normally distributed, histograms were plotted and the Shapiro-Wilk analysis was used [50]. This is a method which tests the null hypothesis that data are normally distributed. A result with a p value of <0.05 allows rejection of the null hypothesis and suggests that the data are very unlikely to be normally distributed.

If data were normally distributed, paired samples were analysed using the paired T test and independent samples were compared using the independent T test. If data

were not normally distributed, paired samples were analysed using the Wilcoxon Signed Ranks test and independent samples were analysed using the Mann-Whitney test.

We carried out power calculations in our studies of microembolisation in dialysis patients. The seminal work in this field was carried out by Rolle et al in 2000 [45]. From their data, the number of microemboli detected prior to haemodialysis was (0 +/- 0) (mean +/- standard deviation) and detected during haemodialysis was (21.6 +/- 4.7), giving an effect size index (d_z) of 4.6. We used these figures during power calculations for microemboli detection.

In our first experiment, we used ultrasound to look for differences in rates of microemboli detection in arteriovenous fistulae prior to and during haemodialysis in the same patient. Using the Wilcoxon signed rank test, with statistical significance set at 0.05 for power of 80%, a sample size of 3 patients would be required. Our next study used transcranial Doppler to look for differences in rates of intracranial microemboli detection before and during dialysis in the same patient. Again, with the use of the Wilcoxon signed rank test, statistical significance set at 0.05 and a power of 80%, 3 patients would be required. A subsequent study compared rates of microembolisation between HD and PD patients during dialysis. With use of the Mann-Whitney test (as they are independent samples), statistical significance at 0.05 and a power of 80%, 4 patients would be required.

Dialysis, PFO and cognitive function

Shapiro-Wilk testing was again used to assess the normality of distribution of the cognitive function data. Median and inter-quartile ranges are given for each of the test results. As the data were not normally distributed, the Wilcoxon test was used to test for statistical significance of paired data whilst the Mann-Whitney test was used for independent data. In a separate analysis looking at cognitive changes in individual patients, we dichotomised results into "decline" or "no decline". Significance in rates of decline between different groups of patients was then assessed using the Pearson Chi-Square test.

The main difficulty in a power calculation for the cognitive function study was that as we used 4 different cognitive tests in the battery, differing results are obtained from each of the different tests. Another difficulty is in the definition of "clinically significant change" with reference to cognitive test results. As we go on to explain in the chapter of cognitive function, we do not define clinically significant change or difference but for the purpose of the power calculations, it is necessary to do so. We elected to use 1 standard deviation as a significant difference between 1 group and another. One of the main aspects of the cognitive function study is in the comparison of HD patients with a PFO against HD patients without a PFO. If we assume that the prevalence of PFO in a dialysis population will be 25%, we would expect to recruit 1 patient with a PFO to every 3 without a PFO.

Digit Symbol

Normative age-matched data from Royer et al show a score of 37.3 +/- 15.6 [51]. To detect a difference of 1 standard deviation between groups using the Mann-Whitney test, with a p value of 0.05 and 80% power, we would require 11 patients with a PFO and 35 patients without a PFO.

Trails A

Normative data for an aged matched population were again obtained from Tombaugh et al and showed a trails A score of 33.2 +/- 9.1 [52]. To detect a difference of 1 standard deviation between groups using the Mann-Whitney test, with p set at 0.05 and 80% we would require 9 patients with a PFO and 27 patients without a PFO.

Trails B

Normative data for an age matched population were obtained from Tombaugh et al and showed a trails B score of 74.6 +/- 19.6 [52]. To detect a difference of 1 standard deviation between groups using the Mann-Whitney test, with a p value of 0.05 and 80% power we would require 9 patients with a PFO and 29 patients without a PFO.

FAS verbal fluency

Normative data for an age matched population were obtained from Tombaugh et al and showed a score of 38.5 +/- 13.7 [53]. To detect a difference of 1 standard

deviation between groups using the Mann-Whitney test, with a p value of 0.05 and 80% power would require 8 patients with a PFO and 26 patients without a PFO.

Digit span backwards

Normative data for an age matched population were obtained from Monaco et al and showed a score of 4.2 +/- 0.9 [54]. To detect a difference of 1 standard deviation between different groups using the Mann-Whitney test, with a p value of 0.05 and power of 80% would require 9 patients with a PFO and 29 patients without a PFO.

Continuous renal replacement therapy and microembolisation

Shapiro-Wilk testing was again used to test for normality of data. As the data were non-parametric, the Wilcoxon Signed Rank test was used to assess for statistical significance in paired data. As no previous research has focused on the creation and detection of microembolic signals during haemofiltration, we used data from microemboli detection during haemodialysis to perform a power calculation. Again using the figures from the Rolle paper [45], for a power of 80% and statistical significance set at 0.05, 3 patients would be required in this pilot study.

Chapter 3 (Prevalence of Patent Foramen Ovale)

Background

Embryology of patent foramen ovale

The primitive embryonic cardiac atrium starts as a single cavity. The septum primum then grows caudally from the top of the atrium down towards the endocardial cushion. The area between the leading edge of the septum primum and the endocardial cushion is known as the ostium primum, which closes when the septum primum fuses with the endocardial cushion. A second orifice, the ostium secundum, develops at the top of the septum primum following the coalescence of multiple small perforations. The septum secundum then develops on the right atrial side of the septum primum. The septum secundum covers the ostium secundum but does not completely divide the atria. This persisting orifice within the septum secundum is the foramen ovale (see figure 2). The foramen ovale allows the shunting of oxygenated blood from the right atrium into the left atrium and thereafter into the systemic circulation. Following birth, the changes in pressure within the atria lead to closure of the foramen ovale in the majority of people. A patent foramen ovale (PFO) results from a failure to close and persists in around 25% of adults [55].
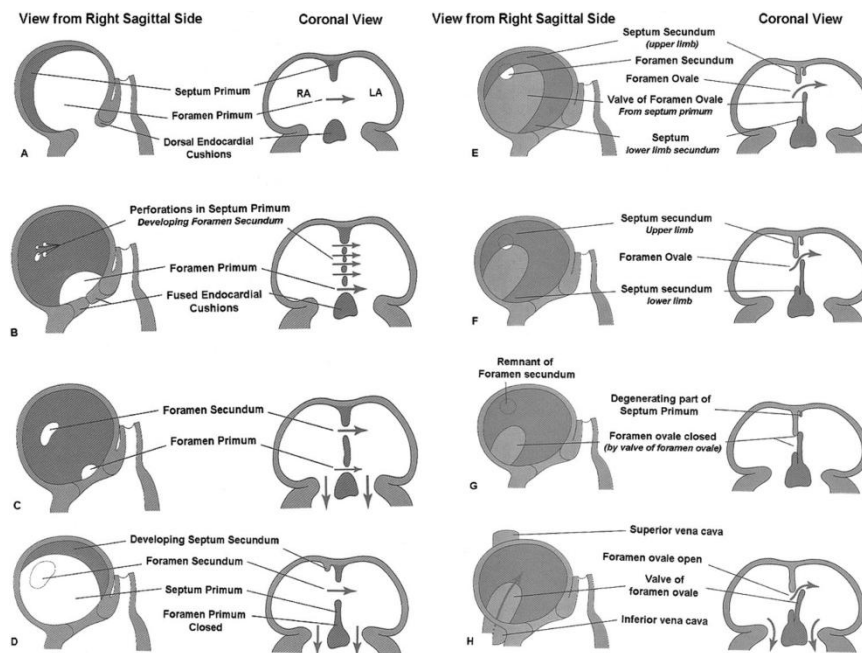
Figure 2 - Development of a PFO (from Hara _et al_ [56])

A PFO is an example of a right-to-left shunt that can allow paradoxical embolisation.
A paradoxical embolus describes any embolus originating in the venous system that
crosses into the arterial system via a right-to-left shunt, bypassing the filtering effect
of the pulmonary vasculature, and causing arterial obstruction. Depending on the
location of the arterial obstruction, damage can result to the brain or other organs.
Embolisation to the brain can result in a transient ischaemic attack (TIA) or stroke.
Various pathologies have become associated with and been attributed to PFO; these
will briefly be discussed here.

Paradoxical embolisation leading to stroke

The first case of pathology resulting from a PFO is thought to have been described
by Julius Cohnheim [57], who in 1877 presented the case of a 35 year old woman

who died after a paradoxical embolus had led to a cerebral embolism causing a fatal

stroke. The most commonly used research definition for different types of ischaemic

stroke was first described in 1993 in the Trial of Org 10172 in Acute Stroke

Treatment (TOAST) [58]. This used both clinical features and imaging results to

divide acute strokes into the following five categories: large-artery atherosclerosis;

cardioembolism; small-artery occlusion (lacune); stroke of other determined

aetiology; and stroke of undetermined aetiology (cryptogenic). A cryptogenic stroke

is defined as an ischaemic stroke not attributable to a source of definite

cardioembolism, large artery atherosclerosis or small vessel disease despite

thorough investigation. A prospective study of 1805 patients in the Stroke Data Bank

in the United States by Sacco *et al* [59] found that up to 40% of acute ischaemic

strokes have no identifiable cause after investigation and are categorised as

cryptogenic.

Individual case reports and series from the 1980s first started to associate PFO and

inter-atrial septal abnormalities with stroke [60][61]. A study by Cabanes *et al* [62]

using transoesophageal echocardiography in 100 consecutive patients aged under

55 with cryptogenic stroke found a significant association with the presence of a

PFO. A large meta-analysis of case control studies carried out by Overell *et al*

confirmed a high prevalence of PFO in patients under the age of 55 with cryptogenic

stroke [63]. De Castro *et al* [64] studied 350 patients with ischaemic stroke and

found that right-to-left shunting at rest via a PFO was a risk factor for recurrence of

stroke. The presence of a PFO as a risk for recurrent stroke had also been shown in

the meta-analysis by Overell *et al* [63]. However a large prospective study by

Homma *et al* [65] found that although PFO was associated with stroke, there was no

increase in the risk of stroke recurrence if patients with a PFO were treated with appropriate medical therapy (aspirin or warfarin). A study by Steiner *et al* [66] also confirmed that patients with a cryptogenic stroke had a higher prevalence of PFO than patients with a stroke of known origin.

The size of the PFO may also be important in determining whether it leads to pathology.   Steiner et al [66] used TOE to identify PFO and to grade according to size (small <2mm, medium/large >2mm). They found that stroke patients with a medium or large PFO had more brain imaging features of ischaemic infarcts than stroke patients with a small PFO. Homma et al had previously investigated patients with a PFO and found that patients with cryptogenic stroke had a significantly larger PFO (2.1mm +/- 1.7mm) than patients with an identifiable cause of stroke (0.57mm +/- 0.78mm) [67]. Lee et al demonstrated that the size of a PFO in patients with a stroke is linked to the risk of recurrence of stroke [68]. They used TOE (with omni-plane imaging) to size PFO in patients with cryptogenic stroke. During follow up of 159 patients, they found that patients with recurrent stroke had a significantly larger PFO (3.9 mm +/- 1.0mm) than those patients without a recurrence of stroke (1.8mm +/- 1.0mm). These finding have not been replicated by all groups. De Castro et al found no difference in the size of PFO as assessed by TOE between patients who had suffered a cryptogenic stroke compared to healthy controls with a PFO [64]. The study also discusses the difficulty in accurately measuring the opening of a PFO, even with the use of high frequency imaging. Kutty et al carried out a study where they assessed PFO morphology by balloon sizing prior to PFO closure [69]. They found no correlation in the maximal potential PFO size and the number of preceding neurological events prior to PFO closure.

In older patients, the association between stroke and PFO is less clear, with several studies showing no association [70][71] whilst a more recent prospective study by Handke *et al* that compared patients with cryptogenic stroke against patients with stroke of known cause found a higher prevalence of PFO in patients with cryptogenic stroke in both young and elderly patients [72].

Until fairly recently, no randomised trials had been carried out to assess for benefit in closure of PFO to prevent recurrent stroke. This changed with the CLOSURE 1 trial [73], a prospective, randomised multicentre trial using the STARFlex septal closure system versus best medical therapy (aspirin or warfarin) in a study involving more than 900 patients. Patients were selected from those presenting with a stroke or TIA and with a PFO on transoesophageal echocardiography. The results of the trial showed no significant benefit in device closure in addition to medical therapy over medical therapy alone in reducing the composite rate of stroke, transient ischaemic attack (TIA), death from any cause (first 30 days) and death from a neurological cause (31 days to 2 years) over 2 years. The trial has however been criticised for both the rates of successful closure (around 85%) and for increased rates of complications such as atrial fibrillation in the device arm.

Two further randomised controlled trials comparing medical therapy with PFO closure in reducing cryptogenic embolism were published in the New England Journal of Medicine in March 2013. Meier et al [74] recruited 414 patients under the

age of 60 with a stroke, TIA or other embolic event with a PFO on TOE with no other identifiable cause for embolism. They were randomised to best medical treatment or PFO closure with the Amplatzer device. After 4 years of follow up, there was no reduction in the composite end point of all cause mortality, stroke, TIA or peripheral embolism. Carroll et al [75] conducted a trial ("RESPECT") involving 980 patients between the ages of 18 and 60 with an ischaemic cryptogenic stroke. In the intention-to-treat analysis, they found no evidence that device closure reduced the rate of a composite endpoint of non-fatal ischaemic stroke, fatal ischaemic stroke or early death after randomisation. In 2009, both the American Heart Association and the American Stroke Association recommended that PFO closure in the setting of cryptogenic stroke should be carried out only in the setting of a clinical trial. It is likely that this advice will remain in place in light of the most recent results.

Less commonly, systemic embolisation via a PFO can result in damage to other organs. A recent review of referrals to a tertiary centre in the USA by Dao *et al* [76] found that over an eight year period, there were 416 patients referred with systemic embolisation due to a PFO. As would be expected, the majority of these patients had had a neurological event with 219 patients having had a stroke and 80 patients having suffered a TIA. There were 12 patients who presented with systemic embolisation to another organ. Of these, 8 patients presented with a myocardial infarction. These patients had evidence of myocardial infarction on biomarkers, electrocardiograms and imaging with echocardiography but no evidence of obstructive disease on coronary angiography. Another 4 patients presented with embolisation to the popliteal, brachial and ophthalmic arteries. There is also an

emerging association between undiagnosed PFO and retinal artery occlusion leading to visual impairment [77].

Decompression sickness in divers

Decompression sickness ("the bends", "the chokes", "the staggers") occurs when gas (principally nitrogen) comes out of solution at lower pressures when ascending, forming bubbles form in the tissues. This can affect those ascending from depth (e.g. divers), those ascending to altitude (e.g. pilots) and those leaving a high pressure environment (e.g. caisson workers). In divers, the risk of decompression sickness is increased by the duration and depth of dives, especially if ascent is carried out without acclimatisation. Decompression sickness can produce a host of symptoms ranging from rashes to permanent neurological disability. Following a study on workers building the Dartford tunnel (a 1430 metre long road tunnel crossing the river Thames and connecting the counties of Essex and Kent in the United Kingdom), Golding *et al* suggested categorisation of the symptoms into type 1 (generally less serious including skin manifestations) and type 2 (generally more serious including permanent neurological injury) [78].

The cardiologist Peter Wilmshurst was amongst the first to suggest a link between right-to-left shunts and decompression sickness. He first published a case report of a diver who had suffered decompression sickness and was subsequently found to have an atrial septal defect [79]. The proposed mechanism is that nitrogen coming out of solution forms bubbles, which are normally filtered by the lungs but in the

presence of a right-to-left shunt are able to bypass the pulmonary bed. This was followed by a study published in 1989 in The Lancet by Moon *et al* [80] which established that the prevalence of PFO was higher in divers who have suffered with decompression sickness than in a control population of healthy volunteers. A study published later that year in the same journal by Wilmshurst *et al* found a higher prevalence of PFO in divers who had experienced decompression sickness compared to divers who have not suffered from these problems, suggesting an association between the two [81]. Another study by Erdem *et al* [82] found that on MRI imaging of the brain, military divers had more cerebral white matter lesions than non-diving controls. A recent study looking at the effect of PFO closure in divers confirmed that closure reduced the risk both of self-reported symptomatic decompression sickness and asymptomatic white matter lesions seen on magnetic resonance imaging (MRI) [83].

Platypnoea orthodeoxia

Platypnoea-orthodeoxia syndrome (POS) is a rare condition characterised by dyspnoea and arterial desaturation in the upright position that is relieved by lying supine. It is associated with the post-pneumonectomy state, aortic aneurysm and cirrhosis of the liver, in the presence of right-to-left intracardiac shunting, usually due to PFO [84]. Although the mechanism by which shunting is present in the upright position but disappears in the recumbent position has not been fully elucidated, there is evidence that closure of the intracardiac shunt relieves symptoms [85].

Migraine with aura

Migraine is a common condition that affects up to 13% of the population aged between 18 and 55 [86]. As well as a health impact on the patient, migraine has a large economic impact on society. A large, Europe-wide cross sectional survey published in 2012 suggests that migraine costs € 1222 per person per year [87]. This includes both direct costs (medications, hospitalisation, investigations) and indirect costs (work absenteeism).   Interest in the role of PFO in migraine first arose when patients who had closure of their PFO for reasons unrelated to migraine noticed an improvement in migraine symptoms. Wilmshurst *et al* carried out a retrospective analysis of patients who had their PFO closed for indications including decompression sickness in divers and cryptogenic stroke and showed that some patients had experienced a significant improvement in concomitant migraine symptoms [88]. The prevalence of PFO also appears to be higher in patients who suffer migraine than in the general population. A cross sectional case-control study carried out by Schwerzmann *et al* used transoesophageal echocardiography to identify a PFO in 47% of patients with migraine with aura compared to PFO in 17% of healthy controls [89]. Other studies have found that migraine with aura is more closely associated with PFO and other right-to-left shunts than migraine without aura [90]. One proposed mechanism of causation is that venous blood contains as yet unknown agents normally filtered by the lungs which enter the cerebral circulation to trigger migraine in those with a right-to-left shunt. One relatively large prospective trial comparing device closure of PFO against an innovative sham procedure in patients with migraine with aura did not show any benefit in the primary end point of complete cure of migraine at 3 to 6 months [91]. However post-hoc analyses with exclusion of outliers did reveal that patients who underwent device closure had a

lower number of total migraine days compared to patients who underwent the sham

procedure.

Diagnosis of PFO

In vivo diagnosis of PFO was difficult until the use of ultrasound and

echocardiography techniques became more widespread. A variety of ultrasound

modalities including transthoracic echocardiography (TTE), transoesophageal

echocardiography (TOE) and transcranial Doppler (TCD) are now available to aid

diagnosis. Inge Edler and Hellmuth Hertz first pioneered the use of ultrasound in

cardiac imaging in the 1950s when they used M-Mode to help diagnose mitral

stenosis [92]. Satomura then introduced Doppler to echocardiography enabling the

measurement of blood flow velocities [93]. In the 1970s, Bom and colleagues

produced the first 2-D images of the heart using ultrasound [94] that made the

technology accessible to many more physicians. Side and Gosling introduced TOE

in 1971, initially to allow measurement of continuous wave Doppler signals [95].

The use of TTE and contrast agents in the diagnosis of right-to-left shunts including

PFO was described by Dubourg and colleagues in 1984 [96]. This was followed by a

description on the use of TOE and colour flow Doppler to diagnose PFO [97]. Later,

TOE and contrast agents were shown to be able to diagnose PFO and other causes

of cardiac emboli following stroke [98].

During TTE, a transducer is placed against the chest wall with the use of ultrasound

jelly to reduce the impedance mismatch between the probe and the patient's skin.

Images are obtained through the chest wall from standard locations (left and right parasternal, apical, subcostal and suprasternal). Image quality can be limited by patient features including obesity, structural chest wall deformities, chronic obstructive pulmonary disease and narrow rib spaces. The inability to obtain adequate echocardiography windows is one of the main limitations of TTE. TTE is performed by a single operator and is non-invasive.

Prior to TOE, the patient is first sedated (usually with an intravenous benzodiazepine) and the throat numbed with a local anaesthetic spray.   The oesophagus is then intubated to allow the ultrasound probe to view the heart from inside the patient's oesophagus rather than from outside the chest wall. The main advantage of this is that as the heart is closer to the probe, higher frequency signals can be used during imaging leading to higher resolution images. It is not possible to use the same high frequencies during a TTE as this would not allow sufficient penetration of the chest wall. Generally, TOE is performed by a team including a clinician, a sonographer and a nurse. During a PFO study, there is an injection of "bubble contrast" (a mixture of saline or colloid, air and blood) into a peripheral vein combined with procedures to increase pressure in the right atrium (Valsalva release, sniff). In both TTE and TOE, visualisation of movement of bubbles from the right atrium (RA) into the left atrium (LA) within 3 cardiac cycles demonstrates the presence of an intracardiac right-to-left shunt.

TCD was first described by Aaslid in 1981 [99]. It was initially used to assess middle cerebral artery blood flow velocity and was helpful in the diagnosis of vasospasm

following subarachnoid haemorrhage. The use of TCD and contrast in the diagnosis of PFO was first described by Teague et al in 1991 [100]. To perform a TCD study, an ultrasound probe is placed against the temporal bone and secured with a headset. Pulsed wave Doppler at a depth of 50-55mm is used to insonate the middle cerebral artery (MCA). "Bubble contrast" is injected into a peripheral vein whilst the MCA is monitored. TCD demonstrates right-to-left shunting (not necessarily intracardiac) by detecting the presence of contrast in the MCA as "hits" (high intensity transient signals).

Sensitivity and specificity of TTE, TOE and TCD

TOE has previously been considered the "gold standard" investigation in the diagnosis of PFO as it has a high sensitivity and specificity [101]. A study by Di Tullio et al in 1993 compared the accuracy of TTE, TOE and TCD in the identification of PFO [102]. They carried out all three investigations in 49 patients being investigated for a PFO following a stroke or TIA. Using TOE and a contrast agent, they identified a PFO in 19 patients. They adopted this as the gold standard before carrying out TTE and TCD with contrast. TCD was able to identify 13 of the 19 patients and had a sensitivity of 68% and a specificity of 100%. TTE with contrast was only able to identify a PFO in 9 patients and had a sensitivity of 47% and again a specificity of 100%. This study seemed to confirm earlier work by Hausmann et al which had also found that TOE with contrast had a higher sensitivity than TTE with contrast in the detection of PFO [70].

The main disadvantage of TOE is that it is an invasive investigation which requires sedation and causes discomfort to the patient. The ability of a patient to perform an adequate Valsalva is also limited both by intravenous sedation and oesophageal intubation [103]. The strain phase of the Valsalva manoeuvre raises intrathoracic pressure (thus both atrial pressures) and compresses the pulmonary veins and the thoracic vena cavae. During the release phase of the Valsalva, the increased systemic venous return precedes the pulmonary venous return and leads to a right-to-left pressure gradient [104]. It is important to carry out a correct Valsalva to increase the chances of successfully detecting a PFO [105]. TOE can also very rarely lead to severe complications. Following a multi-centre survey of 10 419 TOE examinations, Daniel *et al* recorded a complication rate of 0.18% (pulmonary, cardiac or bleeding that lead to early termination of TOE) and a mortality rate of 0.0098% [106].

Later work by Devuyst [107] and Klotzsch [108] suggested that TCD with contrast may be even more sensitive to the presence of a PFO than TOE. In their work, some patients with PFO were identified by TCD where they had previously been missed by TOE. It seemed however that both modalities were superior to TTE in the detection of PFO. This changed with the development of second harmonic imaging (SHI) which has significantly improved the image resolution of echocardiography.

Sound signals, including ultrasound, contain harmonics (multiples of the original frequency) that are produced in addition to the main frequency (the fundamental frequency). Previously, although harmonics were produced during

echocardiography, the ultrasound probes ignored second harmonics and focused instead on the fundamental frequency. During SHI, the image is created using the second harmonic component of the original signal and filters the fundamental frequency. This helps to remove many of the artefacts that are created when the fundamental frequency is used during imaging (usually due to reverberation between the transducer and the ribs). SHI was first developed to improve the resolution in the detection of ultrasound contrast agents [109][110]. It was soon shown that SHI also improved the definition of endocardial borders and myocardium even in the absence of contrast [111]. Disadvantages of SHI include a higher power output requirement and slight alteration of the texture of myocardium and apparent thickness of valves.

With the development of SHI, later studies have suggested that TTE may have a sensitivity at least equivalent to TOE in the detection of PFO. Kuhl et al published work on 111 patients evaluated for a PFO following a cerebral embolic event [112]. After comparing TOE against TTE with SHI, they found that a total of 57 patients had evidence of right-to-left shunting when both investigations were used in complementary fashion. TOE identified 51 patients with PFO and TTE identified 52 patients with PFO, with the authors concluding that both modalities have a comparable yield for the detection of right-to-left shunts. Another multi-centre study by Daniels et al carried out TOE and TTE (SHI) with agitated saline contrast in 256 consecutive patients [113]. A right-to-left shunt was detected in 60 patients overall, 53 by TOE and 55 by TTE, with both modalities missing a few patients that the other modality detected. Again, the authors concluded that TTE (with SHI) was at least as sensitive as TOE in the detection of right-to-left shunting. These finding were mirrored by another study by Thanigaraj et al [114]. After carrying out both TOE and

TTE with contrast in 94 consecutive patients, they found that TTE with contrast had a higher rate of detection of right-to-left shunting than TOE with contrast. Lam et al also confirmed a high sensitivity (94.0%) and specificity (95.2%) with the use of TTE in the detection of PFO [115].

As well as having an equivalent (or better) sensitivity in the detection of PFO, the main advantage of TTE over TOE is that it is non-invasive and does not have the risk that TOE entails. There is also no requirement to sedate the patient with intravenous benzodiazepines and the procedure is much more easily tolerated. The lack of sedation also allows for a more forceful Valsalva manoeuvre during bubble contrast studies. TTE can be carried out by a single operator rather than requiring a team and is therefore cheaper. However, echocardiography can occasionally produce both false positive and false negative results when used to exclude PFO [116].

TCD is another non-invasive investigation which can be used in the detection of PFO and other right-to-left shunts. Teague et al published a study in 1991 that compared TCD with contrast injection against TTE with contrast in 46 patients being evaluated for inter-atrial shunting [100]. They found that contrast could be detected in the MCA in all patients who had a positive TTE. They concluded that TCD is a safe and sensitive alternative method to echocardiography in the detection of right-to-left shunting. A study published in 2009 by Sastry et al also compared TOE against TCD in the diagnosis of right-to-left shunting in 39 young patients with an ischaemic stroke or myocardial infarction [117]. They found that TCD had a sensitivity equal to

TOE in the detection of shunting. This confirmed the work of other groups

suggesting that TCD is sensitive in the detection of right-to-left shunting [118] [119].

The major disadvantage of TCD compared to TOE and TTE is that it cannot

differentiate a right-to-left shunt due to PFO from that due to other causes. Any

right-to-left shunt such as caused by ventricular septal defects or pulmonary

arteriovenous malformations would be detected as contrast in the MCA by TCD

[120]. When carrying out echocardiographic examinations, pulmonary shunting can

be identified by the delay (over 3 cardiac cycles) between contrast being seen in the

right atrium and in the left atrium [121]. Indeed, TTE with saline contrast has been

shown to be an effective screening test in the identification of pulmonary

arteriovenous malformations in patients with hereditary haemorrhagic telangiectasia

[122].

Having considered the relative merits of the different investigations, we chose to use

TTE (SHI) with agitated saline contrast to look for the presence of PFO in our

patients. We also chose to attempt to quantify the size of the potential shunt. PFO

size cannot be accurately measured without pushing the septum primum away from

the septum secundum, either with a balloon in the cardiac catheter laboratory or at

post-mortem [123]. PFO size has been found to range between 1mm to 19mm in

adults at post-mortem, with the average size of PFO being larger in older adults [55].

Potential shunt size however can be estimated using contrast echocardiography by

counting the number of microbubbles that cross from the right to the left atrium. The

definition of a large PFO has varied. Some groups have categorised a PFO as large

if as few as 10 microbubbles are seen to cross the atrial septum [65]. Others have suggested that over 30 microbubbles need to be seen to cross before a PFO is categorised as large [124]. A recent review by Buchholz et al subdivided PFO size further into small (<20 microbubbles), moderate (20-50 microbubbles) and large (>50 microbubbles) [125].

Although still under debate, there is some evidence that a PFO with a permanent right-to-left shunt is more likely to result in pathology than a PFO with a shunt only on Valsalva release. Rigatelli et al compared patients with a permanent shunt against those with only a Valsalva induced shunt [126]. They found that patients with a permanent shunt were more likely to have ischaemic lesions on brain magnetic resonance imaging and were also more likely to have had previous recurrent stroke or peripheral artery embolisation. This was however a small study with novel findings and the work has not yet been replicated.

Aims

The aim of this part of the study was to identify the prevalence of PFO in a population of patients undergoing both haemodialysis and peritoneal dialysis. We also aimed to quantify the size of any potential right-to-left shunt.

Method

Recruitment

Patients were recruited from the Royal Sussex County Hospital Renal Unit based in Brighton, United Kingdom. We invited all 173 patients with end stage renal failure having regular HD treatment and the 101 patients with end stage renal failure having PD treatment at our unit to participate in the study. All patients who expressed an interest in taking part in the study were given a patient information sheet (see appendix 2) and given an opportunity to ask questions. All patients who were interested in taking part were screened against the eligibility criteria set out below.

Inclusion Criteria

Patient with renal failure on HD or PD

Aged eighteen or over

Exclusion criteria

Dementia

Expected life expectancy of less than one year

Mechanical heart valve

Current co-morbid psychiatric or neurological illness

Participant taking sedative or psychotropic medication

In total we recruited 51 HD patients and 29 PD patients who fulfilled our inclusion and exclusion criteria. All patients gave written consent before participating in the research (see appendix 3). Patient characteristics are shown in table 2. The primary cause of renal failure in our study patients is shown in Table 3.

Chapter 3 (Prevalence of Patent Foramen Ovale)

Table 2 – Patient characteristics

	All patients	**HD patients**	**PD patients**
Age in years (median)	64.5 (range 21-84)	63.0 (range 21-83)	65.0 (range 28-84)
Male	54 (67.5%)	35 (68.6%)	19 (65.5%)
Female	26 (32.5%)	16 (31.4%)	10 (34.5%)
Time on dialysis in months (median)	18.0 (range 2-402)	16.5 (range 2-402)	22.0 (range 2-194)

Table 3 - Primary cause of renal failure

Primary cause of renal failure	**HD patients**	**PD patients**
Polycystic kidney disease	8	
Diabetes mellitus	7	5
Obstructive nephropathy	6	3
Unknown cause	6	3
Glomerulonephritis	8	10
Hypertension	4	2
Goodpasture's disease	2	
Renovascular disease	2	2
Myeloma	2	
Primary hyperoxaluria	1	
Tuberculosis	1	
Reflux nephropathy	1	
Waldenstrom's	1	
Alport's disease	1	
Granulomatosis with polyangiitis	1	

Primary cause of renal failure	HD patients	PD patients
Chronic pyelonephritis		2
Scleroderma		2
Polyarteritis nodosa		1
Total	**51**	**29**

Each patient had a transthoracic echocardiogram using a Philips IE33 console (Amsterdam, Netherlands) with bubble contrast injection. After obtaining an apical 4-chamber view, 10ml of a bubble contrast agent composed of 8ml of normal saline, 1ml of the patient's blood and 1ml of air was injected into the venous circulation. The blood helps to emulsify the bubble contrast and allows smaller bubbles to be created which remain suspended for longer. Each patient had up to six injections of contrast in total: two during rest, two during Valsalva manoeuvre and two during sniff. The release phase of the Valsalva manoeuvre and sniff increase pressure and venous return to the right atrium and therefore right atrial pressure, which helps to open an existing PFO. This is a more sensitive method of identifying PFO than injection of contrast agent at rest alone. A PFO was diagnosed if flow of bubble contrast across the atrial septum was visualised within three cardiac cycles of any of the injections. If bubbles were seen to cross a PFO, a visual estimate was made of their number. The size of the PFO was graded into 3 categories depending on the number of bubbles seen in the left atrium. A PFO was categorised as large if there were more than 50 bubbles seen crossing the atrial septum within three cardiac cycles; moderate if 10-50 bubbles were seen to cross the septum; small if less than 10 bubbles were seen to cross the septum. A patient did not have any further injections of contrast if a large PFO was diagnosed at any stage (for example if a

patient had >50 bubbles crossing during injection at rest, no further injections were

carried out).

Results

The results of our investigations can be seen in Table 4 (below). 95% confidence

intervals were calculated to allow comparison with the proportion of the general

population who have a PFO.

Table 4 - Number of PFO

	HD	PD	All patients
PFO	12 (23.5%, 14.0% to 36.8%)	5 (17.2%, 7.6% to 34.6%)	17 (21.2%, 13.7% to 31.4%)
No PFO	39 (76.5%)	24 (82.8%)	63 (78.8%)
Total	51	29	80

Table 5 shows the estimated size of the PFO on echocardiogram. Size estimation

was dependent on the number of bubbles seen to cross the PFO.

Table 5 - Estimated size of PFO

	HD	PD	All patients
Large (>50 bubbles)	8	1	9
Moderate (10-50 bubbles)	3	3	6
Small (<10 bubbles)	1	1	2

The figures below (figures 3-5) show some images from a transthoracic echocardiogram during a bubble contrast study. An apical 4-chamber view is seen prior to and then following injection of bubble contrast into a peripheral vein.
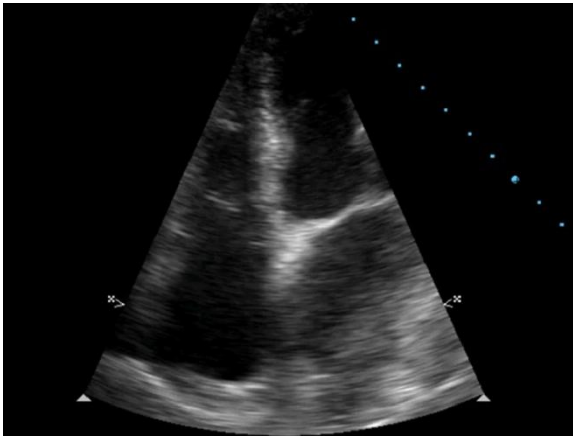
Figure 3- Echocardiogram showing the intra-atrial septum in the apical 4 chamber view

Figure 4 – Apical 4 chamber view showing contrast in the right atrium following injection into a peripheral vein

Figure 5 - Apical 4 chamber view showing contrast in the left atrium and left ventricle after it has crossed a likely PFO

Discussion

We found that out of 51 HD patients, 12 had a PFO whilst in our 29 PD patients, 5 had a PFO. Our study found that the prevalence of PFO in a group of chronic dialysis patients was 17 out of 80 (21.2%). The 95% confidence intervals have a range that includes the documented prevalence of PFO in a post-mortem series of the general adult population [55]. It was important not to assume that this would be the case. In the past, the higher prevalence of PFO in other clinical entities such as migraine was discovered prior to the establishment of an association between the conditions [88]. It may prove to be of relevance that 52.9% of the PFO detected in all patients were found to be large whilst in the HD population 66.7% of the PFO were large.

The main limitation of this study is that although TTE with contrast has a high sensitivity and specificity in the detection of PFO, it is not 100% sensitive or specific. It is possible that a small PFO may not have been identified (a false negative). It is

also possible that the presence of a pulmonary arteriovenous malformation may have lead to a false positive identification of a PFO. Although previously thought to be rare in healthy adults, small pulmonary arteriovenous shunts may be present in over 20% of the population [127]. The methodology used however (with PFO only diagnosed if contrast is seen within 3 cardiac cycles) reduces the risk of a pulmonary shunt being incorrectly classified as a PFO.

A further limitation in our study is that we do not have a control population of normal patients. If we had carried out echocardiography with bubble contrast in a normal population, it would have been possible to compare the detection of PFO using our methods in these patients against prevalence from historical post-mortem and echocardiographic controls. This may have helped to validate our techniques in PFO detection. This may be especially relevant as we found an unusual distribution of small, medium and large PFO shunts.

It is known that patients having HD treatment have an advanced rate of cognitive decline. One possible mechanism for this advanced rate of cognitive decline might relate to ongoing low level microembolisation of platelet aggregates, microbubbles or other microemboli, to the brains of HD patients in the presence of a PFO, occurring during HD sessions. This study confirms that PFO is present in HD patients in the same proportion as would be expected from other studies of non-HD patients.

Chapter 4 (Dialysis and cerebral microembolisation)

Background

Haemodialysis and microembolisation

Problems with air microemboli causing clinical pathology were first described in the field of cardiothoracic surgery [128] during correction of a cleft mitral valve. In 1973, Gallagher *et al* [129] used ultrasound to identify the source of microbubbles in the arterial blood and also showed that they could be detected in the carotid arteries during open heart surgery. They demonstrated that the bubble oxygenators used at the time were responsible for creating the microbubbles. Not long afterwards in 1975, evidence was presented that pulmonary microembolisation is a problem during HD. Bischel *et al* [130] demonstrated that pulmonary microembolisation during dialysis was responsible for ventilation and perfusion abnormalities that manifest as a decrease in arterial oxygenation and a widening of the alveolar-arterial oxygen gradient. The HD circuit returns blood to the patient's venous circulation, carrying microemboli to the lungs. The nature of the microemboli identified during HD was uncertain. Initially the microemboli were thought to be platelet aggregates that could be filtered with the use of different filters although subsequent studies have revealed a more heterogeneous composition.

In an advance in technology, Spencer *et al* [131] showed that Doppler ultrasonography could be used to detect both solid and gaseous microemboli, initially

during carotid endarterectomy. They described the detection of transient signals

lasting 0.01 to 0.1 seconds and over 10 dB greater than the background Doppler

signal during surgery. They speculated that they had detected and managed to

differentiate both gaseous microemboli (characterised by high amplitudes) and

certain formed element embolic signals (usually associated with lower amplitudes).

However, they also acknowledged that they had used the timing of the detected

signals in relation to surgery to help separate solid from gaseous microemboli and

they were unable to confirm the accuracy of this differentiation. Ringelstein *et al*

reported on an international consensus that further delineated the features of a

microembolus as seen on Doppler ultrasonography [132], although they also stated

that TCD was not able to differentiate solid from gaseous microemboli. More

recently, using the criteria proposed by Ringelstein *et al*, the presence of

microemboli has been demonstrated in the drainage vein from arteriovenous fistulae

during HD using Doppler ultrasound by Rolle *et al* [45]. They identified microemboli

during dialysis at a rate of up to 25 hits per minute. These findings were confirmed

by Droste *et al* [49] who went on to show that microembolic signals could be reduced

during dialysis with the use of pre-filled rather than dry dialysers. Both of these

studies were unable to differentiate gaseous from solid microemboli.

The Doppler signal from gaseous emboli is very similar to the signal from synthetic

particles which may be shed from the dialysis machine, with both having a relatively

high amplitude. It is possible to differentiate gaseous emboli from microthrombi or

calcified material of the same size (or smaller) which tend to have a lower amplitude

[133]. Larger solid emboli, however, are difficult to differentiate as they can have a

Doppler signal identical to gaseous microemboli [134]. Initial attempts at

differentiation between solid and gaseous microemboli using differences in amplitude and spectral distribution had relatively low accuracy [135]. Russell et al described a system to better differentiate solid and gaseous microemboli by using a multifrequency TCD system [136]. They insonated the MCA with ultrasound at 2MHz and 2.5 MHz simultaneously using a system named "Embo-Dop" (DWL Elektronische Systeme GmbH, Singen, Germany). Solid microemboli reflect more ultrasound at higher frequencies whilst the reverse is true for gaseous microemboli. By comparing the signals at both frequencies, it is theoretically possible to better differentiate solid and gaseous microemboli. Although their initial report held promise, further work by Markus et al did not fully validate the earlier study [137]. Markus et al used "Embo-Dop" in patients with known carotid artery stenosis (where the microemboli are solid) and in patients with a PFO who underwent a bubble contrast injection (where the microemboli are gaseous). They found that even with multifrequency TCD, the sensitivity and specificity in correctly identifying the different types of emboli were low. The system had a sensitivity of only 50.3% in the correct identification of solid microemboli and a specificity of only 50.3% in the identification of gaseous emboli. This led the authors to conclude that multifrequency TCD was not reliable enough for clinical use in the differentiation of different types of microemboli.

Although not reliable in the differentiation between different types of microemboli, TCD is very reliable in detecting microemboli. The process of recognising "hits" in the MCA was previously carried out by operators using audio and visual data from the TCD and this is still considered the gold standard. The process of separating "hits" representing microemboli from artefact (for example following patient

movement) can be technically demanding as well as time consuming and therefore limited the use of TCD in routine clinical practice. An early study comparing the reliability of automated systems in identifying microemboli correctly found that they had poor correlation with expert consensus with a Cohen's kappa value of less than 0.4 in some systems [138]. This has however improved with time. A study by Cullinane et al compared automated software against a panel of experts in the detection of microemboli in patients with carotid artery stenosis and in patients post carotid endarterectomy [139]. They found that the automated software had a sensitivity and specificity of 85.7% and 88.9% respectively in carotid artery stenosis and a sensitivity and specificity of 95.4% and 97.5% respectively in patients post carotid endarterectomy. More work by Devuyst et al using the "Multi-Dop X4" (DWL Elektronische Systeme GmbH, Singen, Germany) showed that more modern automated software can correctly identify microemboli and has a high level of agreement with human experts [140]. Their automated system had a sensitivity of 97%, a specificity of 98%, a positive predictive value of 99% and a negative predictive value of 94% when compared against expert analysis. TCD with automatic microemboli detection, including with the ST3 Digital TCD System (Spencer Technologies, Seattle, USA) is now routinely used in research practice [141]. There are no studies that directly compare the sensitivity and specificity of the ST3 Digital TCD system against Multi-DOP X4 or Embo-Dop.

The gaseous emboli present in the circuit during haemodialysis take the form of microbubbles. These are very small bubbles thought to originate in the extracorporeal tubing and are more likely to be present in a patient's circulation with increased flow rates [142]. It is thought that microbubbles can cause tissue damage

both by causing physical obstruction and by stimulating an inflammatory response. Microbubbles can abrade the glycocalyx layer that lines capillaries and obstruct blood flow, leading to tissue ischaemia and an inflammatory response that results in aggregation of platelets and clot formation [142].

Haemodialysis and PFO

HD populations are increasing year on year, with 20 972 patients undergoing treatment in 2008 in the UK [143] and 370 274 patients in 2009 in the USA [144]. The prevalence of PFO in HD patients has not been published in the literature but our findings suggest that it is in line with that in the general population. In patients with a PFO, it is possible for microemboli to cross from the venous circulation into the systemic circulation, rather than being filtered out by the lungs. This would allow embolisation into the cerebral circulation where it may contribute to cognitive decline. There has not yet been any study which has shown microembolic signals in the cerebral circulation during HD. However it has recently been shown that in patients undergoing HD, microemboli can be detected in the carotid arteries during treatment [145].

As previously described, the presence of microemboli has been demonstrated in the draining vein of arteriovenous fistulae during HD using Doppler ultrasound [45]. Ultrasound monitoring of the subclavian vein during HD has also revealed the presence of microemboli [49], although these studies were unable to differentiate gaseous from solid microemboli. As a PFO allows paradoxical embolisation from the

right sided venous circulation into the left sided arterial circulation, this means that as well as pulmonary pathology, damage to the brain, eyes, heart, kidneys or other organs could also occur as a result. A recent study by Forsberg *et al* found that during HD, microembolic signals can be detected in the carotid artery using Doppler ultrasound scanning [145]. They found that 38 out of 54 patients (70%) in the study had evidence of microembolisation detectable in the carotid circulation. This is a rate higher than would be expected if carotid microembolisation occurred only in patients with a PFO. The authors of the study suggested that carotid microembolisation may occur even in the absence of a PFO due to incomplete clearance of microemboli by the pulmonary bed. As echocardiography was not carried out in this particular study population, it is unknown how many of them actually had a PFO or other right-to-left shunt.

To our knowledge, no group has published data to confirm if cerebral microembolisation detectable by ultrasound actively occurs during HD. Transcranial Doppler (TCD) ultrasound is a well proven technology to detect cerebral microembolisation in other conditions: for example, microemboli have been detected using TCD in the MCA in patients who have had implantation of a mechanical heart valve [146]. Use of TCD with concurrent injection of bubble contrast into the peripheral venous system is also widespread in the diagnosis of right-to-left shunts [147][125].

Aims

The aim of this part of the project was to identify whether microemboli can be detected in the cerebral circulation using TCD during HD and whether this is linked to the presence of a PFO. A different population of patients were included as a control group. This control group of patients also had end stage renal failure but were having renal replacement therapy in the form of PD rather than HD.

Methods

The recruitment process for this study has already been described (see chapter 2). Each patient had a transthoracic echocardiogram using a Philips IE33 console (Amsterdam, Netherlands) with intravenous bubble contrast injections as previously described. To confirm findings from multiple previous studies that microemboli are generated by the process of HD, eight HD patients had ultrasound scanning of their drainage arteriovenous fistulae using a 2 MHz transducer and emboli detection software both before and during HD treatment for a period of 5 minutes. As a previous study by Rolle et al had shown ultrasound detection of microemboli at a rate of up to 25 hits per minute, 5 minutes was a reasonable period of time over which to carry out ultrasound monitoring. All patients then underwent TCD scanning of their MCA using a ST3 Digital TCD System (Spencer Technologies, Seattle, USA) and microemboli detection software with a 2 MHz transducer. The right MCA was identified in the temporal window using pulsed wave Doppler by arterial flow towards the handheld TCD probe located at a depth of less than 55mm. Signals were identified as microemboli if they conformed to the criteria previously specified by Ringelstein *et al* [132] with all recordings stored digitally. HD patients underwent

TCD scanning for 5 minutes before HD and then for 5 minutes during HD treatment. All patients were asked to perform the Valsalva manoeuvre twice and to sniff twice during TCD monitoring both before and during HD. Our HD unit uses the Fresenius (Bad Homburg, Germany) 4008S machines with a range of dialysers (FX5, FX8, FX10, FX60, FX80 and FX100). PD patients underwent TCD scanning for 2 episodes lasting 5 minutes each. They were also asked to perform the Valsalva manoeuvre twice and to sniff twice during TCD scanning. Finally, as a positive control, we carried out bubble contrast injections into 5 patients with a known PFO (on transthoracic echocardiography with bubble contrast) whilst monitoring their MCA with ultrasound.

Results

The image shown in figure 6 was captured using ultrasound over an arteriovenous (AV) fistula in a HD patient during HD. Embolic signals can be seen. The histograms in figure 7 show the number of hits detected in the draining AV fistulae in 8 patients prior to HD and then during HD.

Chapter 4 (Dialysis and cerebral microembolisation)
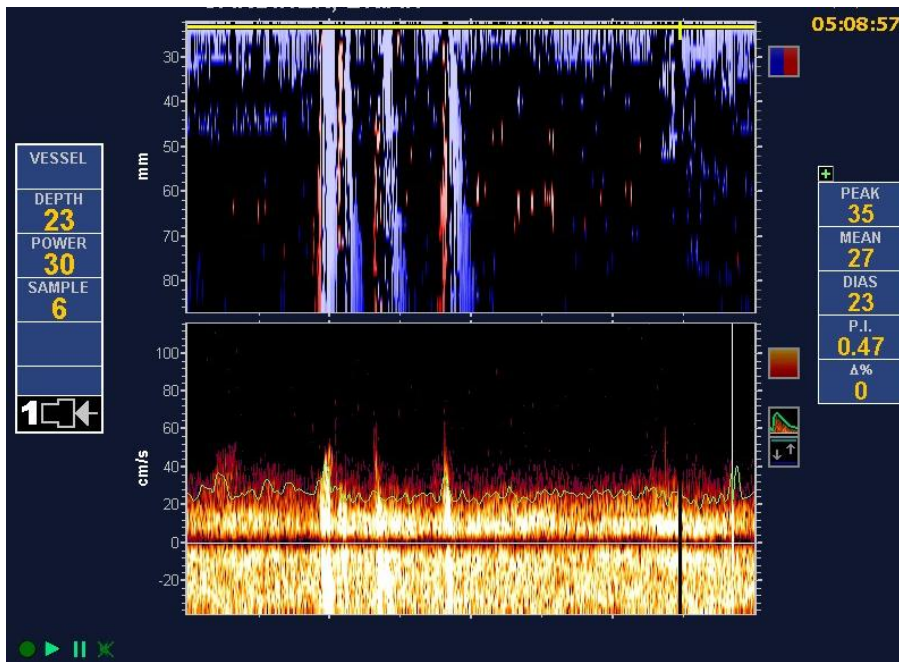
Figure 6 - Monitoring of AV fistula in HD patient during HD showing evidence of microemboli
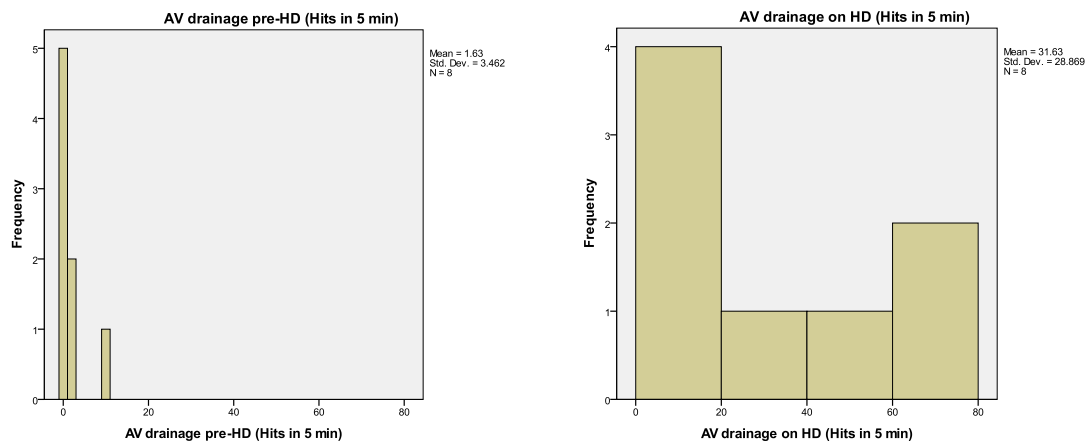
Figure 7 - Histograms showing the number of microembolic hits in the drainage fistulae prior to and during HD

We carried out a Shapiro-Wilk analysis to identify whether the data were normally distributed (table 6).

Table 6 - Shapiro Wilk test of normality of data distribution

	Shapiro-Wilk		
	Statistic	df	Sig.
Hits in fistula pre-HD	.561	8	.000
Hits in fistula during HD	.896	8	.266

The histograms (figure 7) show the distribution of the data. Formal analysis suggested that the number of microemboli signals in the fistula prior to HD was not normally distributed but the number of hits in the fistula during HD was normally distributed. We elected to use non-parametric tests to analyse the data further. Table 7 shows the median and range in the number of hits detected by ultrasound monitoring of the draining arteriovenous fistula during HD in our sample of 8 patients. A Wilcoxon Signed Ranks test was carried out to look for statistical significance.

Table 7 – Microembolic signals in arteriovenous fistulae

HD patients (n = 8)		
Microembolic signals in arteriovenous fistula prior to HD (over 5 minutes) (median, range)	Microembolic signals in arteriovenous fistula during HD (over 5 minutes) (median, range)	P value (Wilcoxon)
0.00 (0 to 10)	24.50 (1 to 26)	**0.012**

Of our 51 HD patients, we were able to isolate the MCA using ultrasound in 40 cases prior to and during HD. The MCA was monitored for 5 minutes before and then for 5 minutes during HD. On each occasion, patients twice performed a Valsalva manoeuvre and twice performed a sniff. Figure 8 shows an image captured during

the monitoring of the MCA in a HD patient during dialysis. The histograms in figure 9

show the numbers of microembolic signals detected before and during HD.
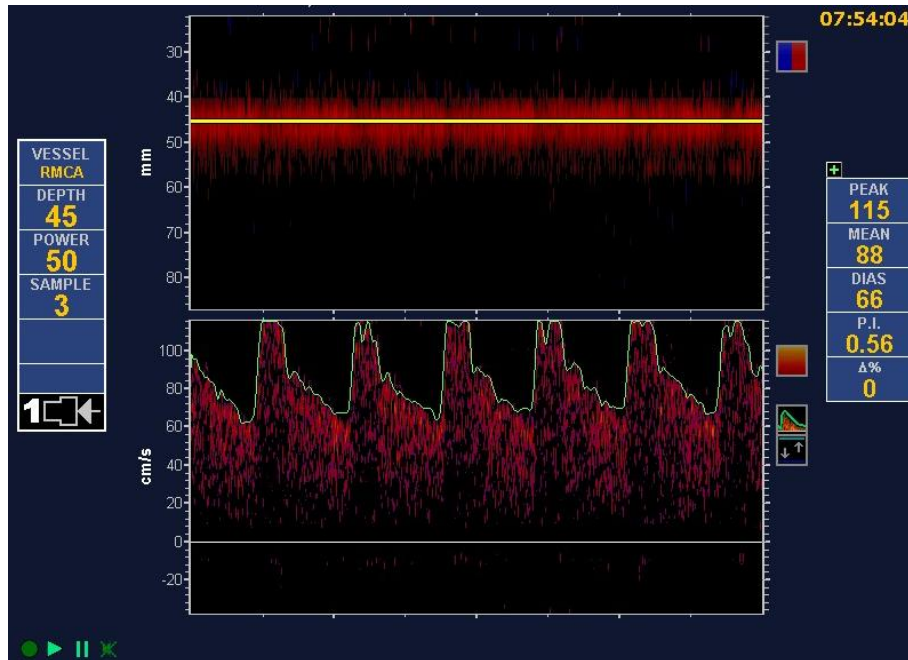
Figure 8 - Monitoring of MCA of a HD patient during dialysis
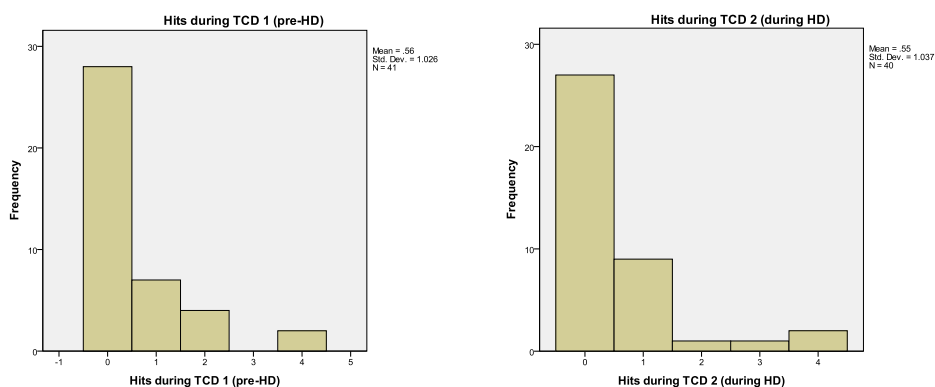
Figure 9 - Histograms showing the number of hits prior to HD and during HD

Shapiro-Wilk testing showed the data were not normally distributed.

Table 8 shows the median number and range of microembolic signals detected in the MCA of these patients. As the data are not normally distributed, we used non parametric tests when looking for statistical significance.

Table 8 – Microembolic signals in the MCA

HD patients (n = 40)		
Microembolic signals in MCA whilst not on HD (over 5 minutes) (median, range)	Microembolic signals whilst on HD (over 5 minutes) (median, range)	P value (Wilcoxon)
0.00 (0 to 4)	0.00 (0 to 4)	0.75

We then compared the rates of detectable cerebral microembolisation between those with (figure 10) and without (figure 11) a PFO, both prior to and during HD.
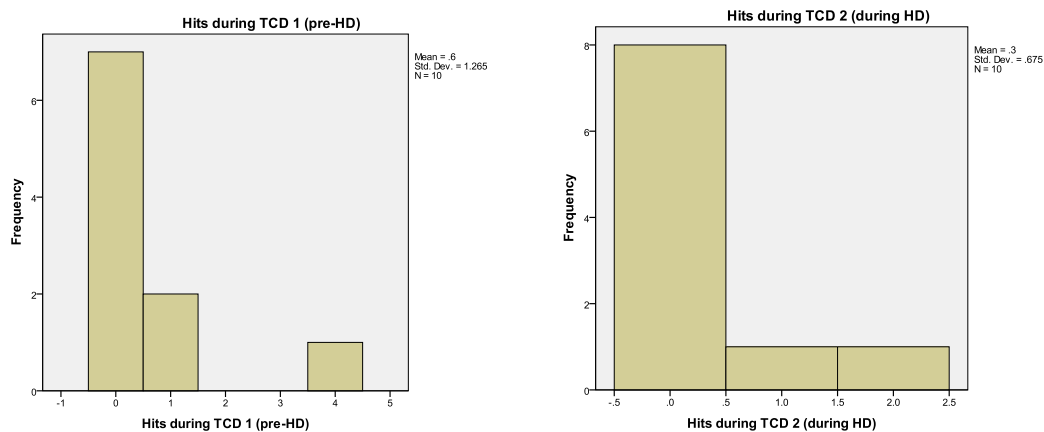
Figure 10 - Histograms showing TCD hits prior to HD and during HD in patients with a PFO

Chapter 4 (Dialysis and cerebral microembolisation)
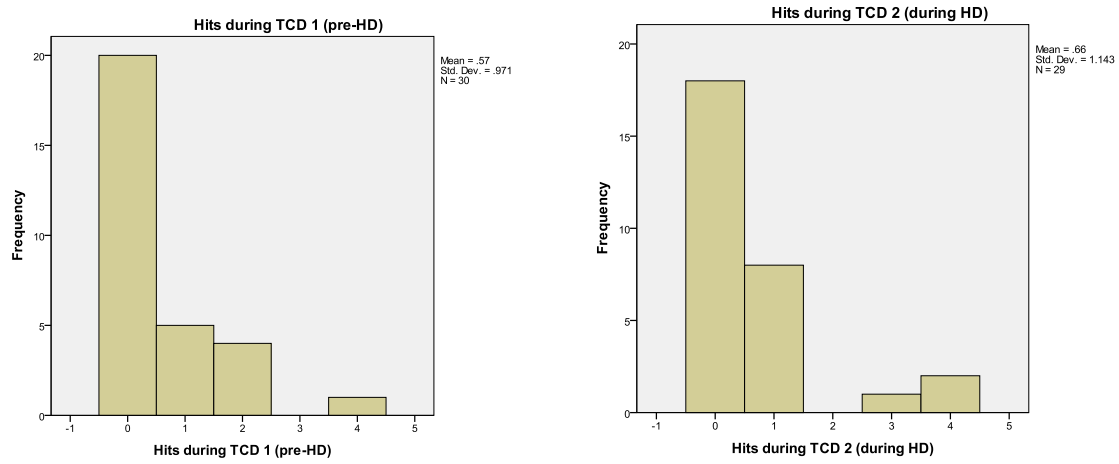
Figure 11 - Histograms showing TCD hits prior to HD and during HD in patients without a PFO

Table 10 shows the relative rates of microemboli detection in the MCA both prior to and during HD between those with and without a PFO.

Chapter 4 (Dialysis and cerebral microembolisation)

Table 8 – Microembolic signals in the MCA in those with and without a PFO

	PFO present	**PFO not present**	**P value (Mann-Whitney)**
Number of HD patients	10	30	
Microembolic signals in MCA whilst not on HD (over 5 minutes)	0.00 (0 to 4)	0.00 (0 to 4)	0.84
Microembolic signals in MCA whilst on HD (over 5 minutes)	0.00 (0 to 2)	0.00 (0 to 4)	0.33

Table 11 shows a comparison of microembolisation rates between HD and PD patients. We again used a Mann-Whitney test to look for statistical significance.

Table 9 – Rates of microembolisation in HD and PD patients

	PD patients	**HD patients**	**P value (Mann Whitney)**
Number of patients	23	40	
Microembolic signals in MCA in period 1 (HD patients not on dialysis)	0.00 (0 to 3)	0.00 (0 to 4)	0.45
Microembolic signals in MCA in period 2 (HD patients on dialysis)	0.00 (0 to 3)	0.00 (0 to 4)	0.63

Bubble contrast injections into 5 patients with a known PFO (identified on
transthoracic echocardiography with bubble contrast) were carried out whilst
monitoring their MCA with ultrasound. This showed concordance between the
results found on transthoracic echocardiography and TCD with multiple "hits"
registered within seconds of contrast injection and confirmed that the TCD is able to
detect embolic signals in the MCA. Figure 12 shows an image captured by the TCD
whilst monitoring the MCA just prior to bubble injection whilst figure 13 shows an
image just after bubble contrast injection. Multiple "hits" can be seen which
represent bubble contrast.
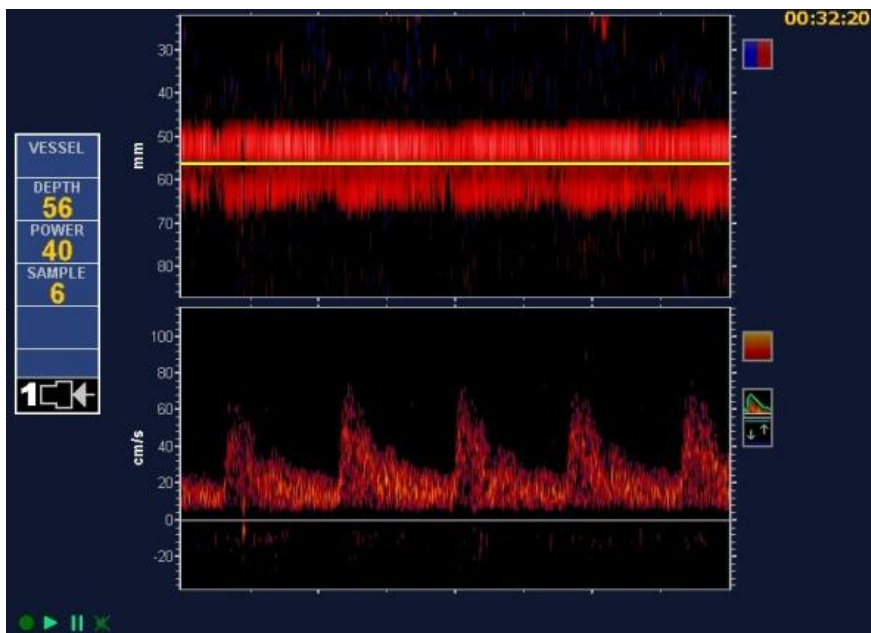
Figure 12 - TCD monitoring of patient with PFO on TTE just prior to contrast injection
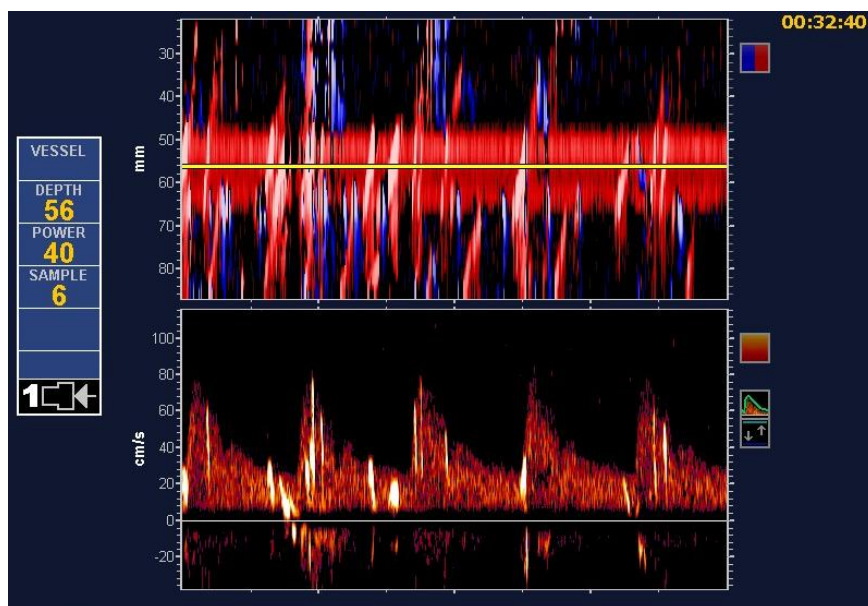
Figure 13 – Multiple embolic hits seen during TCD monitoring just after contrast injection

Discussion

Our study confirmed previous findings by other groups that microembolisation can be

detected in the drainage fistula and veins of patients undergoing HD [45]. A recent

study by Forsberg *et al* [145] has shown that dialysis-related microemboli cross from

the venous (right-sided) into the arterial (left-sided) circulation where they were

detected using ultrasound in the carotid arteries. The study was interesting as it also

found that microemboli could be detected in the carotid arteries of more patients than

would reasonably be expected to have a PFO. The authors of the study suggested

that carotid microembolisation may occur even in the absence of a PFO due to

incomplete clearance of microemboli by the pulmonary bed. It is unknown what

percentage of patients in their study had a PFO or other cardiac or pulmonary shunt.

Although it is likely that the microemboli identified in the quoted study were travelling

up into the cerebral circulation, TCD was not carried out in these patients so cerebral

microembolisation could not be confirmed. Thus it was surprising to us that our

study found no evidence that microemboli could be detected in the MCA of the cerebral circulation during HD. This is especially intriguing in the patients undergoing HD who we had identified as having a PFO. We would have expected that at least some of the microemboli which were shown to be present in the drainage arteriovenous fistulae could cross a PFO and be detectable in the MCA and it is unclear to us why this was not found. One possibility is that as flow across a PFO can only occur when right atrial pressure is greater than left atrial pressure, and as this is an infrequent occurrence, we did not monitor the MCA for a sufficient period of time to allow microemboli to cross the shunt. However this does not explain the lack of cerebral microemboli during sniff and Valsalva manoeuvres, which should have increased right sided pressures sufficiently to allow this to happen.

Nevertheless, the results of our study and the study by Forsberg *et al* [145] are not absolutely contradictory. Both studies confirmed that microemboli are detectable by ultrasound in the arteriovenous fistulae during HD. The Forsberg group then went on to detect microemboli in the carotid arteries during HD but did not examine the MCA. Our own study did not look at the carotid arteries but did look at the MCA, where we did not find any evidence of microembolisation. The result is still surprising as if microemboli can be found in the carotid artery during HD, it was to be expected that they would then travel further into the cerebral circulation.

There were several differences between the groups in the equipment that was used. Our patients underwent dialysis using Fresenius 4008S machines and a range of FX

filters. The Forsberg group patients had dialysis using both Fresenius 4008S/H

machines and Gambro AK200/200S machines with a range of different filters. To

detect the microemboli, our group used an ST3 machine by Spencer Technologies

(Seattle, USA) with microemboli detecting software. To detect microemboli the

Forsberg group used an EMEX 25 by Hatteland Instrumentering (Royken, Norway)

with Emmon W emboli detection software. Both machines use the principle of

pulsed wave Doppler to look for microemboli but it is difficult to know the relative

sensitivities of the machines without doing a direct comparison. We do know that the

emboli detection software used by Spencer Technologies adheres to the guidelines

set by the International consensus group on microembolus detection [132] and we

would imagine that the Emmon W software is similar. It is difficult to know if the

settings for microemboli detection in the studies are identical as it depends on a host

of factors including (quoted from the International consensus group [132]): the

relationship between the backscattered power from emboli and the blood; detection

threshold; sample volume; fast Fourier transform (FFT) frequency resolution; FFT

temporal resolution; the FFT temporal overlap; dynamic range of the instrumentation;

transmitted ultrasound frequency; filter settings.

If microembolisation into the MCA does occur during HD and we were unable to

detect it, it is possible that it may have clinical consequences. In patients with

prosthetic heart valves, cerebral microembolisation has been linked to a deterioration

in cognitive function [148] and it is possible that a similar pathological process occurs

during HD.

Chapter 5 (Dialysis, PFO and cognitive function)

Background

Cognitive function is made up of the many elements that come together to enable us to receive, understand, store, retrieve and use sensory information. To enable us to receive information accurately, we use sensory attention. Attention is the process of selecting one stream of information over another. This can be done either consciously or unconsciously. Another important element in cognitive function is memory. Memory is made up of the series of processes that are responsible for encoding, storing and retrieving information. There are different theories on how the brain manages memory but it is generally accepted that there are distinct processes for information that is stored for only a few seconds or minutes (short term memory) and information that is stored for up to a lifetime (long term memory). A knowledge base is also important for cognitive function. Knowledge is the acquisition of information through education or experience and plays an important part in cognitive function. Finally executive function can be thought of as the overall control mechanism that manages other cognitive processes. It is also used to help in planning and decision making. When testing cognitive function, it is important to test aspects of each of these components.

Patients with chronic renal failure have poorer cognitive function than age and sex matched controls from the general population [3]. Patients who have end stage renal failure and require dialysis treatment are particularly affected. Kurella *et al* [41]

carried out a cross sectional study examining cognitive function in patients with CKD. They used three standardised tests (trail making B, modified mini-mental state examination and California verbal learning trial) to assess different aspects of cognitive function in patients with CKD stage 3 and 4 (who did not require dialysis) and patients with CKD stage 5 (who did require dialysis). They found that all patients with CKD fared significantly worse on all three tests than published norms for age and education matched controls without CKD. Of their patients with CKD, those requiring dialysis treatment had significantly worse scores than those with CKD stages 3 and 4 who did not require dialysis treatment. This was consistent with earlier work by Sehgal *et al* [149] who showed that in a large cohort of patients with CKD requiring HD, 30% had significant cognitive impairment as defined by a modified mini-mental state examination score of less than 24. This is more than twice the prevalence found in an age matched cohort from the general population [150]. Tests of executive function and verbal memory were especially affected, suggesting that frontal brain function is selectively impaired. Another study by Murray *et al* looking specifically at memory, executive function and language in 374 HD patients aged over 55 found that nearly 90% had a degree of cognitive impairment [151]. In 37% of these patients, the impairment was categorised as severe.

The rate of decline of cognitive function is also higher in patients undergoing HD than in other patients. Bossola *et al* used the mini-mental state examination (MMSE) to compare cognitive decline between HD patients and elderly patients without kidney disease [152] over a period of 1 year. The MMSE is a brief 30 point cognitive test first described by Folstein in 1975 [153]. Bossola *et al* found that at 1 year, there

were significant reductions in the median MMSE score in both groups of patients but the decline was significantly greater in HD patients than in the elderly controls. In HD patients, the median MMSE score fell from 24 to 21 over 1 year whilst in the elderly patients, the median score fell from 26 to 25. Although baseline cognitive function in the HD patients had been negatively associated with hypertension, angina and depression and positively associated with education and male gender, they found no independent factors that could predict cognitive decline over the follow up period.

Another investigation by Fazekas *et al* [154] in patients undergoing HD treatment showed that as well as cognitive impairment, these patients have abnormal findings on magnetic resonance imaging of the brain. They demonstrated that HD patients had significantly worse cognitive function measured on mini-mental state examination than a cohort of age and sex matched controls. They also showed that the HD patients had significantly more cerebral atrophy and evidence of confluent white matter hyperintensities on brain scanning. These ischaemic lesions were found in 50% of the HD cohort.

In the general population, it has been well established that dementia is an independent predictor of all cause mortality [155][156]. It is also known from population studies that cognitive impairment, even in the absence of dementia, is a risk factor for death [157]. Unsurprisingly, these relationships are maintained in patients with chronic kidney disease. Griva *et al* showed that cognitive impairment in dialysis patients is an independent predictor of mortality [158]. Their study followed

up 145 dialysis patients over a period of 7 years and found that cognitive impairment even in the absence of dementia and after correction for confounders was predictive of all cause mortality.

The reasons for the relationship between CKD and cognitive impairment are unclear but are likely to be multifactorial. Grimm *et al* [21] showed some improvement in brain function in HD patients when anaemia was treated with erythropoietin. They used a combination of neurophysiological (stimulus related evoked potentials) and neuropsychological (trail making) tests to assess brain function before and after the use of recombinant human erythropoietin. This finding was confirmed by another study carried out by Marsh *et al* [159], who again showed improvement in both neurophysiological and neuropsychological test results after anaemia had been corrected with erythropoietin. This effect may not be due to the reversal of anaemia alone as erythropoietin is known to have a wide range of other biological effects. These include effects on arterial blood pressure, vascular endothelium and the coagulation system [22]. It should also be noted that a recent widely publicised study (CHOIR) found that high target haemoglobins alongside high doses of erythropoietin can lead to an increase in cardiovascular events [29]. This study has led to changes in clinical practice with a lower accepted target range for haemoglobin in patients with renal failure.

Another factor proposed to have an effect on cognitive function is the presence of the non-protein amino acid homocysteine. A prospective observational study carried out by Seshadri *et al* found that there is a strong graded association between high

levels of homocysteine and the risk of developing dementia and Alzheimer's disease [160]. High levels of homocysteine have also been linked with CKD [161][162] and in HD patients, are associated with adverse cardiovascular outcomes [163]. Another factor which can affect cognitive function in older patients is depression [164]. However, in the analysis by Kurella *et al* [41], CKD was associated with worse cognitive function even when corrected for the higher prevalence of depression in patients with CKD. Another putative cause is cerebrovascular disease. Seliger *et al* [23] have shown that dialysis patients are at a much higher risk of stroke compared to a member of the general population. Comorbidities with a high prevalence in CKD such as hypertension [165] and diabetes [166] have also independently been linked to poorer cognitive function. These are likely to be related the common vascular pathologies, and whilst the debate about the two way relationship between vascular disease and renal dysfunction continues [24] a similar argument may be made for vascular disease and cognition. Our study has proposed that cerebral microembolisation may also have a role to play in cognitive decline.

Cognitive ability is made up of different components including episodic memory, long term memory, learning and executive function. Different illness processes can affect different facets of cognitive function; for example Alzheimer's disease typically first affects episodic memory before going on to affect other aspects of cognitive function [167]. This can be explained by considering the underlying neuroanatomical correlation between the areas of the brain affected and their function. In Alzheimer's disease, initial neuronal loss from the temporal lobes affects memory before the damage becomes more widespread and goes on to affect other aspects of cognition.

If cerebral microembolisation does occur during HD, then the pathology seen could be predicted to mimic that seen with cerebral small vessel disease. This term refers to a pattern of clinical and cognitive abnormalities related to pathology of the small arteries and arterioles supplying the brain [168]. Pathology in the vessels that penetrate from the pial surface into deeper brain matter leads to lacunar infarcts, axonal loss, demyelination and periventicular white matter degeneration [169]. When these changes are seen on imaging with computed tomography (CT) or magnetic resonance, they are referred to as leukoaraiosis, from the Greek terms "leuko" (white) and "araiosis" (rarefaction, or reduction in density) [170]. There are a variety of causes of cerebral small vessel disease but the most common are atherosclerosis and lipohyalinosis. Atherosclerosis is a process that involves the hardening of the arteries with the deposition of fat, cholesterol and other material that form plaques. Lipohyalinosis is a process of vessel wall destruction characterised by mural foam cells and fibrinoid necrosis. Other causes of cerebral small vessel disease are thought to include vasculitis, infection, hypoperfusion and embolisation [168]. If cerebral microembolisation does occur during HD, then it is likely to lead to pathology by causing cerebral small vessel disease.

Studies have shown strong links between cerebral small vessel disease and cognitive decline in different patient populations including the elderly [171][172]. Imaging studies have suggested that even asymptomatic members of the population have lesions visible on magnetic resonance imaging that are related to small vessel disease and that these lesions also correlate with levels of cognitive function [173].

Cerebral white matter changes detectable on MRI scanning have also been linked to increased rates of cognitive decline [174]. The pattern of cognitive impairment linked to cerebral small vessel disease is different to that found in Alzheimer's disease [175] with deficits in episodic memory being much less important.

Cognitive testing can be used to selectively measure components of cognitive function such as executive function [176]. Many of the cognitive tests in use in clinical practice have been developed for and used primarily in the context of Alzheimer's disease. One good example is the mini-mental state examination (MMSE), which was originally developed in the 1970's [153]. As we have described above, the disease process in Alzheimer's disease is distinct from that of small vessel disease and it is not surprising that these cognitive tests, with their emphasis on memory are not ideal for assessing executive function and can fail to detect even gross deficits [177]. Classical tests of executive function are extremely time consuming and for this reason, shorter batteries of tests have been developed to measure executive function and its decline.

One of the more commonly used methods was a series of tests that was demonstrated and validated by O'Sullivan *et al* [178]. This battery of tests consisted of: trail making (B-A); digit symbol; digit span backwards; FAS verbal fluency. The trail making test was included to measure set shifting and mental flexibility [179]. The digit symbol test measures both IQ and executive function [180]. It also tests certain aspects of set switching. Digit span backwards tests working memory, an important aspect of executive function [181]. FAS verbal fluency assesses the ability

to generate words [182]. Executive function is an important part of this test as strategies have to be developed to enable word retrieval. They showed these tests to be excellent at discriminating between patients with cerebral small vessel disease and a control group without cerebral small vessel disease. As we wished to look for evidence of cognitive decline caused by microembolisation leading to cerebral small vessel disease, we chose to use this same battery of tests in our own patients.

When analysing the results of batteries of different cognitive function tests, some research groups have chosen to combine the results from multiple tests to give a single score. In an example of this, Newman et al carried out a longitudinal assessment of neurocognitive function in patients undergoing cardiothoracic surgery [183]. During the study, they used the Randt Memory Test, Digit Span Test, the Benton Revised Visual Retention Test, the Digit Symbol Test and the Trail Making Test (B). They then carried out factor analysis with orthogonal rotation and summated the resultant scores into a single continuous measure of cognitive function. This method has the advantage of reducing the number of comparisons and so minimises the risk of a type I error. The major disadvantage of this method is that changes across a range of domains of cognitive function are reduced to a single measure. So for example it is then not possible to separate changes in visual memory from changes in executive function. In our own study, different aspects of executive function would be reduced to a single measure. Another disadvantage of this method is that if a patient is unable to complete one of the cognitive tests in the battery, statistical methods have to be used to estimate a score for that patient to still allow a single continuous measure of cognitive function to be generated.

Due to the disadvantages listed above, we chose to analyse the cognitive test results in our study on an individual basis. It should also be noted that the results of the particular battery of cognitive tests first published by O'Sullivan et al, and used by us during our work, were not combined into a single score in the original paper. No other research group using this battery of tests has attempted to amalgamate the different tests into a single score. Any such score would therefore be difficult to validate.

Another important aspect of cognitive testing is in the definition of a clinically significant decrease in function. The study by Newman et al quoted above chose to define a significant fall in cognitive function as 1 standard deviation (or around 20%) from the baseline figure [183]. There is no universally accepted method of defining clinically significant cognitive decline, even in cardiothoracic surgical patients. Jensen et al carried out a study comparing cognitive decline in conventional versus off-pump coronary artery bypass surgery [184]. They tested cognitive function using 7 separate tests and defined significant decline in cognitive function differently in each of the tests. Similarly in the field of CKD, there is no universal definition of significant cognitive decline. Research groups in CKD have instead studied rates of change in patients with CKD and compared them to patients without CKD. For example, Bossola et al compared rates of cognitive decline between those on haemodialysis and elderly patients [152]. The battery of tests used by O'Sullivan et al also includes no definition of a clinically significant decline [178].

As no clear definition for a significant decline exists, we chose instead to focus on the differences in rates of decline between different groups. Rather than a "clinically significant" decline in cognitive function, our study aims to objectively measure cognitive function and explores patterns of change over time, in the presence and absence of PFO.

Studies looking specifically at cognitive function in patients undergoing HD treatment have suggested that the time at which the testing is carried out has a large impact on the result [185]. Further work by Murray *et al* using tests both of verbal fluency and executive function showed that HD patients perform best in cognitive testing either just before or 1 day after dialysis [44]. It was important not to carry out the cognitive testing during dialysis as patients can experience confusion induced by cerebral ischaemia (commonly referred to as dialysis disequilibrium). For this reason, when carrying out cognitive testing on the HD patients in our study we chose to do so just prior to HD. This relationship to the dialysis cycle was maintained in both the baseline cognitive testing and during the follow up cognitive testing at 1 year.

Aims

The primary aim of this part of the project was to assess the impact of the presence of a PFO on cognitive function in patients undergoing haemodialysis over 1 year by comparing them to patients without a PFO. Our hypothesis was that patients with a PFO undergoing haemodialysis will have a greater rate of cognitive decline than

those without a PFO. A secondary aim was to compare rates of change in cognitive function between all patients undergoing haemodialysis against those undergoing peritoneal dialysis.

Method

The process of recruitment into this study has already been described (see chapter 2). Cognitive testing at baseline using FAS verbal fluency, digit recall backwards, digit symbol, and trail-making (A and B) was carried out soon after recruitment. An outline of the tests is given below whilst the full tests can be found in appendix 1. We carried out cognitive function testing on 80 patients, 51 of whom were having HD and 29 of whom were having PD. All patients carried out the baseline cognitive assessment but 3 of these patients (1 from the HD group and 2 from the PD group) were unable to carry out the "digit symbol" and "trail making" parts of the test due to poor vision. Unfortunately 5 patients (3 in the HD group and 2 in the PD group) died and 1 patient left the country during the 1 year after initial testing and therefore follow up cognitive function testing was carried out on 47 HD and 27 PD patients. All tests were carried out in a quiet side-room.

Digital symbol test (5 minutes)

Each digit from 1-9 is given a unique symbol which is given to the patient as a key. After completing a practice section to familiarise themselves with the key, patients are given a series of numbers and are asked to complete the corresponding symbols

in the spaces below. Patients are given 90 seconds to convert as many numbers into symbols using the key as possible.

FAS verbal fluency (5 minutes)

The participant is asked to name as many different words beginning with particular letters (F, A and S) in 1 minute. The patient is not allowed to use proper nouns and is also prohibited from using the same word with a different ending (for example "fly" and "flying"). The score over the 3 minutes is summated.

Digit span backwards (5 minutes)

Participants are asked to listen to a list of random numbers that are read out to them at a rate of 1 per second and then asked to recall them in reverse order. Initially just 2 numbers are read out, then 3 numbers, and then 4 numbers and so on until the patient makes a mistake. Their score is the maximum number of correct numbers that they successfully recall backwards. All numbers were chosen using "Hotbits" – a computer program that uses radioactive decay to generate random numbers [186].

Trail making B-A (5 minutes)

In part A of the test, the participant is asked to join the numbers in circles in ascending order starting at 1 and finishing at 25. The time they take to do this is measured. In part B of the test, the circles contain both numbers and letters going from 1-13 and A-L. The participant is asked to join letters and numbers in ascending order whilst switching between number and letter (so 1-A, 2-B, etc). The time taken to completion is again measured. The score given to the patient is then calculated by subtracting the time taken for part A from the time taken for part B. The reason

for this subtraction is to try to remove the effects of physical ability (vision, speed of

drawing, etc) and try to isolate executive function. It is possible to do this because

"Trails B" is a more specific test of executive function than "Trails A".

A repeat assessment of cognitive function involving the same battery of 4 tests was

carried out in identical fashion 1 year following the initial test.

Results

The histograms below (figures 14-17) show the distribution of data from the 4

cognitive function tests at baseline and follow up at 1 year in all of our patients.
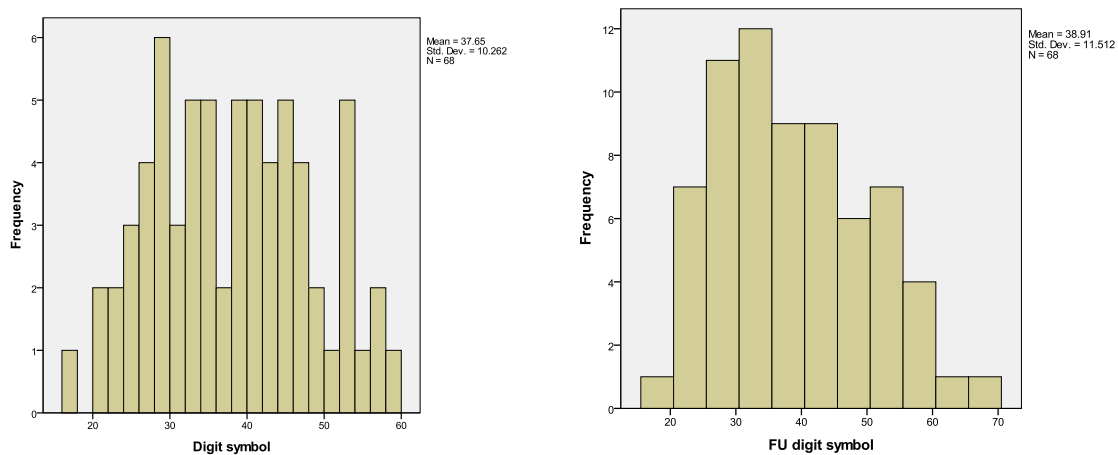
Figure 14 - Results of digit symbol test at baseline and at 1 year follow up

Chapter 5 (Dialysis, PFO and cognitive function)
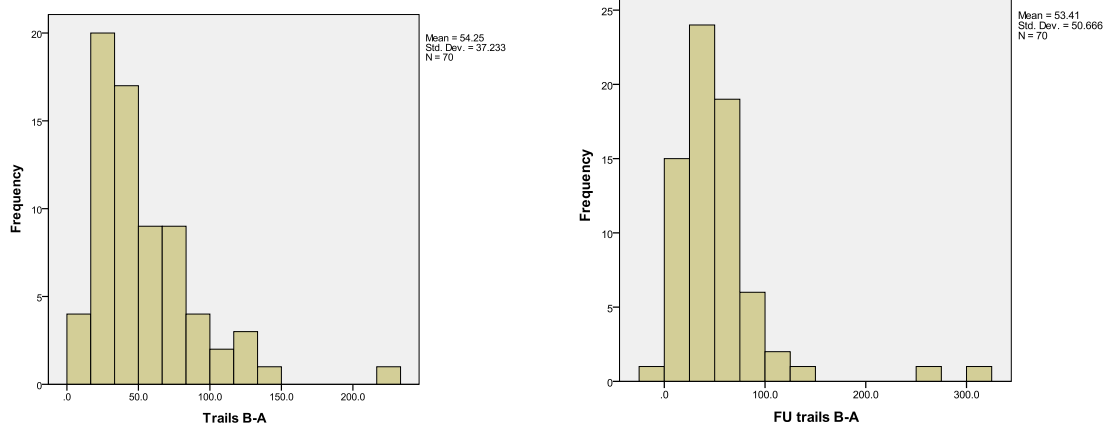
Figure 15 - Results of Trails B-A at baseline and 1 year follow up
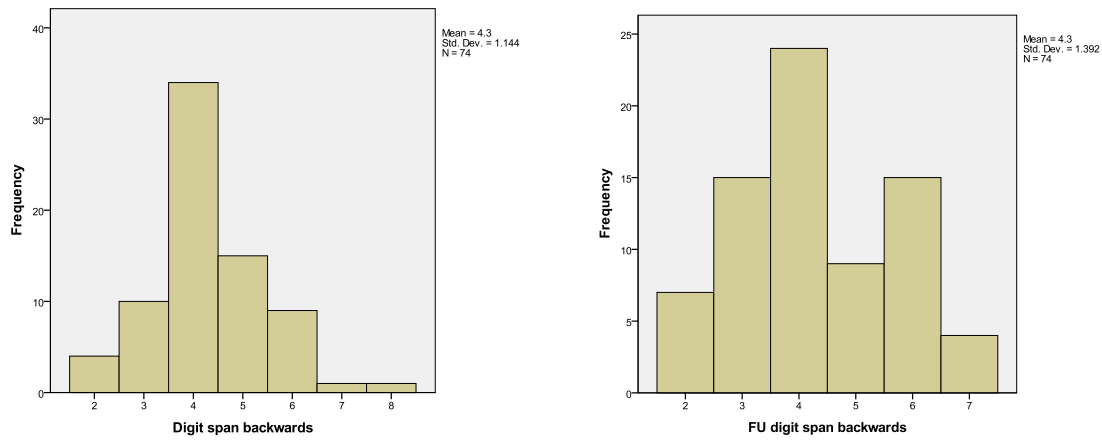
Figure 16 - Results of digit span backwards at baseline and at 1 year follow up
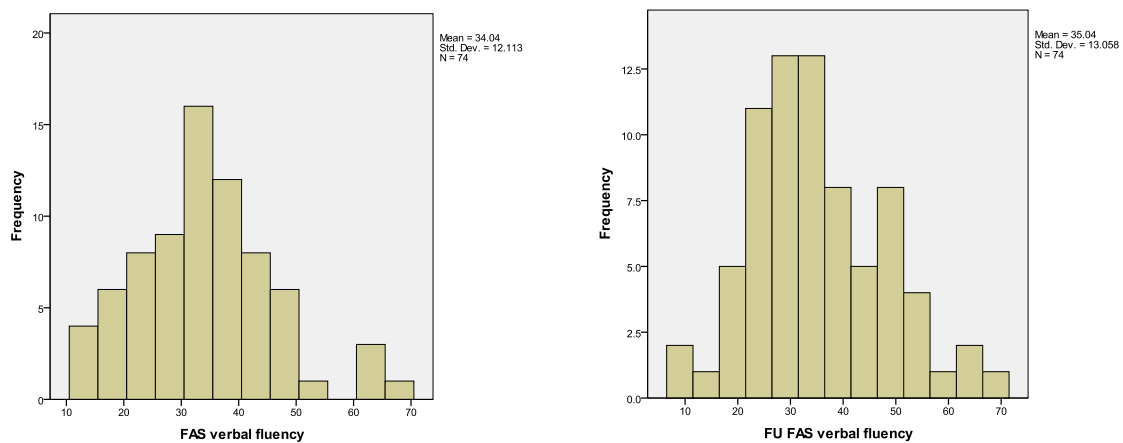
Figure 17 - Results of FAS verbal fluency at baseline and at 1 year follow up

Shapiro-Wilk testing showed that the majority of data were not normally distributed.

Tables 13 and 14 show the median and standard range of the cognitive test results at baseline and at the 1 year follow up.

Table 10 - Results of cognitive function tests at baseline

	Digit Symbol	Trails (B-A)	Digit Span Backwards	FAS Verbal Fluency
N	68	70	74	74
Median	38.00	44.20	4	33.5
Range	17 to 59	9.4 to 223.1	2 to 8	13 to 70

Table 11 - Results of cognitive function tests at 1 year follow up

	Digit Symbol	Trails (B-A)	Digit Span Backwards	FAS Verbal Fluency
N	68	70	74	74
Median	36.50	42.05	4.00	33.00
Range	18 to 70	-0.3 to 322.2	2 to 7	9 to 70

We then carried out an analysis to compare the results of the baselines scores with the follow up scores in each patient. To analyse paired data, we used the Wilcoxon test. The results of this can be seen in table 15.

Table 12 - Wilcoxon test of cognitive function tests at baseline and follow up at 1 year in all patients

Cognitive test	Significance (Wilcoxon)
Pair 1 Digit Symbol	0.28
Pair 2 Trails (B-A)	0.30
Pair 3 Digit Span Backwards	0.94
Pair 4 FAS Verbal Fluency	0.26

We then looked at cognitive function test results at baseline and 1 year follow up separately in the PFO group and the non PFO group. Table 16 shows the baseline and 1 year follow up cognitive function test results in those with a PFO whilst table 17 gives the same information in those without a PFO.

Chapter 5 (Dialysis, PFO and cognitive function)

Table 13 - Baseline and follow up cognitive test results in patients with a PFO

		Baseline (Median, range)	1 year f/u (Median, range)	P value Wilcoxon
Digit symbol	All patients (n=17)	38.00 (24-53)	35.00 (21-54)	0.44
	HD patients (n=12)	33.50 (24-49)	30.50 (21-54)	0.92
	PD patients (n=5)	40.00 (36-53)	41.00 (35-46)	0.34
Trails B-A	All patients (n=17)	44.00 (9.4-133.2)	64.10 (8.3-116.6)	0.06
	HD patients (n=12)	70.25 (9.4-133.2)	66.85 (8.3-116.6)	0.08
	PD patients (PFO) (n=5)	43.20 (38.3-76.0)	35.80 (19.0-79.2)	0.69
Digit span back-wards	All patients (PFO) (n=17)	4.00 (2-5)	4.00 (2-6)	0.67
	HD patients (PFO) (n=12)	4.00 (2-5)	3.00 (2-6)	0.49
	PD patients (PFO) (n=5)	4.00 (2-5)	4.00 (2-5)	0.66
FAS verbal fluency	All patients (PFO) (n=17)	32.00 (15-50)	28.00 (13-51)	0.53
	HD patients (PFO) (n=12)	31.50 (15-50)	27.50 (13-51)	0.43
	PD patients (PFO) (n=5)	32.00 (19-43)	32.00 (17-51)	1.00

Table 14 - Baseline and follow up cognitive test results in patients without a PFO

		Baseline (median, range)	1 year follow up (median, range)	P value
Digit symbol	All patients (n=51)	38.00 (17-59)	37.00 (18-70)	0.23
	HD patients (n=32)	37.00 (17-59)	36.50 (23-70)	0.15
	PD patients (n=19)	40.00 (20-56)	37.00 (18-59)	0.90
Trails B-A	All patients (n=53)	42.60 (15.1-223.1)	41.40 (-0.3-322.2)	0.85
	HD patients (n=33)	34.40 (15.1-223.1)	34.30 (-0.3-322.2)	0.71
	PD patients (n=20)	47.70 (15.7-109.0)	47.00 (15.8-265.9)	0.74
Digit span backwards	All patients (n=57)	4.00 (2-8)	4.00 (2-7)	0.58
	HD patients (n=35)	4.00 (2-8)	5.00 (2-7)	0.36
	PD patients (n=22)	4.00 (2-6)	4.00 (2-7)	0.83
FAS verbal fluency	All patients (n=57)	35.00 (13-70)	34.00 (9-70)	0.59
	HD patients (n=35)	36.00 (14-64)	36.00 (10-65)	0.32
	PD patients (n=22)	32.50 (13-70)	33.00 (9-70)	0.64

We carried out a Mann-Whitney-U test to look specifically at the differences in the change in cognitive function between baseline and follow up at 1 year between those with and without a PFO. Table 18 shows the change in cognitive function in all patients, HD patients and PD patients.

Table 15 - Change in cognitive function in those with and without a PFO

		Change from baseline (median, range)		Mann-Whitney-U
		PFO	No PFO	P value
Digit symbol	All patients	-1.00 (-10.0 to 9.0)	1.00 (-12.0 to 21.0)	0.20
	HD patients	-0.50 (-8.0 to 9.0)	1.00 (-12.0 to 21.0)	0.29
	PD patients	-1.00 (-10.0 to 1.0)	0.00 (-11.0 to 10.0)	0.31
Trails B-A	All patients	-6.60 (-60.8 to 25.4)	-1.20 (-50.8 to 180.0)	0.16
	HD patients	-5.80 (-60.8 to 10.8)	-3.40 (-44.7 to 99.1)	0.17
	PD patients	-8.20 (-19.3 to 25.4)	3.10 (-50.8 to 180.0)	0.73
Digit span backwards	All patients	0.00 (-2.0 to 2.0)	0.00 (-3.0 t 2.0)	0.50
	HD patients	-0.50 (-2.0 to 2.0)	0.00 (-3.0 to 2.0)	0.28
	PD patients	1.00 (-1.0 to 1.0)	0.00 (-3.0 to 2.0)	0.70
FAS verbal fluency	All patients	1.00 (-23.0 to 9.0)	0.00 (-14.0 to 17.0)	0.82
	HD patients	1.00 (-23.0 to 9.0)	1.00 (-10.0 to 17.0)	0.97
	PD patients	0.00 (-4.0 to 8.0)	-1.00 (-14.0 to 9.0)	0.68

We also looked at the effect of the type of dialysis on the change in cognitive function. Table 19 shows the baseline results and the change over the follow up period for patients on HD and those on PD.

Table 16 - Effect of type of dialysis on cognitive function

	HD patients (Median, range)	PD patients (Median, range)	P value (Mann-Whitney-U)
Digit Symbol			
Baseline	36.00 (17 to 59)	40.00 (20 to 56)	0.16
Change at 1 year	1.00 (-12.0 to 21.0)	-0.50 (-11.0 to 10.0)	0.34
Trails (B-A)			
Baseline	41.30 (9.4 to 223.1)	44.00 (15.7 to 109.0)	0.79
Change at 1 year	-5.00 (-60.8 to 99.1)	2.80 (-50.8 to 180.0)	0.30
Digit Span Backwards			
Baseline	4.00 (2 to 8)	4.00 (2 to 6)	0.40
Change at 1 year	0.00 (-3.0 to 2.0)	0.00 (-3.0 to 2.0)	0.91
FAS Verbal Fluency			
Baseline	36.00 (14 to 64)	32.00 (13 to 70)	0.65
Change at 1 year	1.00 (-23.0 to 17.0)	-1.00 (-14.0 to 9.0)	0.27

We then looked at the number of individual patients in each category who had a worsening in their cognitive function in the follow up period. After dichotomising the data by categorising each patient at follow up as "decline" or "no decline", we looked for significance between the different groups using a Chi-square analysis (table 20). "Decline" was defined as any worsening of performance on the cognitive function test at follow-up compared to the baseline result.

Table 17 - Number of HD and PD patients showing decline in cognitive function and relationship to presence of PFO

Dialysis Type			Digit symbol		Pearson chi square
			No decline	Decline	
HD		No PFO	22	10/32	
		PFO	6	6/12	
		All	28	16/44	0.250
PD		No PFO	10	9/19	
		PFO	2	3/5	
		All	12	12/24	0.615
All		No PFO	32	19/51	
		PFO	8	9/17	
		All	40	28/68	0.255
			Trails B – A		Pearson chi square
			No decline	Decline	
HD	PFO	No PFO	20	12/32	
		PFO	8	4/12	
		All	28	16/44	0.798
PD	PFO	No PFO	8	12/20	
		PFO	3	2/5	
		All	11	14/25	0.420
All	PFO	No PFO	28	24/52	
		PFO	11	6/17	
		All	39	30/69	0.433
			Digit span backwards		Pearson chi square
			No decline	Decline	
HD	PFO	No PFO	26	9/35	
		PFO	6	6/12	
		All	32	15/47	0.119
PD	PFO	No PFO	16	6/22	
		PFO	3	2/5	
		All	19	8/27	0.574
All	PFO	No PFO	42	15/57	
		PFO	9	8/17	
		All	51	23/74	0.138
			FAS verbal fluency		Pearson chi square
			No decline	Decline	
HD	PFO	No PFO	22	13/35	
		PFO	8	4/12	

Chapter 5 (Dialysis, PFO and cognitive function)

	Total		30	17/47	0.813
PD	PFO	No PFO	10	12/22	
		PFO	3	2/5	0.557
	Total		13	14/27	
All	PFO	No PFO	32	25/57	
		PFO	11	6/17	0.530
		All	43	31/74	

Figure 18 shows the percentage of patients with a decline in one or more cognitive function tests over the test period. Figure 19 shows cognitive decline by PFO status and dialysis modality.
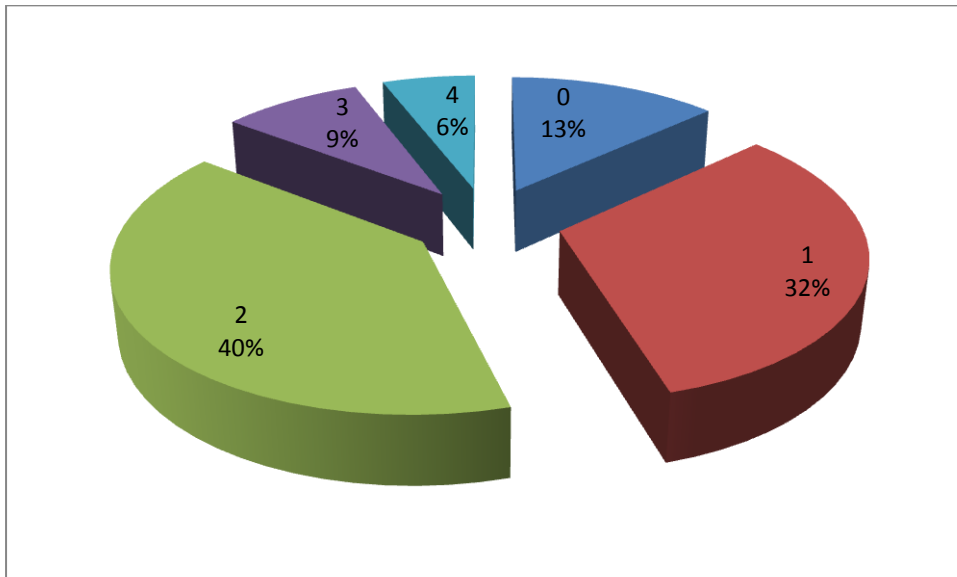
Figure 18 - Percentage of subjects showing cognitive decline across all tests. 0-4 represents no decline to decline in all four cognitive tests.
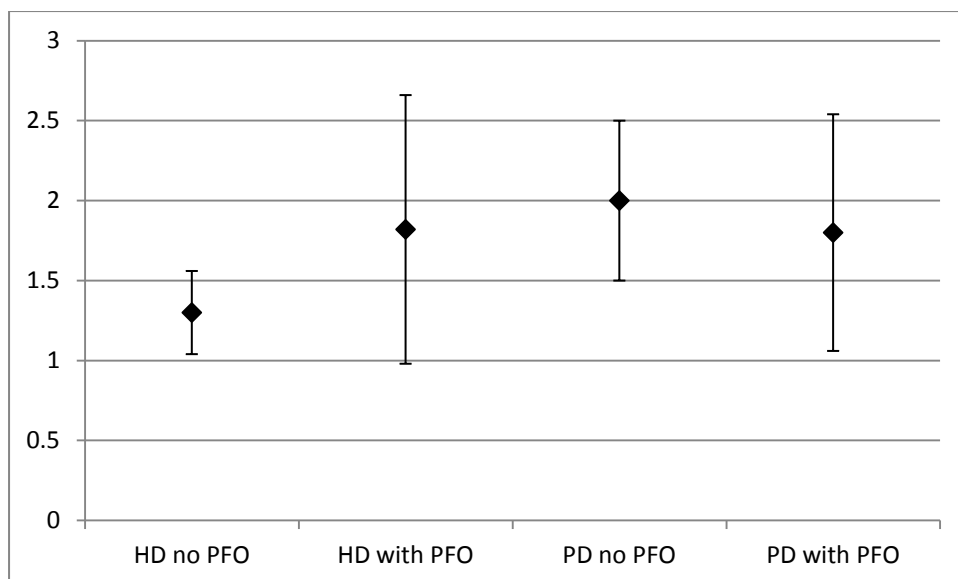
Figure 19 - Overall decline in cognitive function over all 4 cognitive tests

Finally, we carried out an analysis comparing the patients who had died during the

follow up period against patients who had survived. We looked for any differences in

age, dialysis vintage and baseline cognitive function (table 21).

Table 18 - Comparison between patients who died and those who survived

	Alive	Dead	P value (Mann Whitney-U)
N	74	5	
Age (median, range) (y)	63.00 (21 to 84)	75.00 (65 to 83)	**0.01**
Dialysis vintage (median, range) (m)	20.00 (2 to 402)	9.00 (3 to 53)	0.39
Digit symbol (median, range)	38.00 (17 to 59)	38.00 (22 to 43)	0.40
Trails B-A (median, range)	44.20 (9.4 to 223.1)	50.00 (38.0 to 88.0)	0.55
Digit span backwards (median, range)	4.00 (2 to 8)	4.00 (3 to 7)	0.77
FAS verbal fluency (median, range)	33.50 (13 to 70)	35.00 (16 to 54)	0.81

Discussion

Our investigation found no change in cognitive function between the assessments carried out at baseline and the follow up assessments carried out at 1 year in our patients. The histograms featured in figures 14-17 and table 12 (Shapiro-Wilk test) shows that the majority of the data are not normally distributed. A Wilcoxon test showed that there was no statistically significant change between baseline and follow up at 1 year in any of the 4 tests of cognitive function that we used (table 15). Analysis of the cohorts of HD and PD patients show that there is no difference even when these groups are analysed separately.

We then went on to look at the cohorts of patients with a PFO (table 16) and patients without a PFO (table 17). Neither of these groups showed any significant changes in their cognitive test results between baseline and 1 year. Of particular interest to us was the cohort of HD patients who had a PFO. It was our expectation that these patients would have more marked cognitive decline, especially in tests of executive function when compared to the cohorts of patients without a PFO. However, even when the cohort of HD patients with a PFO was analysed separately, they showed no significant changes in cognitive function at the end of 1 year. We carried out a Mann-Whitney-U test to look specifically at the differences in the change in cognitive function between baseline and follow up at 1 year between those with and without a PFO (table 18). This showed that the presence of a PFO makes no significant difference to change in cognitive function. This finding applied both to PD patients (where we would not have expected to find any effect from a PFO) and to HD patients (where we did expect to see an effect).

We also looked separately at the effect of the type of dialysis on cognitive function.

We started by looking at the baseline performance in cognitive function tests by the

HD and PD patients and then at the change in cognitive function over 1 year (table

19). We used a Mann-Whitney-U test to compare the performance of these two

cohorts at baseline. There were no significant differences between the 2 groups at

baseline. Using the same test, there were also no significant differences in the rate

of change over 1 year.

Although there were no changes in the median cognitive function test results in the

differences between baseline and follow up in the different groups, there had been

deterioration in the performance of the tests by individual patients. This can be seen

clearly when the data are individually dichotomised to "no decline" versus "decline"

(table 20). This shows that across both the HD and PD patients, there were a large

number who performed worse at follow up across the 4 different cognitive function

tests. When a chi-square analysis was carried out to look for a difference in the

number of patients with decline between different groups, we found no statistically

significant differences.

Although we did not find any decline in median cognitive function test scores, we did

find that when patients were individually analysed 87% of all dialysis patients

showed some worsening of their cognitive function (figure 18). This was not

necessarily associated with the presence of a PFO, even in the HD cohort (figure

19). The decline in cognitive function over a relatively short period is worrying but

consistent with previous data showing a significant deterioration in cognitive function

as measured by mini-mental state examination (MMSE) even over a period of 1 year

[152]. This is very important as for many patients home dialysis therapies (HD or

PD) are an attractive option, are more cognitively demanding, and may limit or place

at risk the duration of such therapy. Very few (if any) units formally test patients

formally for cognitive decline to ensure that they are capable of continuing to perform

their own therapy safely.

Finally, we compared the characteristics of the 5 patients who died during the follow

up period against the 74 patients who survived (table 21). This showed that there

was a significant difference in the age of the patients who died compared to the

patients who survived (median 63 years versus 75 years). There were however no

differences in the dialysis vintage (median 20 months versus 9 months). There were

also no differences at baseline in the results of any of the 4 cognitive function tests.

This is surprising as we would have expected the patients who went on to die over

the follow up period to have worse baseline cognitive function than those who

survived.

One explanation for these results is that our proposed theory, that cerebral

microembolisation occurs during HD as a result of crossing a PFO, is false. If

cerebral microembolisation does not occur during HD in patients with a PFO then

there is no reason to expect that their cognitive function will decline more rapidly

than those without a PFO. This explanation would be concordant with the results of

our own investigation using TCD scanning where we found no evidence of cerebral

microembolisation. It would however not fit with the previously published literature

which has identified carotid embolisation during HD [145].

Another explanation for the lack of difference in decline in cognitive function between

those with and without a PFO could be if it was the case , as suggested by other

groups [145], that cerebral microembolisation occurs in all HD patients regardless of

the presence or absence of a PFO due to incomplete clearance of microemboli by

the pulmonary bed. This would explain the lack of a difference in change of

cognitive function between those HD with and without a PFO. However if cerebral

microembolisation does occur in all HD patients, then we would have expected that if

microembolisation does have a clinical effect then these patients would have had a

greater decline in their cognitive function than the patients undergoing PD. As we

did not find this, it suggests either that cerebral microembolisation does not occur

during HD or that even if it does occur; it has no effect on cognitive function.

Another reasonable explanation for the lack of change in cognitive function is that we

simply did not follow up these patients for a long enough period of time. Patients

with chronic renal failure may be on renal replacement therapy with PD or HD for

many years and it may be that 1 year was not long enough to detect deterioration in

cognitive function. It was not possible within the timescale of our research project to

follow up these patients over a longer period but it is possible that follow up over

several years may have led to significant changes in cognitive function. As

previously stated however, Bossola *et al* did show significant deterioration in

cognitive function as measured by MMSE in HD patients over a period of 1 year

[152].

Chapter 6 (Continuous renal replacement therapy and microembolisation)

Background

Thus far we have considered patients with chronic renal failure who required HD or PD. Another group of patients who require renal replacement therapy are those who suffer an acute kidney injury. Acute kidney injury has now replaced the phrase acute renal failure and is characterised by a rapid reduction in kidney function resulting in a failure to maintain fluid, electrolyte and acid-base balance. Formal guidelines on the definition of acute kidney injury were published by a network of international experts from the fields of nephrology and intensive care and became known as the RIFLE definition and staging system for acute kidney injury (table 22) [187].

Table 19 - RIFLE staging system for acute kidney injury

	GFR criteria	Urine output criteria	
Risk	Increased serum creatinine x 1.5 or GFR decrease > 25%	UO < 0.5ml/kg/hr over 6 hours	High sensitivity
Injury	Increased serum creatinine x 2.0 or GFR decrease > 50%	UO < 0.5ml/kg/hr over 12 hours	
Failure	Increased serum creatinine x 3.0 or GFR decrease > 75% or serum creatinine > 4mg/dl	UO < 0.3ml/kg/hr over 24 hours or anuria for >12 hours	High specificity
Loss	Persistent acute renal failure = complete loss of kidney function > 4 weeks		
ESKD	End stage kidney disease over 3 months		

This was later updated by another international multi-specialty collaborative group, "Kidney Disease: Improving Global Outcomes (KDIGO)" who produced a staging system that is anticipated to be adopted globally (tables 23 and 24) [188]. They define and grade acute kidney injury as follows:

Table 20 - KDIGO definition of acute kidney injury

Acute kidney injury	-Increase in serum creatinine by >0.3mg/dl within 24 hours; or -Increase in serum creatinine to > 1.5 times baseline which is known or presumed to have occurred within the last 7 days; or -Urine output < 0.5ml/kg/h for 6 hours

Table 21 - KDIGO staging of acute kidney injury

Stage	Serum creatinine	Urine output
1	1.5-1.9 times baseline or >0.3mg/dl increase	<0.5ml/kg/h for 6-12 hours
2	2.0-2.9 times baseline	<0.5ml/kg/h for > 12 hours
3	3.0 times baseline or increase in serum creatinine to >4.0mg/dl or initiation of renal replacement therapy or in patients <18 years, decrease in eGFR to <35ml/min per 1.73m^2	<0.3ml/kg/h for > 24 hours or anuria for >12 hours

Acute kidney injury is a major cause of both morbidity and mortality in critically ill patients [189]. Up to 25% of critically ill patients may develop acute kidney injury and two thirds of these patients may require renal replacement treatment [189]. There are no proven treatments that reverse acute kidney injury and the treatment remains largely supportive [190]. CVVH is an alternative temporary form of renal replacement therapy most commonly used in the intensive care units of most hospitals. It is used almost exclusively in the treatment of acute kidney injury in very unwell patients. The process of CVVH provides support by enabling removal of waste products found in the blood using convection and a semi-permeable membrane where a pressure gradient drives the removal of water and a concentration gradient drives removal of waste products. A double or triple lumen central venous catheter is used both to remove unfiltered blood and to return filtered blood. Blood flow rates tend to be between 100-200ml/min (much slower than HD). CVVH therapy therefore requires the patient to be treated for longer periods of time than HD and is usually continuous. There is some evidence that use of CVVH rather than intermittent HD in acute kidney injury results in fewer patients becoming dependent on long term renal replacement therapy [191]. The authors speculate

that this may be due to the ability to maintain tighter controls of fluid balance using haemofiltration.

As in HD, CVVH involves removal of blood from the body and treatment in an external machine prior to return of the blood to the circulation. CVVH machines have similar air-traps and filters to HD machines to prevent thrombi from being formed and returned to the body. Surprisingly, no published research has been carried out to establish whether CVVH also creates clinically detectable microemboli. It is therefore unknown whether microemboli are created during the process and whether these microemboli can cross from the venous into the arterial circulation and be detected in the cerebral circulation during CVVH. However, it has been established that microemboli can be detected using ultrasound in the subclavian vein of patients undergoing HD [49].

Objectives

The primary purpose of the research was to assess whether treatment of acute renal failure with CVVH on the intensive care unit results in cerebral microembolisation that is detectable during ultrasound scanning of the MCA.

Hypothesis

The null hypothesis was that CVVH does not create microemboli that can be detected in the cerebral circulation using ultrasound. The alternative hypothesis was

that microemboli are created by CVVH and that these can be detected using TCD scanning.

Method

Study type

This was a single centre cohort study.

Study population and sample size

The study involved patients with acute renal failure who require CVVH treatment on the intensive care unit. The inclusion and exclusion criteria are set out below. This was a pilot study with the aim of recruiting 15 participants into the study.

Recruitment

Patients requiring CVVH were identified by a specialist research nurse working on the intensive care unit at Royal Sussex County Hospital, Brighton, United Kingdom. A mental capacity assessment was carried out by Dr George and a member of the intensive care team. If the patient had mental capacity to consent, they were given a participant information sheet (see appendix 4) and given an opportunity to ask questions. They were allowed as long as they felt they needed to decide if they wanted to take part in the study but were given a minimum of 24 hours. If a patient did not have capacity to consent to take part in the study, then we approached the next of kin to act as a consultee. This person was given a consultee information sheet and also given an opportunity to ask questions about the study. If either the

patient or the consultee expressed any wish not to be included in the study then they were not contacted further. Written consent was obtained before patients were entered into the trial (see appendix 5).

Inclusion Criteria

Patient with acute renal failure having CVVH

Aged over eighteen

Exclusion criteria

Chronic renal failure having dialysis treatment

Mechanical heart valve

Study conduct

Following recruitment, participants had their medical notes screened by a specialist research nurse to ensure that they fulfilled the inclusion and exclusion criteria set out above. Participants who met the criteria were enrolled in the study. All patients recruited into the study underwent CVVH using a Baxter Aquarius (Illinois, USA) machine with an Aquamax HF 12 filter as part of their normal care. Participants in the study had ultrasound monitoring of their right MCA using a 2 MHz TCD probe (ST3, Spencer Technologies, Seattle, USA). The right MCA was identified in the temporal window using pulsed wave Doppler by arterial flow towards the handheld

TCD probe located at a depth of less than 55mm. Again, signals were identified as microemboli if they conformed to the criteria previously specified by Ringelstein *et al* [132] with all recordings stored digitally. The MCA was monitored for 10 minutes whilst on CVVH as part of the patient's normal care. Later, when the patients were no longer having CVVH, they had a further 10 minutes of monitoring of their right MCA. The number of microembolic signals detected was recorded on each occasion using signal processing software from Spencer Technologies (Seattle, USA).

Results

In total 17 patients were recruited into the study. It was impossible to identify the MCA using ultrasound in 1 of these patients and this patient was therefore excluded from the analysis of the results of the study. Of the remaining patients, 12 patients underwent TCD scanning during and after CVVH. 4 patients underwent TCD scanning during CVVH but died prior to being scanned again after haemofiltration. Patient characteristics can be seen in table 25 and the reasons for their admission in table 26.

Table 22 - Patient characteristics

Age in years (median) (range)	65.5 (41-86)
Male (%)	12 (75%)
Female (%)	4 (25%)

Table 23 - Reason for admission to intensive care unit

Primary cause of admission to ICU	Number of patients
Abdominal aneurysm repair	5
Sepsis	5
Hepatorenal syndrome	1
Coronary artery bypass grafting	1
Renal failure due to prostatic carcinoma	1
Pneumonia	1
Pulmonary oedema	1
Post surgery for cord compression	1

The numbers of microembolic signals detected during CVVH and after

haemofiltration are shown in the histograms below (figure 20).
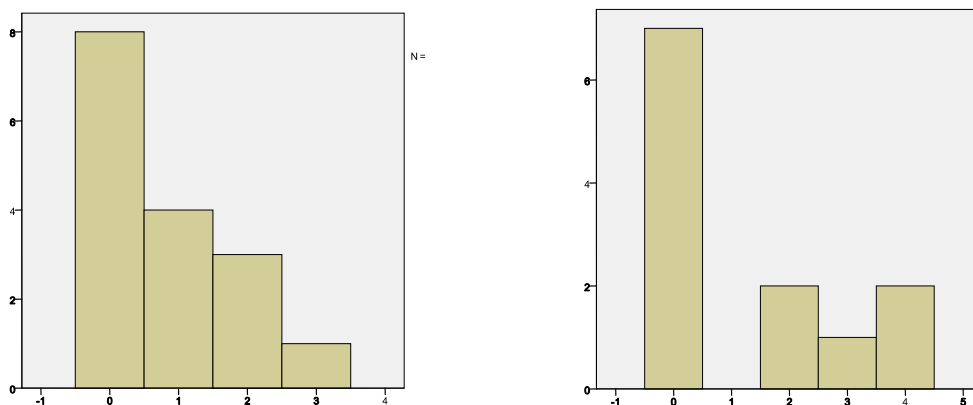
Figure 20 - Histograms showing the number of microembolic signals detected in the MCA during and after CVVH

Statistical analysis revealed that the data are not normally distributed.

Microembolisation was not detected either prior to or during CVVH

Table 27 – Microembolisation in the MCA on and off CVVH

Haemofiltration patients		
Microembolic signals in MCA whilst on CVVH (n=16) (Median, range)	Microembolic signals whilst not on HD (n=12) (Median, range)	P value (Wilcoxon)
0.50 (0 to 3)	0.00 (0 to 4)	0.47

Discussion

The results of our pilot study suggest that there is no evidence of detectable microembolisation in the MCA during CVVH treatment. We found no evidence of microembolisation either during a period on or a period off CVVH. During haemofiltration, the median number of microemboli detected in the MCA was 0.50 (range 0 to 3). Off CVVH, the median number of microemboli detected in the MCA was 0.00 (range 0 to 4). There was no statistically significant difference between these rates using a Wilcoxon test. The reason for the very small number of hits in some patients both on and off CVVH is likely to be due to artefact with movement of either the probe or the patient during monitoring.

It is reassuring that significant numbers of microemboli were not detectable during CVVH in the MCA. It is possible that microemboli are simply not created by the process of CVVH. A review of the published literature reveals that there are no available studies that either confirm or refute this at the present time. Our own study

does not however exclude the possibility that microemboli are created during CVVH. It is possible that microemboli are created by CVVH but do not cross from the right sided venous circulation into the left sided arterial circulation. To completely exclude microemboli formation, it would be necessary to carry out a study where the draining venous system is monitored for microemboli during CVVH. This is likely to prove technically challenging as CVVH catheters are traditionally sited in or just above the right atrium and it would be difficult to monitor downstream of this catheter using ultrasound. It is possible that these are the difficulties which have prevented such a study from previously having been carried out.

Another explanation for the reason that microemboli were not detected in the MCA could be that in our small pilot study, we did not include any patients who were known to definitely have a PFO or other form of right-to-left shunt. To have detected a PFO in our patients, we would have had to carry out a transoesophageal echocardiogram (as a TTE without patient cooperation during Valsalva etc would not have been diagnostically reliable) and we did not feel this invasive procedure could be ethically justified. As previously stated, a large post-mortem study by Hagen et al found a prevalence of PFO of around 25% in the general population [55]. However there is no published research on the prevalence of PFO in a population of patients in the intensive care unit with an acute kidney injury. We would estimate that there were 3 patients in our cohort with a PFO but due to the small sample size, there may actually have been no patients with a PFO. Finally, it is acknowledged that as our earlier work involving HD patients with known PFO did not reveal microemboli in the MCA, it was unlikely that our work on a population of patients with unknown PFO

status undergoing a process (CVVH) which is not known to definitely produce

microemboli would have detected microembolic signals in the cerebral circulation.

Chapter 7 (Discussion)

The results from our first study using transthoracic echocardiography and bubble contrast suggests that the prevalence of PFO in patients with renal failure undergoing dialysis treatment is similar to that found in the general population. The prevalence of PFO in our group of patients with chronic renal failure undergoing dialysis was 21.25% (95% CI 13.71-31.42) whilst the prevalence found in the general population in a large post-mortem study was 27.3% [55]. Although there is no reason to have suspected that the prevalence of PFO in dialysis patients should be any higher than in the general population, it was important to establish that this was actually the case. The number of patients around the world undergoing dialysis is increasing year on year and if PFO was to be associated with pathology during dialysis, it would be important to have an accurate estimate of the size of the burden in this very select population. For example, prior to the studies on prevalence of PFO in patients with migraine [89], it was not known that there was an association between these conditions.

Our study did contain a number of limitations. Although transthoracic echocardiography with second harmonic imaging and bubble contrast is a sensitive method of identifying PFO [112][113][114], post mortem studies have a slightly higher sensitivity still. It is therefore possible that our study slightly underestimates the true prevalence of PFO in those patients undergoing dialysis treatment. We sought to minimise the risk of a false positive diagnosis of PFO by looking for evidence of contrast in the left atrium within 3 cardiac cycles but could not absolutely

exclude the presence of a pulmonary shunt. In vivo, TOE with bubble contrast is still considered by some groups to be the gold standard diagnostic method for the detection of PFO [192][119][193]. We chose TTE with bubble contrast as it has a high sensitivity and specificity in the diagnosis of PFO and does not require the use of intravenous sedation (making Valsalva more difficult) or entail the (admittedly low) risks associated with TOE.

Our study also aimed to quantify PFO shunt size by estimating the number of bubbles that were seen to cross from the right atrium into the left atrium. This can be difficult and there is also no standardised method of quantifying shunt size. Cabanes et al also demonstrated that there is significant inter-observer and intra-observer variability in the diagnosis of PFO and in the quantification of shunt size when 3 different echocardiographers reviewed 100 videotaped TOE contrast studies [194]. To further complicate matters, an elegant study by Devuyst et al showed that the shunt size as quantified by the number of microbubbles detected by TCD can be a function of the duration and strain pressure of the Valsalva manoeuvre as well as the physical size of the PFO [195]. They demonstrated that Valsalva manoeuvre with a target strain pressure of 40 mmHg was associated with the presence of a higher number of microembolic signals crossing a PFO and measured by TCD compared with a target strain pressure of 20 mmHg. This is not surprising as other groups have shown that the pressure gradient between right and left atria during provocational procedures is directly related to the level of target strain pressure [103].

Although we found a prevalence of PFO in the dialysis population that is similar to the general population, we did have an unusual distribution of PFO sizes with more patients than expected in the "large PFO" group and fewer than expected in the "small PFO" group. This was on the basis of numbers of bubbles seen to cross the inter-atrial septum. As can be seen above however, the numbers of bubbles are not always an accurate reflection of true anatomical PFO size. Our study may also have been stronger if rather than using historical echocardiography and post-mortem controls, we had carried out transthoracic echocardiography on a group of normal subjects to help validate our results in the dialysis patients.

Our subsequent investigations involving TCD ultrasound scanning of the MCA during HD showed no evidence of cerebral microembolisation into the MCA [196]. This is an unexpected result as other studies have shown evidence of microemboli both in the draining arteriovenous fistulae and in carotid arteries during HD [45] [49] [145]. It is intriguing that our results do not correlate fully with other published work. We have convincingly excluded technical issues with our equipment as a potential cause of this result. For example, monitoring of the MCA using the ST3 (Spencer Technologies) in 5 of the HD patients with a PFO (previously identified on TTE) during bubble contrast injection detected a large number of hits within seconds of bubble contrast injection. We were also able to confirm the result from previous studies that large numbers of microemboli are detectable in the arteriovenous drainage fistulae during HD. Further work is needed to investigate this apparent discrepancy in the context of the protocols used by different research groups, who have shown evidence of microemboli in the carotid circulation, looking at the cerebral

circulation during dialysis. The clear evidence from our study, however, is that

cerebral microembolisation does not occur during HD

One criticism of our work may be that we failed to monitor the MCA for a sufficient

period of time with TCD. Our methodology specified that the MCA was monitored for

5 minutes prior to dialysis and 5 minutes during dialysis. If microemboli are

infrequent occurrences, then a longer period of monitoring may have detected them

during dialysis. However, the evidence from previous research studies in this area

clearly shows that microemboli are created (and detected) at a high rate during

haemodialysis. Rolle et al used ultrasound to monitor the subclavian vein during

haemodialysis and detected microemboli at rates of up to 21.6 +/- 4.7 hits per minute

[45]. The study by Forsberg et al which demonstrated that microemboli can be

detected in the carotid arteries during haemodialysis also only monitored their

patients with ultrasound for 5 minutes [145]. These studies suggest that 5 minutes

should have been sufficient time to detect microemboli in the MCA using TCD.

Our analysis of cognitive function showed that a majority of dialysis patients showed

some deterioration in their test results between baseline and follow up testing at 1

year. However, there was no decline in the median average cognitive function in

different groups over the year. This was the case across the full range of tests used

to capture different aspects of cognitive function [197]. This may suggest that the

deterioration seen in many of the individual patients may be as a result of variability

in the test results rather than a true decline in cognitive function. In keeping with

this, we also found that there was no difference in the rates of change in cognitive

function between those with and those without a PFO, and between those having HD and those having PD. There are several explanations that we have considered for this finding. One possibility is that, as we found no evidence of cerebral microembolisation during HD, the presence of a PFO does not make any significant difference to rates of cognitive decline. However, as we also found no significant change in the median cognitive function between baseline testing and follow up testing, it is also possible that one year was simply not a long enough period of time to see any statistically significant changes in the cognitive tests that we had chosen. This is contrary to the findings of groups such as Bossola *et al* who did find significant deterioration in cognitive function in HD patients over a period of 1 year [152]. It should be noted however that whilst we used a battery of tests looking especially at changes that may be associated with embolic small vessel disease, their group used the more generic MMSE to assess cognitive function.

A further criticism of our work on cognitive function in dialysis patients may relate to the way in which our groups of patients were simply categorised into patients with a PFO and patients without a PFO. An alternative method would have been to use only patients with a large PFO and to compare these patients against patients with no PFO, therefore ignoring patients with a small or medium sized PFO. The rationale for this would have been that some studies have suggested that patients with a large PFO suffer more clinical consequences than patients with a smaller PFO [65][66][68]. However these findings have not been universally validated [64][69]. We have also already discussed the problems associated with accurately assessing PFO shunt size (see above). Finally, it should be noted that choosing to use only patients with a large PFO in our work would have significantly reduced the number of

patients eligible to be studied. With a fixed population of haemodialysis patients at our unit, this would have made the recruitment of sufficient numbers of suitable patients impossible.

Our work on cognitive function focused on looking at changes over time within and between different groups. We looked for significance by comparing rates of change between baseline results and follow up results within the same group and also compared rates of change in cognitive function between different groups. We deliberately did not attempt to define "significant cognitive decline" as there is no accepted standardised measure for this. Other research groups have chosen to define significant decline in cognitive function during their studies, for example using standard deviations from the mean [183][184]. However this approach does not correlate with any real world level of function and does not reflect the ability of patients to carry out various activities of daily living. As our cognitive tests are only a proxy measure of how patients handle the cognitive demands of day-to-day living, we chose to attempt to study patterns of change rather than to focus on defining clinical significance.

During our study of cognitive function in dialysis patients, we chose to compare the results of our 4 different cognitive function tests separately rather than to amalgamate them to produce a single score. Although this would have helped to reduce the likelihood of a type 1 error (or incorrect rejection of the null hypothesis), this approach would have had important limitations.   One of the major drawbacks is that although our tests of cognitive function are measuring similar things, they are

not all measuring the same aspect of cognitive function. Amalgamating the scores reduces the ability to differentiate between subtly different aspects of cognitive function deficit.

It is reassuring that our work did not identify microemboli in the cerebral circulation in dialysis patients. If evidence had been found of a link between PFO and cognitive decline due to cerebral microembolisation, then a clinical trial would urgently have been required to elucidate whether PFO closure could halt or slow the intellectual decay associated with HD

Finally, our pilot study into CVVH in the intensive care unit in patients with acute renal failure found that microemboli cannot be detected in the MCA during CVVH. This is important as procedures where microemboli are created and then detected in the cerebral circulation tend to be associated with cerebral injury [198]. However, we cannot fully exclude the possibility that microemboli are created by CVVH and simply do not cross into the left sided arterial circulation. It was a limitation of our work that we do not know how many of the patients who underwent CVVH had a PFO or other right-to-left shunt. It is possible (but unlikely) that none of the patients included in our study had a PFO or other right-to-left shunt. Work is therefore still required before it can be concluded that microemboli are not produced by the process of CVVH.

Future work

It would be interesting if future studies could look to correlate microembolic signals in the carotid circulation with signals in the cerebral circulation in patients undergoing HD treatment. If these further studies were to confirm that microemboli can be detected in the carotid artery and not in the MCA it would be difficult to explain what becomes of the microemboli. We believe that there is scope for further work in this area before cerebral microembolisation can be excluded with certainty during HD.

Another avenue of future research is the use brain scanning with magnetic resonance imaging (MRI). Previous work by Reul *et al* using MRI to image the brains of amateur deep sea divers has shown that there are more hyperintense lesions in the subcortical cerebral white matter in those who dive regularly compared to age and sex matched controls who participate in sports other than diving[199]. As these hyperintense lesions were largely found in the subcortical white matter and basal ganglia, a vascular aetiology has been hypothesised. Subsequently, a non-randomised study by Billinger *et al* showed that divers who have their PFO closed and continued to dive have both fewer asymptomatic cerebral white matter lesions and fewer episodes of major decompression sickness than divers where the PFO is left open whilst continuing to dive [83]. If cerebral microembolisation does occur during HD, it is possible that the brains of patients with a PFO and undergoing HD treatment could show similar changes in the cerebral white matter. One possible future investigation would be a cohort study comparing MRI brain scan images of those on HD with a PFO against those on HD without a PFO. If there were more white matter lesions in the brains of patients undergoing HD who had a PFO

compared to those who did not have a PFO, it may again suggest an embolic

aetiology mediated by the PFO.

More work is also needed to look at cognitive function and decline in patients with

end stage renal disease having dialysis treatment. Although it has been clearly

established that these patients have cognitive impairment when compared to the

general population, rates of decline in different aspects of cognitive function are less

clear. Our own study found no decline in cognitive function over 1 year using a

battery of tests (digit symbol test, FAS verbal fluency, digit span backwards, trail

making B-A) looking specifically at impairment likely to be caused by small vessel

disease. A longer term study using the same group of tests may help to reveal rates

of decline in these particular aspects of cognitive function.

The pilot study on the intensive care unit did not identify cerebral microembolisation

during CVVH in patients with acute kidney injury. As mentioned earlier, the

positioning of the venous access line makes it impossible to visualise microemboli in

the draining circulation. It would, however, be interesting to scan the carotid arteries

during CVVH to look for any microemboli that are present. This would help to clarify

both if microemboli are created by the process of CVVH and whether they are able

to cross from the right sided circulation into the left sided circulation.

This case-control study of patients with end-stage renal failure has shown that there

is no increased prevalence of PFO compared to the general population, and that the

presence of a PFO is not correlated either with cerebral microembolisation or with cognitive decline in the context of HD. This is important in the context of an ever-increasing HD population, and will hopefully pave the way toward further studies into the physiology and pathology of the dialysis-dependent patient.

Publications

Papers

"Does a patent foramen ovale influence cognitive function in dialysis patients? –
George S, Holt S, Medford, N, Hildick-Smith D, Nephron Clin Pract. 2013 Jun
1;123(1-2):1-6

"Patent foramen ovale, dialysis and microembolisation" – George S, Holt S, Hildick-
Smith D, Nephrology, 2012 Aug;17(6):569-74

"Patent foramen ovale: diagnosis, indications for closure and complications" –
George S, Hildick-Smith D, Br J Cardiol 2011;18:219–22

Abstracts

"Patent foramen ovale, dialysis, microembolisation and cognitive function" – Renal
Association Meeting, Gateshead, June 2012

"Patent Foramen Ovale And Haemodialysis - Is there evidence of cerebral
microembolisation?" – Journal of the American College of Cardiology, March 27,
2012, Volume 59, Issue 13

"Patent foramen ovale and microembolisation in haemodialysis" – Renal

Association/British Renal Society Meeting, Birmingham, June 2011 (available at

http://www.renalarchive.org/FetchDoc.aspx?id=6352)

Oral presentations

1st prize for oral presentation of research at the Brighton and Sussex Medical School

postgraduate student research symposium – May 2011

Posters

"Prevalence of patent foramen ovale in haemodialysis patients" – Medical Research

Society at the Royal College of Physicians, London, February 2011

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Appendices

Cognitive tests (appendix 1)

Digit symbol

1	2	3	4	5	6	7	8	9
∨	⊐	÷	∧	X	⌐	⊏	⊤	⌐

2	1	3	1	4	2	1	3	5	3	2	1	4	2	1	3	1	2	4	1
⊐	∨	÷	∨	∧															

1	2	3	4	5	6	7	8	9
∨	⊐	÷	∧	X	⌐	⊏	⊤	⌐

2	1	3	1	2	1	3	1	4	2	4	2	5	1	4	3	5	2	6	2

1	6	5	2	4	7	3	5	1	7	6	3	8	5	3	6	4	2	1	8

9	2	7	6	3	5	8	3	6	5	4	9	7	1	8	5	3	6	8	2

7	1	9	3	8	2	5	7	4	1	6	7	4	5	8	2	9	6	4	3

FAS Verbal Fluency

A letter of the alphabet is given to the patient. They are then asked to think of as many words as possible beginning with that letter in one minute. They are not allowed to use proper nouns. They are also not allowed to use the same word with a different ending (for example "run" and "running"). This is done with three letters- F, A and S with a minute for each letter.

Digit span backwards

Participants are asked to listen to a list of random numbers that are read out to them at a rate of 1 per second and then asked to recall them backwards. Initially two numbers are read out, then three, then four and then so on until the participant makes a mistake. Their score is the maximum number of numbers that they can correctly recall backwards. The numbers were generated using a computer program prior to the test.

19

534

7209

98745

947929

8618358

18983931

109165466

1649868005

51003943067

721955708203

Appendices

Trail Making Test Parts A & B

In Part A, the circles are numbered 1 – 25, and the participant is asked to draw a line to connect the numbers in ascending order. In Part B, the circles include both numbers (1 – 13) and letters (A – L). The participant is asked to draw a line to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The patient will be instructed to connect the circles as quickly as possible, without lifting the pen from the paper. The time taken to complete the trail will be measured. If the patient makes an error, this will be pointed out and the patient asked to correct it. Both parts A and B will be demonstrated to the patient using the sample below.

Trail making test Part A - demonstration
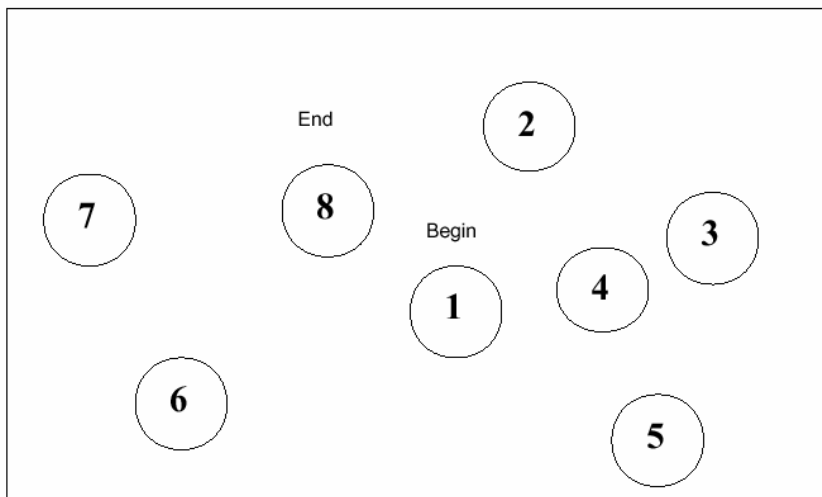
Trail making test Part B -demonstration

Appendices
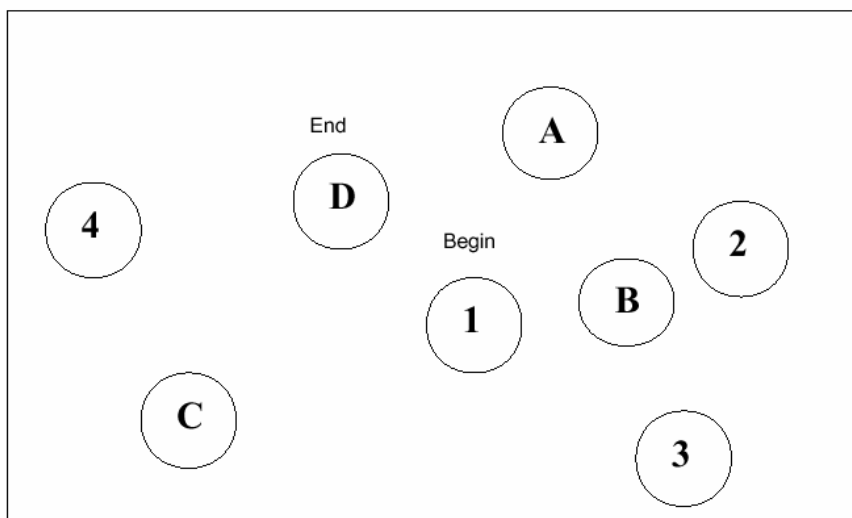
Trail making test Part A

Trail making test Part B

Participant information sheet for dialysis patients (appendix 2)

Study title: Patent foramen ovale and cognitive function in dialysis

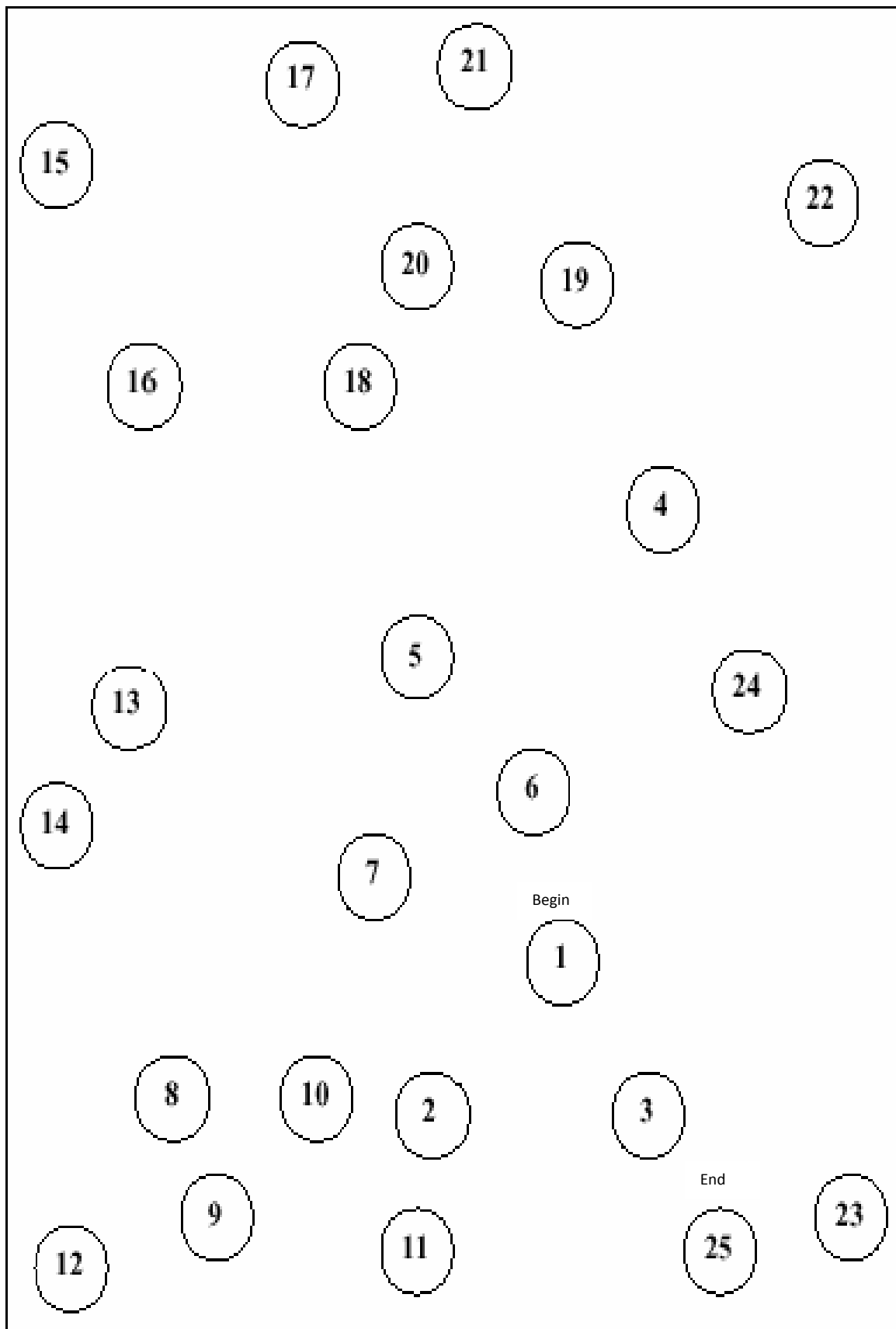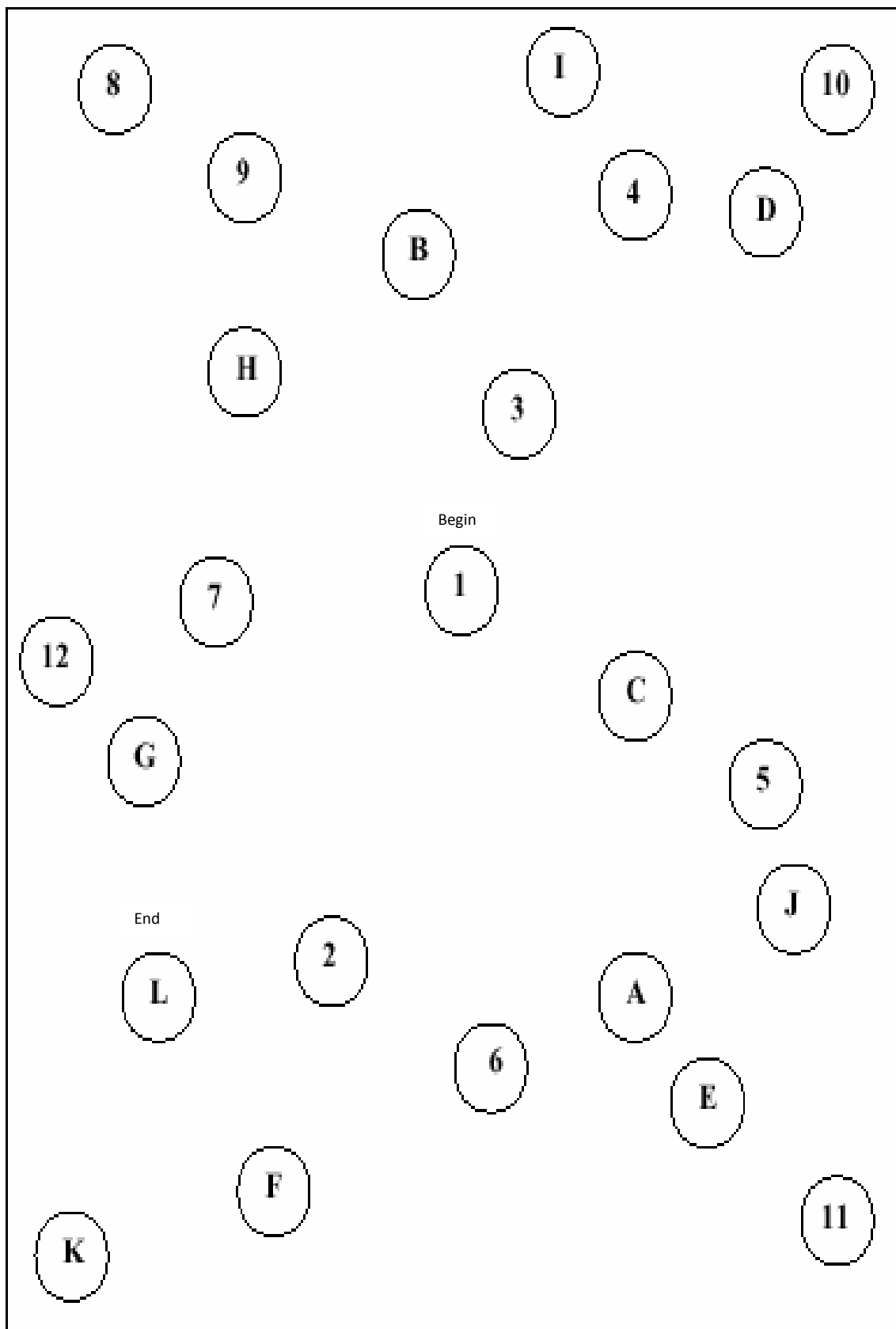*We would like to invite you to take part in a research study. Before you decide if you are interested, we would like you to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.*

What is the purpose of the study?

It is known that people who have kidney failure, and especially those who need dialysis have a decline in their ability to concentrate, remember and process information. Nobody knows exactly why this happens. One suggestion is that this might be related to whether there is a small have a small hole in the heart (called a patent foramen ovale). This is a very common finding and affects up to one in four people. In most people this patent foramen ovale does no harm at all, but in a small number, it can lead to a stroke or mini-strokes. Nobody knows what happens to this small hole in dialysis patients. We are doing a study to detect this small hole and to see if this affects people on dialysis.

Why have I been invited?

You have been invited because you have renal failure and are having haemodialysis treatment.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I decide to take part?

You will first be asked to sign a consent form to say that you are happy to take part in this study. The next time that you come for dialysis, we will ask you to have a scan of your heart called an echocardiogram. We will use a small plastic tube to inject some bubbles of air mixed with salt water and a bit of your blood into the vein through which you are going to have your haemodialysis and then scan your heart with an ultrasound probe. This uses sound waves and is harmless. It does not use x-rays. During this scan, we will ask you to blow into a plastic syringe to increase the pressure in your lungs.

This test takes around twenty minutes and will help us to see whether you have a patent foramen ovale. If the study shows you have a small hole, you will be told, but it will not change your medical management in any way.

Echocardiogram being performed

On another day, just before you have dialysis, Dr George will carry out some thinking tests. These involve various written and spoken tasks that take around twenty minutes in total. You might find these dull or interesting, hopefully the latter! The

next time that you come for dialysis after that, we will ask you to have a non-invasive test called a transcranial Doppler. This is a test which uses sound waves to look at blood flow to the brain. It also helps us to see if there are any small bubbles or tiny clots travelling through the blood vessels in your head.

During this scan, we will again ask you to blow into a plastic syringe. The scan will take around forty five minutes in total.

Transcranial Doppler scan being performed

Finally, we will contact you in one year to repeat the thinking tests to see if there are any changes in your results.

What are the disadvantages and risks of taking part?

It will however cost you extra time and some inconvenience. We need to do the bubble echocardiogram, thinking tests and transcranial Doppler scan on everyone who agrees to take part in the study. The bubble echocardiogram is an invasive test, but as far as we know, there are no known extra risks. Since you have haemodialysis we will arrange to do the tests on days when you come into hospital to have dialysis.

Are there any advantages in taking part?

There will not be any direct advantages to you in taking part in this study. We hope that by doing this study, we will be able to learn more about brain function and dialysis in patients with kidney failure. This may help patients in the future with kidney failure.

What if new information becomes available?

If any new relevant information becomes available during the study, then we will phone you or write to you to let you know about it and we can discuss whether you would like to continue to take part in the study.

Will my taking part in the study be kept confidential?

All information collected about you will be kept strictly confidential. Your name will not be used at any time. You will be identified by a study code. We will store data from the study on the secure hospital computer system and filed securely on hospital premises until the study is completed. All data from the study will be destroyed within one year of completion of the study. If you choose to withdraw from the study at any time, we will destroy any personally identifiable data about you. We will write to your GP to let them know that you are taking part in this study.

What if something goes wrong?

If you have any problems, concerns, complaints or other questions about any aspect of the way you have been approached or treated during the course of the trial then please contact the chief investigator Dr David Hildick-Smith on 01273 696955, ext 4049. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Brighton and Sussex University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

What will happen with the results of the project?

It is hoped that the results from this study will be published in a cardiology or kidney journal. You would not be identified in any way in the publication.

Who has reviewed the project?

The project has been reviewed by the Local and Regional ethics committee. Their role is to check that the study is acceptable from an ethical and safety point of view in the interests of the patients participating.

Can I find out the results of the study?

If you are interested in finding out the results of the study, please inform Dr George when you sign the consent form and we can arrange to send you a final report from the study in around eighteen months time.

Where can I get further information?

Thank you for taking the time to read the information sheet. If you are interested in taking part, please contact Dr Sudhakar George on 01273 696955 ext 3665. This study is being sponsored by the Brighton and Sussex University Hospitals NHS Trust. There are no private companies who are sponsoring the study or who will benefit from it.

Consent form for dialysis patients (appendix 3)

Name of Researcher: Dr Sudhakar George

Please initial box

1   I confirm that I have read and understood the information
    sheet dated 01.03.10 (version 2.0) for the above study

    and have had the opportunity to ask questions.

2   I understand that my participation is voluntary and that I am
    free to withdraw at any time, without giving any reason, without my

    medical care or legal rights being affected.

3   I understand that sections of any of my medical notes may
    be looked at by responsible individuals from regulatory

    authorities where it is relevant to my taking part in research as well as
    members of the research team. I give permission for these individuals to
    have access to my records.

4   I confirm that I am happy for my GP to be informed that I am taking part in this
    study

5   I agree to be contacted in one year by post or telephone

6   I agree to take part in the above study.

_____   _____   _____

Name of Patient   Signature   Date

_____   _____   _____

Name of Person taking consent   Signature   Date

Participant information sheet for haemofiltration study (appendix 4)

Study title: Does haemofiltration cause cerebral microembolisation?

We would like to invite you to take part in a research study. Before you decide if you are interested, we would like you to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Patients who are very ill and develop kidney failure often have a treatment called haemofiltration which acts to get rid of some of the waste products that the body produces. Haemofiltration is a very safe and effective treatment and can be lifesaving.

We would like to know whether the haemofiltration machine produces very small blood clots called microemboli that can be detected in the arteries supplying the brain. Microemboli can be detected using ultrasound, a safe and non-invasive technology.   Our study aims to see if haemofiltration produces microemboli by doing an ultrasound scan of an artery supplying the brain whilst patients are having their haemofiltration treatment.

Why have I been invited?

You have been invited because you have kidney failure and are having haemofiltration treatment. Haemofiltration is a safe treatment that is being carried out as part of your normal medical care.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I decide to take part?

You will first be asked to sign a consent form to say that you are happy to take part in this study. The next time that you are having haemofiltration, we will carry out a non-invasive test called a transcranial Doppler. This is a test which uses high frequency sound waves (ultrasound) to look at blood flow to the brain. It also helps us to see if there are any signals travelling through the blood vessels in your head.

The scan will take around 10 minutes and involves a probe on a headset being placed over the head. We will also repeat the scan for a further 10 minutes later on when you are no longer having haemofiltration.

Transcranial Doppler scan being performed

What are the disadvantages and risks of taking part?

We will take up 20 minutes of your time in total. As ultrasound is a non-invasive test, there are no risks in taking part in the study.

Are there any advantages in taking part?

There will not be any direct advantages to you in taking part in this study. We hope that by doing this study, we will be able to learn more about haemofiltration. This may help patients in the future with kidney failure.

Will my taking part in the study be kept confidential?

All information collected about you will be kept strictly confidential. Your name will not be used at any time. You will be identified by a study code. We will store data from the study on the secure hospital computer system and filed securely on hospital premises until the study is completed. All data from the study will be destroyed within one year of completion of the study. If you choose to withdraw from the study at any time, we will destroy any personally identifiable data about you. We will write to your GP to let them know that you are taking part in this study.

What if something goes wrong?

If you have any problems, concerns, complaints or other questions about any aspect of the way you have been approached or treated during the course of the trial then please contact the chief investigator Dr David Hildick-Smith on 01273 696955, ext 4049. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Brighton and Sussex University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

What will happen with the results of the project?

It is hoped that the results from this study will be published in a kidney or intensive care journal. You would not be identified in any way in the publication. You will be offered a summary of the results of the study if you would like to see them.

Who has approved this project?

The project has been approved by the National Research Ethics Service Committee London – South East. Their role is to check that the study is acceptable from an ethical and safety point of view in the interests of the patients participating.

Can I find out the results of the study?

If you are interested in finding out the results of the study, please inform Dr George when you sign the consent form and we can arrange to send you a final report from the study in around eighteen months time.

Where can I get further information?

Thank you for taking the time to read the information sheet. If you are interested in taking part, please contact Dr Sudhakar George on 01273 696955 ext 3665. This study is being sponsored by the Brighton and Sussex University Hospitals NHS Trust. There are no private companies who are sponsoring the study or who will benefit from it.

Participant consent form for haemofiltration study (appendix 5)

Does haemofiltration cause cerebral microembolisation?

CONSENT FORM

Name of Researcher: Dr Sudhakar George

Please initial box

1   I confirm that I have read and understood the information sheet
       "Participant information sheet (dated 01/05/11, version 1) for the above
       study and have had the opportunity to ask questions.

2   I understand that sections of any of my medical notes may be looked at by
       responsible individuals from regulatory authorities where it is relevant to
       my taking part in research as well as members of the research team.

3   I confirm that I am happy for my GP to be informed that I am taking part in
       this study.

4   I agree to take part in the above study.

_____   _____   _____

Name of patient   Signature   Date

_____   _____   _____

Name of person taking consent   Signature   Date