When working towards an optimised Carotid stenting procedure does patient, procedural and pharmacological selection impact on outcome.

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Abstract

Carotid intervention for stroke prevention in the form of carotid artery stenting has been performed in clinical trials since the beginning of the 1980's. Improvements in the operative technique and pharmacology used during the procedure have reduced complication rates since stenting began. Long term follow up of the Sheffield data set has shown that patient selection has the most impact on reducing adverse outcomes from the procedure.

Multivariable analysis of the Sheffield database detailed in this thesis has shown that retinal events have a lower risk of adverse outcomes than cerebral events, and clopidogrel use at the time of stenting prevents adverse events. Recurrent stenosis of more than 50% also increases risk for recurrent stroke in the long term and therefore warrants a long term surveillance programmes in stented patients. It was possible to show from the same analysis that stenting patients as a routine procedure prior to cardiac surgery had no immediate benefit in reducing operative stroke risk from the procedure.

The EPICAS study was developed from these initial observations to investigate the effects of variability in response to clopidogrel on outcomes. Using transcranial Doppler detected embolic events as a clinical endpoint for comparison with the clopidogrel dependant pathways of platelet activation the study hoped to show a direct effect of the degree of platelet inhibition on the number of emboli detected. This was not shown to be the case in the patients studied as part of this work. Patients classified as non responders to clopidogrel had no difference in emboli counts before and after the stenting procedure ($p = 0.24$). The results suggest that a variance in response to

clopidogrel is unlikely to be clinically significant even in larger cohorts of patients.

Contents

Summary

Carotid angioplasty and stenting to treat carotid artery stenosis in symptomatic and asymptomatic patients has been developed by vascular interventionalists from the early 1980's as an alternative technique to the proven benefits of carotid endarterectomy. The Sheffield Vascular Institute and Department of Neurology were one of the earliest adoptees of angioplasty and stenting of the carotid artery in the UK and have adapted and studied the technique since inception. Much of this work has been published by the department.[1-7] However, despite extensive research in the field of carotid stenting the procedure continues to have a stroke risk of between 3 % and 5 %. This thesis represents continued work to improve the safety of the procedure using an extended multivariate analysis of previously unpublished 30 day and long term outcomes in symptomatic stenting, a cohort analysis into the benefits of asymptomatic intervention prior to cardiac surgery, and a detailed experimental assessment of the impact of clopidogrel resistance during carotid stenting.

Several of the randomised trials comparing carotid endarterectomy and carotid stenting have now published 30 day outcomes that were not available at the start of this period of research. Recruitment and randomisation into these trials has been performed over many years during which the procedure of carotid stenting has been developed. These changes have included the routine implementation of additional medical therapies, developments of device characteristics and the additional perceived impact of an operator learning curve in the stenting arm of these trials. A multivariate analysis of the Sheffield stenting database was conceived as an initial screen to detect elements impacting on outcomes in the Sheffield patients, and the preliminary analysis was presented at the world congress of neurology in 2005. An analysis of this sort has not

previously been undertaken in an entirely symptomatic group of patients. The maintained and modified analysis of the data is presented in its most recent form in chapter 2.

The Sheffield Vascular Institute has also served as a quaternary referral centre for carotid stenting procedures prior to coronary artery bypass grafting or valve replacement. This procedure has become a common request from cardiothoracic surgeons in patients who have bilateral high grade carotid artery stenosis. The theoretical benefits of performing a staged carotid stent prior to cardiothoracic surgery were felt to reduce peri operative stroke or death risk. In a review of the Sheffield carotid stenting procedure to improve its overall safety the optimising selection of patients was of interest, and if no benefit could be shown from the combined approach then stenting these patients posed unnecessary risk. The analysis published [8] and detailed in chapter 3 was performed to fill a void in the literature for the overall efficacy of the combined procedures. The safety and clinical benefit of this combined approach remains in question today.

The first randomised study of the benefits of combined antiplatelet therapy in carotid stenting using clopidogrel and aspirin was performed in a cohort of Sheffield patients by researchers from the department on neurology. The outcomes of this study were analysed and published during the planning stages for the EPICAS study and showed a clear benefit for combined antiplatelet therapy.[9] The risk of stroke or adverse event in patients treated without clopidogrel was significantly higher and was confirmed in the initial assessments of 30 day outcomes of the stenting database detailed in chapter 2. The EPICAS study was undertaken in response to a perceived risk of significant harm to patients who were, "clopidogrel resistant." Clopidogrel resistance presented a clear

clinical risk if up to 20% patients were truly clopidogrel resistant then 20% of all stent patients were effectively undergoing carotid stenting without adequate antiplatelet cover as suggested by our randomised trial. Alternative antiplatelet agents have been investigated in addition to the combination of clopidogrel and aspirin, but have been shown to be too high risk to be used in all patients undergoing carotid stenting. The EPICAS protocol was designed to address this issue by trying to identify if patients classified as resistant to clopidogrel on platelet testing had a clinically higher risk of peri operative stroke. This would then allow judicious use of intravenous antiplatelet agents in this high risk group in the peri operative period. The methodology and results of this study are detailed in the remaining chapters of this thesis.

The results of the EPICAS study have suggested that the variation in response to clopidogrel and its impact on platelet function may not be as significant as we had predicted, with the majority of the outcome measures assessed showing no statistically significant trends. The relevance of our findings in the light of more recent research from other centres published during this trial is reviewed in the closing chapter.

1 Introduction

i) Carotid Intervention from Endarterectomy to the birth of Stenting.

This thesis describes work undertaken to improve clinical outcomes from patients undergoing carotid artery stenting for secondary stroke prevention in a tertiary teaching hospital setting. The prospectively maintained Sheffield carotid stenting registry has been used in conjunction with the ethics approved Effective Platelet Inhibition in Carotid Artery Stenting (EPICAS) study to identify specific targets for optimising the stenting procedure (www.nrr.nhs.uk ID: N0059132292). Review of the benefits of carotid stenting procedure in "asymptomatic," patients prior to cardiac surgery was also assessed as part of our optimisation of the stenting procedure in our institution.

History of carotid intervention

The clinical relevance of sustained, unimpeded blood flow through the carotid artery was first commented on by the physicians of ancient Greece. Hippocrates (460 to 370 BC) original descriptive writings of his neurological assessments in patients lead us to deduce that he was aware that the, "convulsions and paralysis of the limbs," he described could follow injury to the brain. What is not clear from the writings of the ancient Greeks is if they were aware of exact causation of stroke. It was not until writings by Rufus of Ephesus (98- 117 AD) describing external compression of the carotid vessels inducing, "karos," in certain susceptible individuals that the impact of the damage to the carotid artery was realised. The Greek word karos or karotide literally means to stupefy, or plunge into a deep sleep, and it is the derivation of this

word now that names these essential blood vessels which form the focus of this thesis.[10, 11]

Causation of stroke or, "apoplexy," by atheroma and thrombosis of the carotid arteries was discussed by Johann Webfer in 1675 in his work, "Observationes anatomicae, ex cadaveribus eorum, quos sustulit apoplexia," and this is probably when direct causation of stroke by internal carotid artery disease was first considered. However, despite the knowledge of causation it did not lead to physicians taking the next step and operating on the carotid artery until 1798. Initial attempts at carotid surgery were simple ligation procedures to stem blood flow and save life, invariably following trauma, and often with variable degrees of success.[12] The notion of operating on a diseased artery in a non life threatening situation to prevent future ischaemic cerebrovascular events was not considered until the publications by Miller Fisher in 1951, and again in 1954 examining the relationship between frequency of carotid artery disease and cerebrovascular insufficiency.[13, 14] Routine successful surgery on carotid arteries to treat carotid artery stenosis in patients with a history of intermittent cerebral dysfunction and carotid disease begins in 1953.

An American surgeon, Michael Ellis De Bakey, carried out the first successful carotid endarterectomy whilst head of surgery at the Baylor College of Medicine in Houston. The operation was highly successful with his patient making an uneventful post operative recovery, and is later described with 19 yrs follow up and no further cerebrovascular events until the patients death from coronary artery disease in 1972.[15] The original procedure described was novel but applied techniques learned for peripheral vascular disease in the carotid arteries. It was undertaken without any preoperative imaging, and was based only direct clinical assessment and an assumption

of finding a pathological lesion based on knowledge from previous descriptions of stroke and post mortem studies. Thankfully, in this case the evidence free approach was successful and encouraged development of the basic technique still used today in surgical carotid intervention. However, as this case was not reported for several years the expansion in carotid surgery that occurred shortly afterwards cannot be attributed to it. The greatest impetus to carotid surgery development was most likely Eastcott's group at St Mary's Hospital, London in 1954.[16] The number of procedures performed for, "carotid insufficiency," then grew exponentially as various groups reported successful outcomes from the operations with low self reported mortality figures of between 2 % and 3.3%.[17, 18] Comparative data from the time suggested that untreated patients had mortality rates approaching 20%, and therefore surgical intervention was considered a favourable option.[19] Various carotid reconstruction techniques were assessed, but the technique of endarterectomy, originally described by Cid Dos Santos[20] in peripheral arterial disease, and used by De Bakey became the eventual technique of choice.

Operative and post procedural complications will occur, no matter how meticulously the operator applies a technique. The variability in the numbers of adverse clinical outcomes due to endarterectomy seen between countries and at different centres within individual countries led to questions about the appropriateness of these procedures, and lead to the first randomised trial of carotid surgery which was published in 1984 from a single UK centre. [21] Continued uncertainty surrounded the role for surgical intervention and recruitment was slow. With only small numbers recruited the outcome tended failed to support surgical intervention over the limited medical therapies of the time. Whilst controversial, in the opinion of some observers, it provided impetus for larger randomised trials investigating the benefits of surgical carotid intervention to prevent

stroke and TIA over and above the benefits of medical therapies of the time. Antiplatelet agents had increasing evidence for a definitive role in treating patients with recent stroke and TIA which it was hypothesised negated the benefits of surgery. Confirmation of the benefits of antiplatelet therapy in secondary prevention of stroke came later with the publication of the two large antiplatelet trials (IST1 and CAST).[22-24]

The North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the European Carotid Surgery Trial (ECST) collaborative groups on either side of the Atlantic developed large randomised trials to confirm the benefits of surgical carotid intervention in addition to medical therapies. The trials initially used differing methods to measuring the degree of carotid stenosis in the internal carotid and had slightly differing inclusion criteria but both achieved similar outcomes. Carotid endarterectomy was beneficial over and above best medical therapy for significant high grade carotid stenosis greater than 70 % when based on the NASCET measuring criteria.[25, 26] These trials have undergone further review in combined meta analysis and the results are unchanged.[27] What has also been demonstrated by the trials is that intervention, while remaining beneficial at 6 months after the initial presenting minor stroke or transient ischaemic attack (TIA), has greater benefit the sooner the procedure is performed. These pivotal trials continue to provide insight into the optimal management of carotid arterial disease, including suggesting that the severest stenoses with near or total occlusion gain no benefit from surgery.[28] The trial results have also been shown to be reproducible in non selected populations outside of a trial setting.[29]

Interestingly, it was patients with near or complete occlusions that were chosen to be the first patients to undergo procedures back in 1953. The numbers of patients involved in these and subsequent carotid intervention trials has ensured that the procedure of carotid

intervention is one of the most robust, evidence based, vascular surgical procedures performed to date, and the trials involving carotid stenting procedures continue to add to this strong evidence basis.

Development of Carotid Angioplasty and Stenting.

The procedure of endovascular carotid intervention was proposed in the early 1980's as an alternative to endarterectomy to provide similar stroke prevention benefit, and the first carotid angioplasty balloon was used on a common carotid lesion during an open endarterectomy.[30] The development of balloon angioplasty of the neck vessels was slow in the first instance due to competition from the robust carotid endarterectomy procedure it was being compared to. The initial angioplasty procedures were not performed on atherosclerotic plaques but on critical stenosis of the internal carotid arteries in patients with fibro muscular dysplasia,[31, 32] and vertebral artery origin stenosis.[33] Concerns about cholesterol plaque embolisation were the limiting factors in the development and acceptance of the procedure. With no direct control over distal blood flow in the carotid the risk of embolisation was felt to be higher in carotid angioplasty. Therefore, endovascular techniques were used only on lesions felt to be non operable by conventional means, either due to their location or a previous failure with the endarterectomy technique e.g. Takayasu's arteritis,[34] and subclavian steal.[35]

The first successful descriptions of angioplasty performed for atherosclerotic lesions were published by American authors in 1983,[36, 37] and a further European group in 1984.[38] The procedure of primary transluminal catheter angioplasty (PTCA) was chosen in these selected patients as an alternative to endarterectomy due to high surgical risk from the medical co morbidities of the patients involved. Of the eight patients described in the three initial reports only one had an immediate post procedure neurological complication. Despite these initial successes in highly selected groups of patients the technique remained controversial and was not universally accepted as a promising alternative to endarterectomy. The reluctance to undertake the developing

procedure explains why it took a further two years for the European group to perform 8 procedures in total,[39] and the first true assessment of the endovascular technique was not undertaken until the CAVATAS investigators began randomisation into the endarterectomy vs. angioplasty trial for symptomatic atherosclerotic lesions in 1991.[40]

In the absence of a randomised trial several groups continued to perform balloon angioplasty in selected cases for atherosclerotic disease outside of a controlled trial setting. In an identical situation to the initial endarterectomy procedures individual groups reported complication rates of permanent neurological deficit of between 0% and 9.5% and those for transient neurological deficits between 0% and 57%. [41-45] Closer inspection of these figures reveals that between 1986 and 1990 the number of patients published in peer reviewed series of endovascular carotid intervention for all indications was 59 worldwide,[41-45] and did not increase until 1991 when a German group published their own experience with 38 carotid territory angioplasties and reviewed 177 carotid territory angioplasties from the literature.[46] Combined all indication, all cause, complication rates of 1.7% major complications, defined as death or permanent disability, and minor complications of 2.3% were quoted by this publication, and the data was used by the authors to suggest possible equivalence between angioplasty and endarterectomy. This claim met with considerable opposition from vascular surgeons. Publication bias clearly has a significant influence on the number and type of outcomes in case series dramatically skewing the data in these small numbers so it became an unreliable indicator of the true safety profile of these initial procedures.

The CAVATAS trial began in 1992 to prove equivalence of endovascular intervention with endarterectomy. Interim results were published in 1996 and 1999, but its long

term follow up was not complete and published until 2001.[40] The vacuum of knowledge throughout the 1990's was supplemented with data from short case series, individual patient reports and small single centre studies. Results from these publications supported continued use of the procedure in selected high risk patients outside of a randomised trial when carotid endarterectomy was not an option.[47] However, these studies lacked the independent neurological assessment which the CAVATAS trial design allowed for, and has since been postulated to have resulted in a more accurate determination of the true neurological complication rates of these procedures'.

The first data from CAVATAS trial patients was published in 1996 when 193 patients had been randomised.[6] At the same time other groups in Europe and America were publishing larger, self reported, single centre, non randomised case series, with good clinical outcomes.[48-51] What is important to note, and relevant to the design of the research on which this thesis and the EPICAS study is based, is how rapidly the angioplasty procedure was changing even within the few months that these publications were released over. Of particular interest is the dramatically differing procedural methodology especially in the use of anti-thrombotic measures by different clinicians. The technique of carotid angioplasty and stenting was being developed with no clear consensus on an optimal treatment approach. Rapidly changing technology and the lack of a unified technique requires historical literature to be reviewed critically to determine the impact any of these procedural modifications may have had on outcome. Several modifications to the stenting technique when critically assessed have a limited evidence basis, and therefore when designing a trial investigating the role of platelet agents these factors need to be taken into account. Published examples of differing practice serve to highlight this.

The CAVATAS data published in 1996 was comprised mainly of simple balloon angioplasty procedures with occasional stent placements using modified non carotid stents as permitted in the trial protocol from 1994 onwards. Two American series published at the same time used stents for the majority of their patients. Patients from the CAVATAS group all received heparin for 48 hours post procedure in addition to an oral aspirin loading dose of 300mg. In contrast one American group gave no post operative heparin relying solely on Aspirin to prevent thrombotic events,[48] another American group and a European group routinely used Ticlopidine in place of Aspirin in patients undergoing angioplasty,[49, 50] and a French group reported the use of routine Ticlopidine and subcutaneous heparin as an anti-thrombotic in all patients receiving stents.[51] This variability in technique prevents combined analysis of the data from the initial angioplasty procedures to be undertaken with any meaningful results, and the multivariate cohort analysis detailed in this thesis was designed to address some of these issues.

ii) Optimising carotid stenting the drive to modify the technique.

Achieving adequate cerebral protection became the driving force behind rapid changes in the methods used for endovascular carotid intervention. Fears surrounding the safety of balloon angioplasty had focused on the risk of distal embolisation into the cerebral circulation from cholesterol crystals, plaque debris and platelet aggregations derived from the carotid plaque under treatment.[52, 53] The type, and size, of embolic material has been shown to be closely related to the stage of the angioplasty procedure being undertaken,[54] and operators began modifying their practice to reduce the risk of embolic complications occurring at each of these stages. Developments in stenting technology provided single wire delivery systems and low profile dilatation balloons to reduce the risk of dislodging embolic material from numerous wire changes passing through the offending lesion, and protection devices were developed to catch debris.

Surgeons undertaking carotid endarterectomy have the benefit of being able to reduce cerebral embolic load by clamping distal to the lesion, or by shunting to bypass the operative field and protect the brain from distal embolisation. Initial therapeutic adaptations focussed on a perceived requirement for distal protection during carotid angioplasty and stenting and distal balloon occlusion devices discussed in Theron's paper in 1987[44] were the first modifications adopted by some interventional radiologists. His ideas were universally adopted by most other operators who developed the technique and catheters to suit their methods.[55] Although a good idea in theory the use of these devices was never subject to a randomised study before universal implementation. Methods then focussed on three protection techniques including distal balloon protection, proximal flow occlusion and flow reversal.[56-60] Theron's large series published in 1996 made a strong case for routine cerebral protection with his

distal protection technique having no embolic complications among the 136 patients he treated despite there being no control arm in his series.[51, 61] The benefits of cerebral protection devices were finally given limited scientific justification when a randomised trial was published in the late 1990's.[62] This trial had been stopped early due to a significant number of adverse events in the stenting arm which had failed to use cerebral protection devices. However, even the balloon protection devices, which have been shown to be highly efficient at collecting debris from the procedure, are not able to reduce intra-operative complications entirely due to incomplete collection of all particulate matter.[63, 64] In most series peri-operative complication rates with balloon occlusion devices are between 1.6% and 2.3%.[65, 66] These occlusion devices are also limited by the requirement to have a functioning circle of Willis to avoid cerebral hypo perfusion complications during device inflation.[67]

As an alternative to balloon occlusion filter devices were developed to capture particulate debris from the carotid artery preventing stroke. Initial in vitro experimental studies suggested that they may be at least 80% efficient in stopping all particulate matter from the procedure.[68] Initial experiences published as case series suggested the filters were highly effective with intra-operative complication rates of 0% and 1.2% encouraged widespread use of the devices.[69-71] A recently published follow up of these devices in regular use has confirmed these operative complication rates can be maintained, and has shown no statistically significant difference between the design and type of device used.[71] However, the increasing use of these filter devices in routine practice has required optimisation of intra procedural antiplatelet and antithrombotic therapies to prevent filter clotting while the filters are in place and may add to bleeding risk.[72]

Only one true randomised trial of protection verses no protection has been carried out and this is sub set analysis of an ongoing trial not yet published. Trials who have recruited patients early in the development of stenting when protection use was not widespread and historical analysis of registry data of patients treated with and without protection is the only clinical data of the true benefit of these technologies we have available to us. One of the largest of these registry studies confirmed a significant difference between the protected (0.9%) and unprotected groups (2.3%) but this difference was statistically insignificant (p= 0.15), due to the small numbers of unprotected patients.[71] In one study the complication rates for major stroke were 0% in both protected and unprotected groups and the rates for minor stroke were 3.2% unprotected vs. 0.7% protected. [73] Other large series have stroke and death rates of 1.1% and all complication rates of 3.4%.[74] A systematic review of all cases published in the literature shows rates for the outcome of all stroke and death to be 5.5% unprotected vs. 1.8% protected.[75] This observational evidence prevented proper randomised studies being undertaken and changes to the protocols for the trials of endarterectomy vs. stenting studies make this unlikely in the future. The Endarterectomy Versus Angioplasty in patients with Symptomatic Severe carotid Stenosis (EVA-3S) trial for example was modified to prevent patients having CAS performed without protection due to abnormally high complication rates in procedures performed without a protection device in place.[76]

No protection option can provide one hundred percent protection from embolic events, clinical strokes and transient ischemic attacks still occur with their use but less frequently and when they do occur are often after the initial operative period. Magnetic Resonance Imaging (MRI) studies have confirmed the presence of clinically silent

ischaemic events on diffusion weighted imaging occurring after the stenting procedure has been completed.[77, 78]

These changes have been seen in cases with and without cerebral protection [79, 80] and could represent events occurring after the protection device has been removed and therefore the role of the protection device in preventing these silent events is difficult to ascertain.[81-83] One method to optimise the degree of cerebral protection offered in particular to patients, with poor collateral flow via the circle of Willis has been to use two protection devices in tandem in a, "seat belt and airbag," approach.[84] Analysis of the Sheffield data in this thesis failed to provide clarification for a role of the protection device in routine use.

Implementing the routine use of stents, already shown to be efficacious in the coronary arteries, was the next logical step in controlling complications. The first experimental use of stents in animals was performed in 1987 with three carotid stents successfully inserted.[85] The first human use of stents in the carotid artery was reported in 1994 in two patients who had suffered carotid dissection and a further case in an Italian patient.[86, 87] The first reports of stenting atherosclerotic lesions in 1996 used Palmaz biliary stents adapted for use in the carotid circulation.[88, 89] Initial successes with lower post operative complication rates using these non specialised stents was supported by prospective non randomised studies into 30 day neurological outcomes with their use. The effect on 30 day outcomes was likely due to the lower embolic potential of stent insertion when compared to angioplasty, and was later confirmed in experiments performed on human cadavers 2 years after their initial routine use in patients.[90] Imaging follow up of these successful stent procedures demonstrated that some stent designs deformed when used in carotid vessels and in particular the Palmaz stent was

withdrawn from use. This was replaced by the self expanding Wallstent in the majority of the remaining patients in early published series.[50, 91] Cases of deformed carotid stents continued in the literature into 1998 and in addition to the Palmaz stent, the Strecker stent and Be Stent were also noted to have this problem.[92, 93] The risk of deformation lead to the withdrawal of these stents from routine use but the durability of the procedure has been shown to be equivalent in patients with and without deformed stents.[94] Dedicated stent systems were in routine use at completion of the initial randomisation phase of the CAVATAS study in 1999. Since then stent technology has advanced with the introduction of self expanding nitinol stents which have superior outcomes compared to the original stainless steel stents.[95, 96] The increased use of intracerebral monitoring by transcranial Doppler (TCD) has also confirmed that stenting appears to be safer than balloon angioplasty from a purely athero-embolic risk perspective.[97]

Despite the technical adaptations carotid stenting and carotid endarterectomy continue to have 30 day complication rates of around 6% in randomised trials. The CAVATAS study results were first released in abstract form with both the carotid endarterectomy and angioplasty / stenting arms showing equivalent complication rates of 6% at 30 days for disabling stroke or death.[98] It must be remembered that the CAVATAS study was performed on the background of continued development of both techniques. Throughout the study surgeons were able to develop the carotid endarterectomy techniques making them more durable using patching material to prevent restenosis, [99-103] and the radiologists developed protection devices and stents during the trial. Yet despite these developments the 30 day complication rates remained similar to the original NASCET, and ECST trials performed 5 years prior to these developments. Final results of the CAVATAS study were published in 2001 showing no significant

difference between endovascular and surgical carotid procedures at 30 days.[40] The maximum safety level of the procedures appeared to have been achieved and it did not appear at this point that complication rates could be lowered further. This thesis contains work that was designed to seek ways to improve stenting outcomes further.

iii) The role of antiplatelet agents, drug resistance and the rationale behind present anti-platelet therapies in carotid stenting.

Medical therapy and specifically antiplatelet therapy prior to, during, and after carotid stenting has been shown to have a significant impact on the outcome from stenting procedures because the insertion of a metallic stent into an artery will always lead to activation of platelet dependant thrombotic pathways.[104] Uncontrolled platelet activation will result in aggregation and thrombus formation with distal embolisation or stent occlusion in any patient undergoing stenting procedures. Platelet aggregation, the crucial step in thrombus formation, is mediated by the binding of activated platelets to fibrinogen through the platelet integrin glycoprotein IIb/IIIa. Antiplatelet regimes used in stenting were developed to prevent this activation.

Aspirin was administered to all patients to effectively inhibit collagen and arachidonic acid induced platelet aggregation and therefore thrombus formation within the stent. As aspirin only affects a single pathway the IIb/IIIa platelet activation pathway is still capable of trigger thrombosis and stent related complications. Thienopyridines such as Ticlopidine and Clopidogrel used synergistically with aspirin block ADP-induced activation of the IIb/IIIa receptor and consequently platelet aggregation. These two therapies are key parts of the antiplatelet regime prior to stenting. The combination prevents stable thrombus formation at the site of stent deployment and vessel endothelial injury and reduces embolic stroke risk.[105] Patients undergoing coronary and carotid stenting are now routinely pre-treated with dual antiplatelet therapy consisting of aspirin and clopidogrel to reduce the impact of platelet activation on clinical outcomes. The benefit of this combination was first demonstrated clinically in cardiac stenting procedures but combination antiplatelet therapy has now also become the minimum

standard for endovascular carotid stenting procedures, and has been confirmed to be beneficial in randomised controlled trials.[9, 106]

Dual antiplatelet therapy is administered prior to the stent insertion and is continued for 4 weeks following insertion. In theory this provides all patients with adequate suppression of platelet activation to prevent thrombotic-embolic complications from occurring in the immediate post operative period. However, the large follow up series that have already been discussed show that cerebrovascular complications continue at a rate of 2 – 3% at 30 days follow up. The terms, "aspirin resistance," and "clopidogrel resistance," have been used to describe sub groups of patients in whom varying levels of inhibition from these agents has been shown despite a standardised dosing regime.[107, 108] It has been assumed that patients with high levels of, "resistance," to the drug are those more at risk of embolic complications but this has yet to be proven in carotid stenting. In a clinical setting demonstrating an increased risk using clinical outcomes as end points would require a trial of several thousand patients to provide any statistical power. Even if a higher risk group can be identified it is not clear what alternative agents could have a role in replacing aspirin and clopidogrel in these, "non responders."

Several alternative antiplatelet agents have been assessed for this purpose to date. The antiplatelet agent Dextran 40 given as a continuous infusion has been assessed as part of the Leicester randomised trial between carotid endarterectomy and angioplasty.[62] Patients in this trial were treated with Dextran 40 if transcranial Doppler (TCD) monitoring detected more then 25 embolic signals occurring in any 10 minute period of monitoring for the first 6 hours after the procedures.[109-112] Dextran works by reducing platelet adhesion, factor VIII activity, and increases clot lysis making it ideal for use in patients with active clot formation on stents. Unfortunately the Leicester trial was

terminated early due to unacceptable complication rates in the endovascular arm of the trial and Dextran has not been trialled since except as a rescue medication in patients with antiplatelet agent failure in carotid stenting. One other case report has been published using dextran 40 as a successful rescue medication in a patient suffering a stroke whilst undergoing stenting; this patient had not received pre procedure clopidogrel and was in the non treatment arm for an alternative antiplatelet agent trial.[113] This patient had been taking S-nitrosoglutathione (GSNO) as an alternative agent to clopidogrel as part of a randomised trial. S-nitrosoglutathione has been shown, like dextran 40, to have considerable success when assessed by transcranial Doppler (TCD) at reducing or eradicating embolic signals but has not been expanded to larger patient study.[114]

Abciximab, a glycoprotein IIb/IIIa (Gp IIb/IIIa) receptor inhibitor, was first used in carotid stenting in 2000 by two groups in America and Israel. The abciximab infusions were given peri-operatively and post operatively for 12 -16 hrs in place of routine clopidogrel in patients thought to have high risk arterial anatomy.[115, 116] In both these studies no significant post operative neurological or bleeding complications occurred prompting further trials with Gp IIb/IIIa receptor blockers. The later trials with these agents used them in addition to clopidogrel and aspirin in a," belt and braces," approach to ensure platelet deactivation because of the perceived risk of clopidogrel resistance.[117, 118] However, with larger studies it became apparent that these drugs lead to increased rates of serious intracranial bleeding complications, and their place in routine procedures has now been questioned.[119-121] However, we must consider that these agents may be useful as a rescue therapy for the limited cases where rescue of a thrombosing stent is required, or embolic complications from a stent are occurring clinically on transcranial Doppler recordings.[122, 123] They may also have a role in

patients where filter devices cannot be used safely.[124] However, even in these circumstances it has been shown that they are not as efficient as embolic protection devices in reducing emboli numbers.[125]

The limitations of other agents mean that the mainstay of antiplatelet therapy for prevention of stent thrombosis and thrombotic embolic events in carotid stenting procedures remains aspirin and clopidogrel. Therefore, clopidogrel resistance could have a more significant impact on outcomes from carotid stenting procedures than has been seen in coronary stenting because most cardiologists employ a triple antiplatelet therapy approach. To detect clinical differences in outcome from stenting procedures due to minor variations in clopidogrel platelet inhibition would have required hundreds of patient hours of follow up to demonstrate statistically significant outcomes. The Effective Platelet Inhibition in Carotid Artery Stenting (EPICAS) study documented in this thesis was designed to utilise the previously described techniques of transcranial Doppler emboli detection as a surrogate endpoint for cerebrovascular damage in combination with platelet function assessments to ascertain what impact clopidogrel resistance may have clinically.

iv) Transcranial Doppler Ultrasound assessments during carotid stenting.

Transcranial Doppler ultrasound (TCD) was first described in 1982 for monitoring intracerebral blood flow [126] and detecting cerebral vasospasm in patients undergoing catheter angiograms following subarachnoid haemorrhage.[127] TCD use in carotid intervention was first documented in 1986 for monitoring middle cerebral artery (MCA) velocity during carotid endarterectomy, and later for the detection of cerebral micro embolic signals (MES) [128] in the Doppler wave form suggesting cerebral embolisation actively occurring from the operative field.[129] Patients that suffered cerebrovascular complications with transcranial Doppler monitoring for embolic signals in place during and after carotid endarterectomy were shown to have higher numbers of embolic signals.[130] It was suggested that embolic signal numbers represented a surrogate indicator of risk of ischemic complications from the procedure.[131] Standard criteria for the detection of embolic signals were then agreed to allow this technique to be used reproducibly.[132]

The first report of the use of transcranial Doppler ultrasound during endovascular carotid intervention was from Markus et al, London in 1994.[133] They were able to demonstrate that micro embolic signals (MES) occurring during the procedure [128] could continue in the post operative period in the 10 patients they studied. They postulated that TCD could therefore be used to monitor for the effects of medication on these events to reduce risk from the procedure. Later reviews from the same group, and confirmed by other authors, suggested that endovascular procedures had higher rates of emboli in comparison with endarterectomy, but that clinically and in neuropsychological testing in a small series it was impossible to detect any difference in outcome from the two procedures.[134-137]

The timing of embolic events during catheter procedures has shown that a significant number of operative events associated with embolic showers occur intra operatively during wire and stent manipulation.[138] These signals are most likely to be solid cholesterol emboli and it is difficult to see how these embolic events can be affected by antiplatelet therapies used in carotid artery stenting. It is in the post operative period that the presence of embolic signals is likely to be due to platelet rich emboli and the benefits of antiplatelet agents in reducing the frequency of these signals can be studied. Transcranial Doppler has also been used to demonstrate differences between embolic loads dependant on the protection device type used during the procedure. Filter devices have significantly higher operative embolic loads than balloon type occlusion devices.[139] Filter devices also generate higher numbers of embolic particles than procedures without embolic protection,[140] but these particles are of a smaller diameter and are postulated to be less likely to cause neuronal damage.[141] The clinical significance of this finding is still as yet undetermined. In the post operative period transcranial Doppler has confirmed that carotid stents are not a potent embolic source in medium (6 months) and long term follow up (12 months) after insertion.[142]

Transcranial Doppler detected embolic signal counts have been used successfully in carotid stenting trials to compare outcomes from new agents with routine therapy.[114] The techniques described allowed a new agent S-Nitrosoglutathione (GSNO) to be assessed against the standard regime of aspirin and heparin. TCD endpoints demonstrated the superior efficacy of this agent by reducing post operative embolic signals.[114] Micro embolic signal (MES) detection has also been used successfully in single and multicentre studies to monitor the effects of antiplatelet therapy in patients with symptomatic carotid artery disease by demonstrating significant reduction in the

number of MES in patients on differing antiplatelet regimes.[143-147] The largest of these studies a multicentre randomised controlled study the Clopidogrel and Aspirin for Reduction of Emboli in Symptomatic carotid Stenosis (CARESS) trial [143] was published after the instigation of the EPICAS study in Sheffield but it provided reassurance that the methodology of the ongoing study was sound.

v) Scientific justification for the EPICAS study.

The benefits of dual antiplatelet therapy over anticoagulation for prevention of thrombotic events has been confirmed in several cardiological and one carotid stenting trial to date. The FANTASTIC study [148] and the SARS study [149] demonstrate the benefits of dual antiplatelet regimes over anticoagulation with warfarin to prevent adverse events during coronary stenting and this was later confirmed by the CLASSICS study. [150] Randomised controlled trials later confirmed the benefits of the same dual antiplatelet regimes in carotid stenting and clopidogrel and aspirin are now considered a minimum standard of care for carotid and cardiac procedures.[9, 106] However, despite a tested standardised regime with a sufficient pre treatment window with the anti platelet agents' stroke and transient ischemic attacks due to embolic events continue to occur post procedurally in carotid stent patients.

Some of these events are due to dislodged cholesterol emboli and carotid plaque material embolising after removal of the embolic protection device. However, most of these events are likely to be athero embolic due to platelet aggregates. This suggests that the either the doses of the agents used are insufficient, or an individual's response to a standard dose varies dramatically from patient to patient allowing variable aggregation. Maximum inhibition of the ADP mediated platelet aggregation pathway is not always possible in all patients even when they are pre loaded with clopidogrel. Pre loading with 75mg of clopidogrel for one week or using a single loading dose of 300mg at least six hours prior to an endovascular procedure has been shown to be effective in the majority of patients, but not all.[151-153] Consequently, some authors advocate using higher doses of the drug in a single

loading dose if a full weeks preloading regime cannot be used.[154-157] However, despite suggestions to try a higher single loading dose general agreement exists that using a preloading regime of one week's therapy prior to the procedure will result in the maximum achievable platelet inhibition in each patient and these regimes are now recommended for all elective procedures.

When developing the EPICAS study protocol it was hypothesised that an individual patient's response to clopidogrel or aspirin would result in stenting failure and complications. Multiple studies of in vitro platelet function testing of patients undergoing stenting procedures had suggested that between 10 – 20 % of all patients given dual therapy may respond poorly to clopidogrel.[108, 158-161] This would mean that up to 20% of patients may be at increased risk of complication from the procedure. However, assessing the effect that the varying degrees of platelet inhibition on a clinically relevant end point such as stroke, or transient ischemic attack or stent thrombosis remains difficult due to the low frequency that these events occur in stenting. Carotid artery stenting provided an opportunity to study the impact of variable inhibition using transcranial Doppler (TCD) micro embolic signals as a surrogate end point for ischemia. The accessibility of the carotid artery meant it could also serve as a model for thrombotic events occurring in all types of stenting procedures.

It is well documented that micro embolic signals are often detected in the first 24 hours after angioplasty, stenting and carotid endarterectomy.[133, 137, 162, 163] This is the period when platelet micro aggregates are most likely to have formed on the stent surface. They are also are more frequent immediately after the procedure particularly within the first few hours. Combining this information with evidence that suggests the number of

embolic signals detected can act as a positive predictor of future stroke risk [131, 164, 165], and data suggesting that clinical complication rates from angioplasty and stenting may be highest in the first 24 hours, we predict that eliminating embolic signals should act as a surrogate marker for reducing complication risk. Previous studies have already demonstrated that this approach was beneficial to examine the effects of new medications used during carotid procedures.[114, 143-147] The EPICAS study aimed to see if these techniques were useful in determining the effects of, "clopidogrel resistance," on embolic counts.

All patients continue on antiplatelet therapy for a period of four weeks after the stenting procedure until the stent is presumed to have endothelialised. It has been suggested that endothelialisation may take up to 96 days [166], and therefore a period of clopidogrel therapy given for only 4 weeks may be insufficient. Previously published data has already shown that at six months the stent no longer acts as a source of micro emboli [142], but at present no information is available to suggest an appropriate length of time for the clopidogrel therapy to continue. Performing TCD after discontinuation of dual antiplatelet therapy may show recurrence of embolic events suggesting longer therapy windows should be investigated and therefore was investigated as part of the EPICAS protocol.

Combining the techniques of platelet function testing and transcranial Doppler ultrasound to develop a model that allows pre procedure platelet analysis to predict possible post procedure complication risk was the primary aim of this research. The study protocol was designed by Dr M.Randall and with assistance from Prof G. Venables and Prof P. Gaines and Dr R Storey. This thesis contains the outcomes of this research. The preliminary analysis of the Sheffield cohort data highlights patient

selection and correct indication for stenting in the case of the pre operative stents for cardiac bypass as primary effectors of outcome. The EPICAS study then focused on the use of clopidogrel highlighted in this preliminary work as an opportunity to improve outcomes.

2 Lessons from the Sheffield endovascular cohort – Multivariate analysis of a prospectively maintained database of 30 day and Long term outcomes.

Introduction

Cerebrovascular disease costs the economies of the European Union €34 billion per annum, with 62% of these costs directly related to health care expenditure.[167] Carotid artery disease is implicated in approximately 25 % of ischemic stroke cases.[168] NASCET and ECST trialists demonstrated the benefits of surgery over best available medical therapy at the time to prevent recurrent carotid territory ischaemic events.[26, 169] Carotid stenting, an alternative to carotid endarterectomy, is undergoing evaluation in 4 multinational randomised controlled trials.[170-173] Analysis of the 30 day outcomes of EVA-3S, and SPACE [170, 173, 174] has been published and follow up analysis of both these trials is in press. The 30 day outcomes from ICSS have been presented at the European Stroke conference.[175, 176] When combined with the meta-analysis of carotid angioplasty trials we have reasonably robust data on possible risks from the stenting procedure.[177] The SAPPHIRE and CARESS investigators suggest non inferiority of the stenting procedure in short and long-term follow up when investigating mixed cohorts of symptomatic and asymptomatic patients.[178-180]

The clinical trial data suggests that carotid stenting is as durable as carotid endarterectomy in the long term although may have higher risks peri procedurally at 30 days. In addition to trial data, cohort studies allow outcomes to be analysed from representative clinical practice. All previous cohort studies contained both symptomatic

and asymptomatic patients, the majority being asymptomatic patients.[181-185] To address this we devised our cohort analysis on prospectively collected, audit department approved, stenting data of recently symptomatic patients only. We attempted to assess if procedural factors, patient factors, or restenosis had any impact on long term outcomes from stenting procedures. We focused on those highlighted by previous work from the ECST dataset [186] and mixed cohorts of symptomatic and asymptomatic patients undergoing stenting.[181, 183, 187-189]

Methodology

Patients with recent carotid territory, cerebral or retinal ischaemia, and carotid artery stenosis greater than 70% (NASCET criteria) on angiogram, have been considered for endovascular carotid intervention in our centre since 1993. Treatment recommendations are made by a neurology / vascular surgery / vascular radiology multidisciplinary team. All patients are screened with carotid duplex and brain CT. Patients with carotid artery stenosis of greater than 60% on duplex undergo arch aortography,[190] or magnetic resonance angiography (MRA) to confirm the stenosis and anatomy of the carotid vessels origins. Patients suitable for either endarterectomy or stenting are invited to participate in randomised intervention trials. Patients unsuitable or unwilling to undergo carotid endarterectomy are offered carotid stenting outside of these trials.

Prospective data collection is performed by a neurologist for each patient, including a pre operative assessment by a neurologist (MSR, FMcK, SK or GSV). The database is maintained in accordance with the National Health Service and Sheffield Teaching Hospital Clinical Effectiveness Unit Service Evaluation Audit policies (database registration 2212). Stenting procedures are performed by interventional vascular radiologists (TJC, PAG). The techniques have been described in previous publications from our centre.[4, 9] From 1996 to 2006 our stenting technique has developed to include the use of cerebral protection devices whenever possible, and mandatory use of dual antiplatelet therapy.[9] Dual therapy with clopidogrel and aspirin continues for 28 days after stenting, before reverting to aspirin or since 2004 aspirin and dipyridamole. A small number of patients' stented before 2002 received only a 14 day course of dual therapy.

Post stenting neurological assessment was performed by a neurologist at 24 hours or discharge if earlier. Neurological complications were classified as ; Amaurosis fugax - monocular visual loss less than 24 hours, transient ischaemic attack - new neurological deficit less than 24 hours, Minor stroke - new neurological deficits more than 24 hours but less than 7 days, and Major stroke - new neurological deficit persisting more than 7 days. The Oxford modified handicap score was used to categorise non-disabling (score 0-2) from disabling stroke (score ≥3).[191]

30 day and yearly follow up was performed in specialist clinics by a neurologist, or stroke specialist nurse / radiologist with neurological support. All patients underwent duplex ultrasound at 30 days, yearly follow up visits or at time of recurrent event when possible to assess for restenosis. Validated criteria for restenosis have been used.[192] Patients missing or, "lost to follow up", were traced and contacted by telephone. Deaths were confirmed from medical records, or death certificates obtained from the General Register Office (www.gro.gov.uk). Data collection was not routinely performed in all patients after 5 years follow up and therefore was censored at this point for the purposes of analysis.

Statistical Analysis

Statistical analysis was performed using SPSS 15.0.1 software package. Event rates per thousand patient years of follow up were calculated. Univariable analysis was performed using Kaplan Meier and Cox survival analysis. A Cox's proportional hazards model for multivariable analysis using a 0.10 cut off after co-linear variables had been screened and the least significant variable discarded was then used. The analysis was repeated with year of procedure forced into the model to see if the global

effect of improved technique and experience impacted on the outcomes. Restenosis was entered as a time dependant variable in the modelling after assessment of residuals, age and year of procedure were similarly assessed for effect of time and they were not shown to have a significant time dependant element after assessment. Analysis was performed for end points of ipsilateral stroke, ipsilateral stroke or vascular death, and all stroke and death.

Possible predictors of 30 day outcomes were examined using Chi square for discrete data and student's t test for continuous data before undertaking multivariable analysis using multiple logistic regression. This analysis has been tabulated.

Results

Between March 1996 and August 2008 562 carotid stenting procedures were performed on symptomatic carotid vessels, with follow up until Sept 2008. The mean follow up was 3.5 yrs, the median 4 yrs, and the range 30 days - 5 years. The presenting complaint was a retinal event in 144 (25.6%) cases. Baseline characteristics and rates of medication use at baseline of the patients treated are shown in **Table 1**. Hypertension was defined by internationally recognised criteria.[193] Clopidogrel was used in addition to aspirin in 413 patients (73.6%).

Table 1 Baseline Characteristics

		n = 563
Mean Age [Range]		68.5 [42 – 89]
Patients aged over 80 years old (%)		65 (11.5%)
Male (%)		381 (67.6%)
Carotid treated Left : Right		282 : 281
Mean time from event (months) [Range]		3.2 [0 – 13]
Treated within 1 month of symptoms		141 (25.1%)
Presenting event Retinal : Cerebral		144 : 419
Hypertensive *		376 (67%)
Ischaemic heart disease		199 (35.5%)
Hypercholesterolemia †		442 (78.8%)
Diabetes		99 (17.6%)
	Type II diabetes	87 (15.4%)
Smoking history		427 (75.7%)
	Current smoker	128 (22.8%)
Medication use at time of stent		
	Clopidogrel	413 (73.6%)
	Statin	381 (67.9%)
	Beta Blocker	77 (13.7%)
	ACE inhibitor / AII blocker	145 (25.7%)
Stent design	Open : Closed	92 : 470
	Carotid Wallstent	439 (77.9%)
	Precise	38 (6.7%)
Protection device used	Any device	362 (64.3%)
	Filter EZ devices	289 (51.2%)
Total amount of follow up		1945 patient years

* British Hypertension society guidelines (grade 1) [193] or on medication

† Total cholesterol > 5mmol/l

At four years of follow up a total of 34 ipsilateral strokes had occurred. Of these events 17 patients had suffered a minor ipsilateral stroke, 12 patients' major ipsilateral strokes and 5 stroke deaths had occurred. Applying Kaplan Meier survival analysis to the entire data set allows for censored and missing data to be taken into account and predicts ipsilateral stroke rates % (±SE) of 4.8% (±0.9), 7.0% (±1.1), 8.0% (±1.2), 8.5% (±1.2), 9.5% (±1.3), and 10.7% (±1.5) at 30 days, one, two, three, and four. and five years respectively. Stroke rates were then re calculated for the, "optimised," therapy of clopidogrel, statin and embolic protection as well as for each of the elements individually. The rates for patients considered high surgical risk by the SAPPHIRE trial criteria were also calculated and no difference was seen between groups considered to be high and low risk (p=0.592). The results are tabulated in **Table 2**.

Table 2 Kaplan Meier calculated Ipsilateral stroke free survival rates. (Comparison with Mantel Cox Log Rank test.)

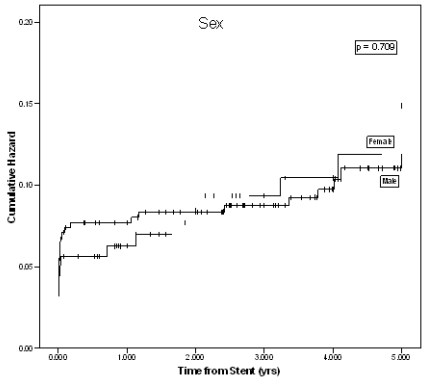
	Number of patients	30 days Stroke free survival % (SE)	1 year Stroke free survival % (SE)	4 years Stroke free survival % (SE)	Log rank p
Overall	563	95.2 (0.9)	93.0 (1.1)	90.5 (1.3)	
Optimal	296	97.3 (0.9)	95.9 (1.2)	95.2 (1.2)	**< 0.001**
Sub Optimal	267	92.9 (1.4)	89.9 (1.9)	85.8 (2.2)	
Protected	362	96.7 (0.9)	95.3 (1.1)	94.1 (1.3)	**< 0.001**
Unprotected	201	92.5 (1.9)	89.0 (2.2)	85.0 (2.6)	
Statin	381	97.4 (0.8)	95.2 (1.1)	93.8 (1.3)	**< 0.001**
No Statin	180	90.6 (2.2)	88.3 (2.4)	84.1 (2.8)	
Clopidogrel	413	97.3 (0.8)	95.6 (1.0)	94.1 (1.3)	**< 0.001**
No clopidogrel	148	89.2 (2.6)	85.8 (2.9)	81.3 (3.2)	
High Surgical Risk	240	95.4 (1.4)	94.1 (1.5)	91.3 (2.0)	0.592
Low Surgical Risk	323	95.7 (1.1)	92.2 (1.5)	89.9 (1.7)	
Trial patients	227	92.1 (1.8)	91.1 (1.1)	90.0 (2.0)	0.741
Non trial patients	336	94.6 (1.2)	94.3 (1.3)	90.8 (1.8)	

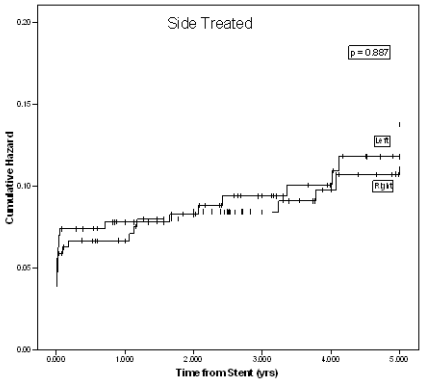
To compare with previous groups univariable survival analysis for end points of stroke, or stroke and vascular death, was performed using Kaplan Meier and Cox proportional hazards analysis (**Table 3)**. Crossing hazards on the Kaplan Meier plots were tested for chance by testing for an interaction with time, no interaction was seen. Statistically significant variables on the outcome of recurrent ipsilateral stroke included; mode of presentation (p= <0.001), presence of hypercholesterolemia (p=0.003), the use of clopidogrel (p=0.001), statins (p= <0.001), and protection devices (p= <0.001), the presence of a recurrent stenosis of >50 % (p=0.008) analysed as time dependant, and the calendar year of treatment (p=0.004). **Figure 1** shows the Kaplan Meier curves for these outcomes. Analysis was repeated for recurrent ipsilateral stroke or vascular death, and ipsilateral stroke or any death as outcome measures (**Table 3**).

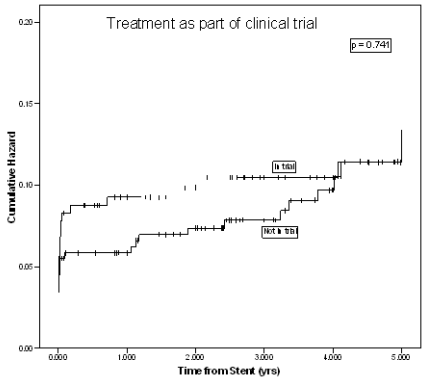
Table 3 Univariable assessment causing recurrent ipsilateral stroke, stroke and vascular death and stroke or any death with time post stent insertion. Kaplan Meier analysis for nominal variables, COX analysis for continuous and time dependant variables.

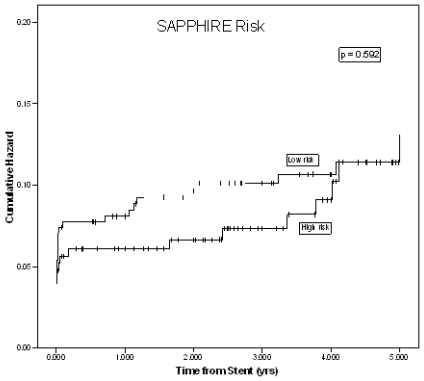
Variable		N	Ipsilateral Stroke Event rate / 1000 pt years follow up	p	Ipsilateral Stroke or Vasc. Death Event rate / 1000 pt years follow up	p	Ipsilateral Stroke or Any Death Event rate / 1000 pt years follow up	p
Sex	M : F	380 : 182	0.07 : 0.17	0.709	0.11 : 0.22	0.762	0.17 : 0.32	0.602
Side Treated	L : R	282 : 281	0.10 : 0.10	0.887	0.14 : 0.15	0.732	0.24 : 0.20	0.354
Clinical trial	Y : N	227 : 363	0.13 : 0.08	0.741	0.19 : 0.12	0.648	0.26 : 0.19	0.680
SAPPHIRE risk	Low : High	324 : 239	0.09 : 0.11	0.592	0.11 : 0.20	0.225	**0.15 : 0.35**	**0.004**
Age	<80 : >80	497 : 65	0.06 : 0.36	0.406	0.08 : 0.90	0.517	**0.11 : 1.91**	**0.002**
Presentation	Retinal : Cerebral	144 : 419	**0.05 : 0.08**	**0.001**	**0.10 : 0.12**	**< 0.001**	**0.22 : 0.18**	**< 0.001**
Stent design	Open : Closed	91 : 470	0.27 : 0.06	0.541	0.46 : 0.09	0.958	0.53 : 0.14	0.261
Hypertension	Y : N	376 : 185	0.07 : 0.17	0.497	0.11 : 0.23	0.793	0.17 : 0.34	0.948
IHD	Y : N	199 :362	0.15 : 0.08	0.718	0.24 : 0.10	0.284	**0.42 : 0.14**	**0.008**
Hypercholesterolemia	Y : N : DK	442 : 86 : 33	**0.05 : 0.68 : 1.73**	**< 0.001**	**0.06 : 0.89 : 2.38**	**< 0 .001**	**0.11 : 1.44 : 3.03**	**< 0.001**
Smoking history	Y : N	427 : 134	0.06 : 0.24	0.484	0.09 : 0.32	0.667	0.15 : 0.45	0.948
Diabetes	Y : N	99 : 462	0.46 : 0.05	0.056	**0.73 : 0.08**	**0.002**	**1.03 : 0.12**	**0.002**
Clopidogrel	Y : N	413 : 148	**0.04 : 0.33**	**< 0.001**	**0.07 : 0.41**	**< 0.001**	**0.14 : 0.55**	**0.002**
Statin	Y : N	381 : 180	**0.04 : 0.25**	**< 0.001**	**0.07 : 0.35**	**< 0.001**	**0.12 : 0.51**	**<0.001**
Beta Blocker	Y : N	77 : 484	0.50 : 0.06	0.404	0.55 : 0.08	0.925	0.82 : 0.13	0.948
ACE / AII inhibitors	Y : N	145 : 416	0.13 : 0.07	0.098	0.21 : 0.10	0.148	0.32 : 0.16	0.089
Protection Device	Y : N	362 : 208	**0.05 : 0.20**	**< 0.001**	**0.08 : 0.28**	**< 0.001**	**0.15 : 0.37**	**0.007**
		Mean	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Age	Years	68.53	0.994 (0.965 – 1.024)	0.708	1.020 (0.994 – 1.046)	0.129	**1.037 (1.016 – 1.059)**	**0.001**
Delay to treatment	Months	3.24	1.101 (0.980 – 1.237)	0.106	1.079 (0.976 – 1.194)	0.137	1.024 (0.940 – 1.114)	0.589
Restenosis > 50 %			**0.412 (0.215 – 0.789)**	**0.008**	**0.511 (0.270 – 0.965)**	**0.038**	1.626 (0.886 – 2.985)	0.117
Year of treatment			**0.843 (0.770 – 0.924)**	**< 0.001**	**0.860 (0.796 – 0.929)**	**< 0.001**	**0.928 (0.873 – 0.987)**	**0.017**

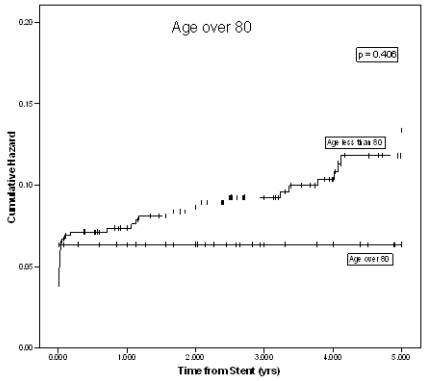
Figure 1 Kaplan Meier Curves of variables assessed in Univariable analysis for the outcome of recurrent ipsilateral stroke. Comparison between variables with Log Rank statistic

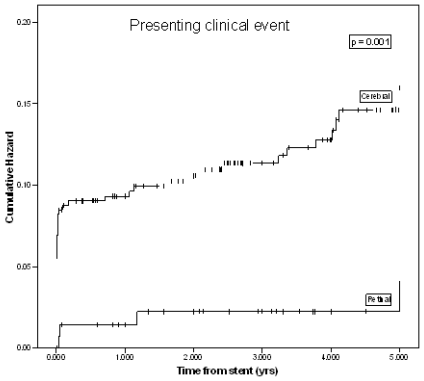


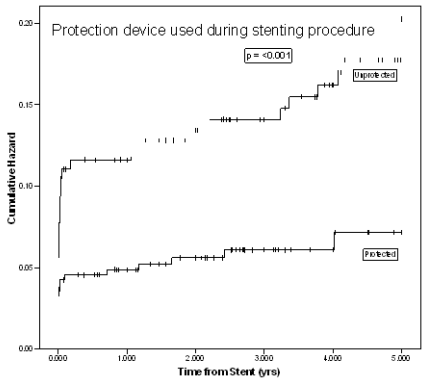


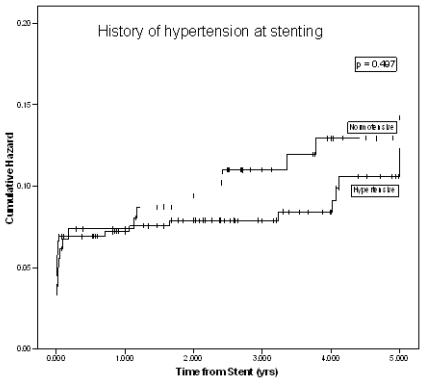


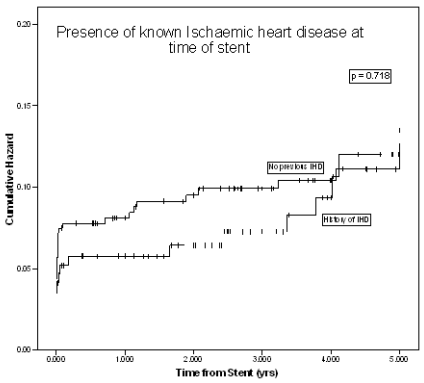


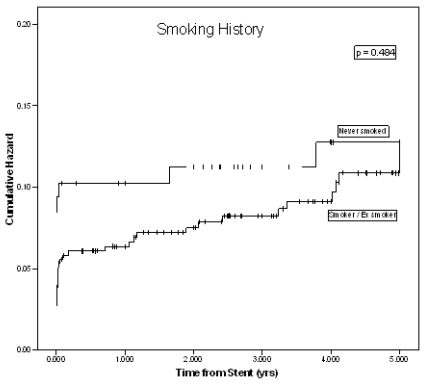


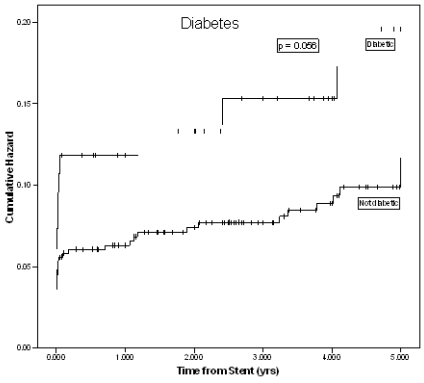


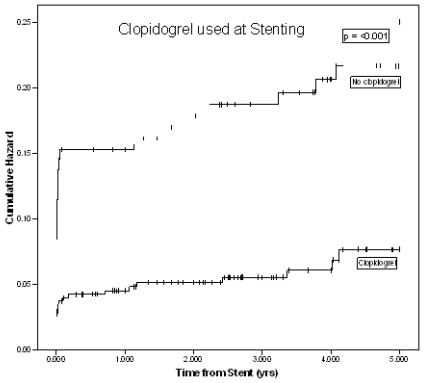


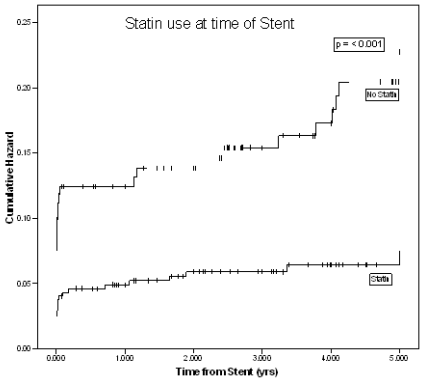


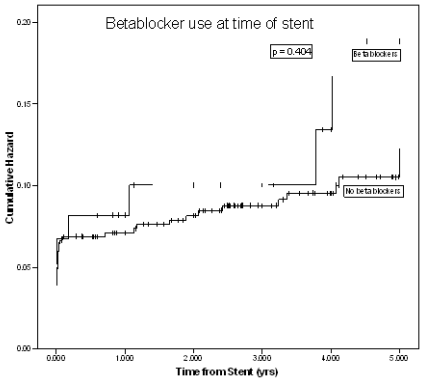

Presence of known Ischaemic heart disease at time of stent
p = 0.718
No previous IHD
History of IHD
Cumulative Hazard
Time from Stent (yrs)

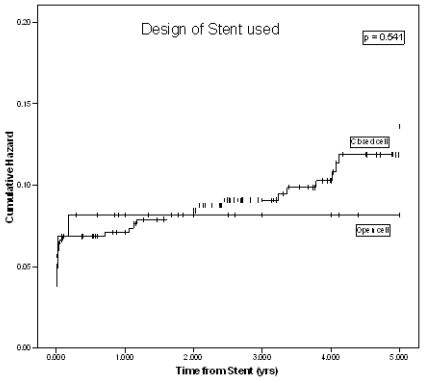

Smoking History
p = 0.494
Never smoked
Smoker / Ex smoker
Cumulative Hazard
Time from Stent (yrs)

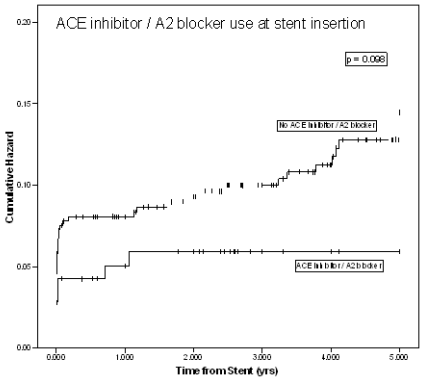

Diabetes
p = 0.066
Diabetic
Not diabetic
Cumulative Hazard
Time from Stent (yrs)


Clopidogrel used at Stenting
p = <0.001
No clopidogrel
Clopidogrel
Cumulative Hazard
Time from Stent (yrs)


Statin use at time of Stent
p = < 0.001
No Statin
Statin
Cumulative Hazard
Time from Stent (yrs)


Betablocker use at time of stent
p = 0.404
Betablockers
No betablockers
Cumulative Hazard
Time from Stent (yrs)


Design of Stent used
p = 0.541
Closed cell
Open cell
Cumulative Hazard
Time from Stent (yrs)

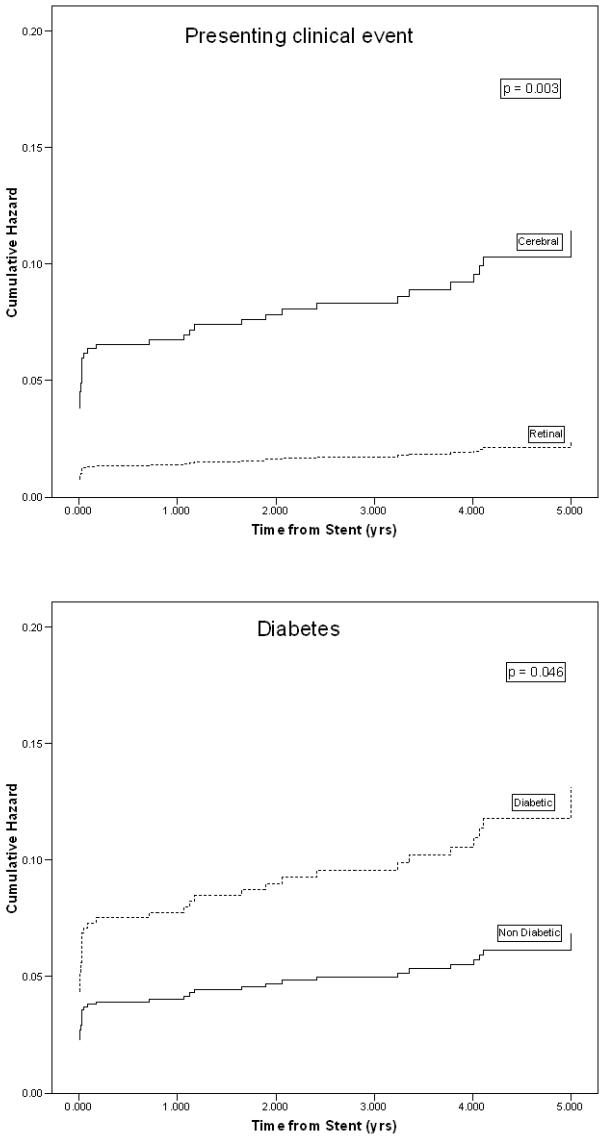

ACE inhibitor / A2 blocker use at stent insertion
p = 0.098
No ACE inhibitor / A2 blocker
ACE inhibitor / A2 blocker
Cumulative Hazard
Time from Stent (yrs)

Multivariable analysis was then applied to all the variables included in table 3 after screening for confounders a $p \leq 0.10$ was used as the cut off for backward selection, the remaining significant variables that were reassessed are shown in (**Table 4)**. Retinal presentations (HR = 0.228, CI 0.082 – 0.632, p = 0.004) had lower risk of recurrent stroke, and the presence of persistent or recurrent stenosis of greater than 50% (HR = 2.187, CI 1.173 – 4.078, p = 0.014) was a risk factors for recurrent ipsilateral stroke. Clopidogrel use during the procedure has an impact on reducing the risk for recurrent ipsilateral stroke (HR = 0.318 CI 0.185 – 0.545, p = <0.001). A further analysis was undertaken with calendar year of the procedure forced into the model but made no difference to the outcome.

Table 4 Multivariable assessment using Cox proportional hazards assessment of independent contribution towards further ipsilateral stroke, stroke or vascular death and stroke and any death post stent insertion.

Variable	Ipsilateral Stroke			Ipsilateral Stroke or Vascular death			Ipsilateral Stroke or death		
	HR	95% CI	P	HR	95% CI	p	HR	95% CI	p
Age				1.019	(0.994 - 1.045)	0.139	**1.031**	**(1.010 - 1.053)**	**0.004**
Retinal Presentation	**0.228**	**(0.082 - 0.632)**	**0.004**	**0.278**	**(0.128 - 0.605)**	**0.001**	**0.460**	**(0.271 - 0.780)**	**0.004**
Diabetes	1.693	(0.919 - 3.118)	0.091	**2.091**	**(1.267 - 3.451)**	**0.004**	**1.921**	**(1.269 – 2.908)**	**0.002**
Clopidogrel	**0.318**	**(0.185 – 0.545)**	**< 0.001**						
Statin							**0.424**	**(0.296 - 0.608)**	**< 0.001**
Beta Blocker	1.655	(0.805 – 3.403)	0.170						
Restenosis > 50%	**2.187**	**(1.173 – 4.078)**	**0.014**	1.148	(0.603 – 2.186)	0.674	**0.370**	**(0.177 – 0.775)**	**0.008**

Figure 2 Multivariable COX analysis of variables impact on long term recurrence of ipsilateral stroke.



Inspecting the Kaplan Meier curves suggests that 30 day outcomes have significant impact on long-term outcomes in common with previous publications.[174, 187, 188] Univariable and multivariable analysis of 30 day outcomes was performed to identify these variables. The results of the univariable analysis can be seen in **table 5** Multivariable logistic regression analysis showed cerebral presentation to have a hazard 6 times greater than retinal presentation (HR 6.668 CI 1.498 – 26.689, p = 0.013). Clopidogrel use (HR 0.291 CI 0.100 – 0.841, p = 0.023) was shown to be an independent variable lowering ipsilateral stroke recurrence at 30 days with Diabetes increasing the risk of an adverse event (HR 2.361 CI 1.052 – 5.302, p = 0.037) (**Table 6**).

Table 5 Univariable assessment of variables influence on 30 day outcomes from stroke and stroke and vascular death. (Chi square, Fishers and Student T test)

Variable		Number of patients	Ipsilateral Stroke		Ipsilateral Stroke or Vasc Death		Ipsilateral Stroke or any Death	
			% stroke rate	p	% stroke rate	P	% stroke rate	p
Sex	M : F	380 : 182	6.8 : 5.5	0.587	7.4 : 6.0	0.723	7.6 : 6.6	0.731
Side treated	L : R	282 : 281	7.1 : 5.7	0.606	7.8 : 6.1	0.507	8.5 : 6.1	0.331
Clinical trial	Y : N	227 : 363	7.9 : 5.4	0.226	8.4 : 6.0	0.311	8.4 : 6.6	0.415
SAPPHIRE risk	Low : High	324 : 239	7.1 : 5.5	0.489	7.4 : 6.3	0.737	8.0 : 6.3	0.513
Age	<80 : >80	497 : 65	6.5 : 6.2	0.927	6.9 : 7.7	0.795	7.5 : 6.2	0.704
Presentation	Retinal : Cerebral	144 : 419	**1.4 : 8.1**	**0.003**	**1.4 : 8.8**	**0.001**	**2.8 : 8.8**	**0.015**
Stent design	Open : Closed	91 : 470	6.7 : 6.4	0.891	7.0 : 6.7	0.904	6.7 : 7.4	0.795
Hypertension	Y : N	376 : 185	6.7 : 5.9	0.855	7.5 : 5.9	0.598	7.7 : 6.5	0.731
IHD	Y : N	199 :362	5.1 : 7.2	0.372	6.1 : 7.5	0.605	6.6 : 7.7	0.735
Hypercholesterolemia	Y : N : DK	442 : 86 : 33	**4.5 : 11.6 : 18.2**	**0.001**	**5.0 : 11.6 : 21.2**	**< 0.001**	**5.2 : 12.8 : 21.2**	**< 0.001**
Smoking Hx	Y : N	427 : 134	5.4 : 9.7	0.104	**5.6 : 11.2**	**0.038**	6.3 : 10.4	0.128
Diabetes	Y : N	99 : 462	**11.1 : 5.4**	**0.043**	11.1 : 6.1	0.083	10.1 : 6.7	0.285
Clopidogrel	Y : N	413 : 148	**3.6 : 14.2**	**< 0.001**	**4.1 : 14.9**	**< 0.001**	**4.4 : 15.5**	**< 0.001**
Statin	Y : N	381 : 180	**3.9 : 11.7**	**0.001**	**4.5 : 12.2**	**0.001**	**4.7 : 12.8**	**0.001**
Beta Blocker	Y : N	77 : 484	6.6 : 6.4	0.954	7.0 : 6.6	0.887	6.6 : 7.4	0.789
ACE / AII block	Y : N	145 : 416	4.1 : 7.2	0.239	4.1 : 8.0	0.133	4.8 : 8.2	0.200
Protection device	Y : N	362 : 208	**4.2 : 10.4**	**0.006**	**4.7 : 10.9**	**0.009**	**5.0 : 11.4**	**0.006**
Age	Years			0.243		0.200		0.259
Delay to Tx	Months			0.301		0.245		0.228
Year of treatment				**0.010**		**0.017**		**0.001**

Table 6 Multivariable analysis of variables effect on 30 day outcome from stroke, stroke and vascular death and stroke and death. Multiple logistic regression analysis.

Variable		Number of patients	Ipsilateral Stroke		Ipsilateral Stroke or Vascular		Ipsilateral Stroke or any Death	
			% stroke rate	p	% stroke rate	P	% stroke rate	P
Sex	M : F	380 : 182	6.8 : 5.5	0.587	7.4 : 6.0	0.723	7.6 : 6.6	0.731
Side treated	L : R	282 : 281	7.1 : 5.7	0.606	7.8 : 6.1	0.507	8.5 : 6.1	0.331
Clinical trial	Y : N	227 : 363	7.9 : 5.4	0.226	8.4 : 6.0	0.311	8.4 : 6.6	0.415
SAPPHIRE risk	Low : High	324 : 239	7.1 : 5.5	0.489	7.4 : 6.3	0.737	8.0 : 6.3	0.513
Age	<80 : >80	497 : 65	6.5 : 6.2	0.927	6.9 : 7.7	0.795	7.5 : 6.2	0.704
Presentation	Retinal : Cerebral	144 : 419	**1.4 : 8.1**	**0.003**	**1.4 : 8.8**	**0.001**	**2.8 : 8.8**	**0.015**
Stent design	Open : Closed	91 : 470	6.7 : 6.4	0.891	7.0 : 6.7	0.904	6.7 : 7.4	0.795
Hypertension	Y : N	376 : 185	6.7 : 5.9	0.855	7.5 : 5.9	0.598	7.7 : 6.5	0.731
IHD	Y : N	199 :362	5.1 : 7.2	0.372	6.1 : 7.5	0.605	6.6 : 7.7	0.735
Hypercholesterolemia	Y : N : DK	442 : 86 : 33	**4.5 : 11.6 : 18.2**	**0.001**	**5.0 : 11.6 : 21.2**	**< 0.001**	**5.2 : 12.8 : 21.2**	**< 0.001**
Smoking Hx	Y : N	427 : 134	5.4 : 9.7	0.104	**5.6 : 11.2**	**0.038**	6.3 : 10.4	0.128
Diabetes	Y : N	99 : 462	**11.1 : 5.4**	**0.043**	11.1 : 6.1	0.083	10.1 : 6.7	0.285
Clopidogrel	Y : N	413 : 148	**3.6 : 14.2**	**< 0.001**	**4.1 : 14.9**	**< 0.001**	**4.4 : 15.5**	**< 0.001**
Statin	Y : N	381 : 180	**3.9 : 11.7**	**0.001**	**4.5 : 12.2**	**0.001**	**4.7 : 12.8**	**0.001**
Beta Blocker	Y : N	77 : 484	6.6 : 6.4	0.954	7.0 : 6.6	0.887	6.6 : 7.4	0.789
ACE / AII block	Y : N	145 : 416	4.1 : 7.2	0.239	4.1 : 8.0	0.133	4.8 : 8.2	0.200
Protection device	Y : N	362 : 208	**4.2 : 10.4**	**0.006**	**4.7 : 10.9**	**0.009**	**5.0 : 11.4**	**0.006**
Age	Years			0.243		0.200		0.259
Delay to Tx	Months			0.301		0.245		0.228
Year of treatment				**0.010**		**0.017**		**0.001**

iv) Discussion of the Sheffield analysis.

Publications in press from the randomised controlled trials suggest that in the long term carotid stenting appears to be as durable as carotid endarterectomy in preventing future vascular events despite a higher 30 day risk.[175, 176] They highlight that 30 day peri-operative event rates contribute to the majority of the excess recurrence in stenting.[175] Our Kaplan Meier stroke free survival rates for the complete dataset 4.8% (±0.9), 7.0% (±1.1), 8.0% (±1.2), 8.5% (±1.2), 9.5% (±1.3), and 11.3% (±1.5) at 30 days, one, two, three, four. and five years respectively compare favourably with the data from the RCT'S of carotid stenting and surgery, suggesting the durability of the procedure can be maintained when patients not conforming to RCT entrance criteria are included in outcome analysis.[26, 40, 175, 176, 185]

Outside of a randomised controlled trial patient selection is less rigorous and exclusive, and comparative cohort studies such as this one are useful to support data from RCT's in real life situations by including patients not eligible for trials. To date one other cohort study stenting purely symptomatic patients, with a mean follow up 25 months, has been published.[183] The focus of this study was on the impact of stents to prevent restenosis in place of angioplasty and not to identify the alternative risk factors undertaken in this analysis. Stent use did significantly reduced rates of restenosis of >70% but did not affect long-term outcome measures.[183] However, the role of restenosis on recurrent outcomes in stented patients has yet to be proven, and the SPACE and SAPPHIRE groups disagree over its rate in comparison to endarterectomy.[180]

The purpose of our analysis was to look at multiple factors affecting long term stroke free survival, using elements suggested previously to affect outcome to see if they are true independent predictors of recurrent events.[186] Our long-term experience of stenting symptomatic carotid vessels presented here provides evidence for the combined benefits of the individual elements now making up the perceived minimum standard of care for a stenting procedure, but individual effects of pharmacological agents on recurrent events were of particular interest.[9, 194] Numerous modifications to the carotid stenting procedure have occurred in a short space of time making determination of the individual changes difficult to detect any other way. It would now be impossible to reverse developments and assess each change individually. The heart protection study in 2002 [195] showed that all stroke or TIA patients, and therefore any patient requiring carotid stenting for a symptomatic carotid stenosis, should be offered statin therapy. Therefore, the effects of statin therapy on the long term outcomes from stenting can now only be assessed with this form of multivariable analysis not a randomised controlled trial. Dual antiplatelet therapy with aspirin and clopidogrel is another example that has become standard therapy at our institution from 2002 after clear benefit of the regime on 30 day outcomes was shown in a randomised trial comparing with aspirin and heparin.[9]

Multivariable analysis in this case showed only clopidogrel had clear independent benefit on the outcomes of ipsilateral stroke and vascular death in the long term follow up, despite statin and clopidogrel therapy appearing to be beneficial in univariate analysis. We have not, as we had hoped, been able to separate out individual effects of the other pharmacological therapies and this may never be possible as most were introduced around the same time. The impact of clopidogrel on long term outcomes is likely to be due to its dramatic effects at 30 days. This raises questions about the

possible benefits of prolonging post stenting clopidogrel therapy to see if this benefit can be extended longer.[152]

In common with the endarterectomy trials we have confirmed that retinal events have a significantly lower risk of future recurrence than cortical events.[186, 196] A significant proportion of this is due to the effect on 30 day outcomes shown here and by other authors.[187] Our analysis has also highlighted that recurrent stenosis of greater than 50% is likely to contribute as an independent risk factor for an event. This effect appears to date from the time of the procedure in some cases and therefore must represent residual stenosis from the procedure as restenosis should not have developed in this time. Although this is likely to be the case we cannot call this residual stenosis as we have no data to show that the stenosis dates exactly from the time of the procedure because post stenting angiograms were not reviewed as part of the analysis. The first imaging data we have available on the patients is at 30 day follow up and it is conceivable although unlikely that stenosis developed in this short time. In the case of the early stents, which were not carotid specific and have been shown to have a liability to collapse this could be the case. [92, 93]

If this can be confirmed in analysis of another co-hort it suggests very strongly to all carotid stenting practitioners that they should not leave a high grade residual stenosis. It is also noted that statistical analysis of our patients is suggesting that restenosis may be protective against the combined end point of recurrent stroke and all death. However, this is most likely a statistical anomaly and probably reflects the fact that death from other cause is affecting a greater proportion of these patients at 4 year follow up diluting the impact of the restenosis on the outcome of stroke.

This analysis has investigated variables only previously subjected to univariable analysis in other studies. The impact of pre existing ischaemic heart disease on any of the outcome measures suggested by previous studies [182] has not been demonstrated. Increasing age has also been previously shown to be a risk factor for 30 day adverse events, [174, 197] but this was not confirmed in our series analysed as either a continuous or nominal (age >80) variable for short and long-term outcomes in common with another recently published series.[189] What is clear from this analysis is that detection of effects from individual changes in procedural technique is not likely to be possible with data available to us. However, when the therapies are analysed as a combination the impact on outcome is dramatic in the long term. Prior to all patients receiving dual antiplatelet therapy, statin therapy and the routine use of a protection device 7.2 % of patients experienced recurrent ipsilateral stroke at 30 days, when these therapies became standard the rates reduced to 2.6%. The benefits are then continued into long term follow up and impact on the Kaplan-Meier calculated outcomes at one, two and three years. It is therefore important that the effect of these procedural developments is considered when analysis of the randomised trials is performed and may explain some of the differences demonstrated between endarterectomy and stenting at 30 days in SPACE, EVA3S and the International Carotid Stenting Study (ICSS) recently presented at the European Stroke Conference.

Previous cohort studies had limitations that we attempted to minimise in our analysis by using larger numbers, purely symptomatic patients, and longer follow up. Our study analyses symptomatic patients and in a centre performing endovascular carotid intervention from 1993 and carotid stenting from 1995. This minimises the, "learning curve effect," on the outcomes in most of the patients described here.[198, 199] The year the procedure was undertaken was forced into the multivariable analysis to account for

learning and changing technique and was not shown to be independently significant. Our study is not without limitations. Prospectively collected data in sequential patients overcomes recall bias, but missed patients introduce selection bias. Our cohort also includes symptomatic patients felt to be unsuitable for carotid endarterectomy due to high surgical risk, anatomical or morphological reasons, but this did not seem to affect outcomes when analysed by inclusion in a trial or by SAPPHIRE risk.[174-176] Our patients were reviewed by a neurologist, not the interventionalists, after the procedure to reduce under reporting of outcomes and may have lead to higher detection rate of minor neurological events.

This study is presently the most extensive multivariable cohort analysis of purely symptomatic patients under follow up and has attempted to assess the impact of restenosis and time to restenosis. Patients treated as part of this cohort study have been treated at varying time points from the time of the event. Patients treated more recently have been treated within 4 weeks, but we do accept that some of the earlier patients treated were several months after the time of their event and in a more modern classification would be considered to be asymptomatic which may have an impact on the outcome rates. However, the study remains the largest of its kind currently published and highlights some interesting points to be investigated in the larger cohorts in the randomised trials.

The effects of individual procedural changes are too small to detect but it suggests optimal patient selection and combination therapy has the most significant impact on long-term outcome and 30 day complications. The issue of residual or restenosis of greater than 50% has been highlighted as a possible risk factor for recurrent stroke

events and needs more investigation, but in the first instance practitioners should ensure that they leave as little residual stenosis as possible.

3 Routine stenting of asymptomatic carotid stenosis pre CABG - Safety and suitability of this procedure from analysis of the Sheffield registry.

i) Introduction

Perioperative stroke and ischaemic encephalopathy are two well recognised complications of coronary artery bypass grafting and heart valve replacement surgery. The risk of stroke in the perioperative period has remained around 2% for the past three decades. The presence of carotid disease in patients undergoing coronary artery bypass surgery has been shown to increase this risk of perioperative stroke from the cardiac procedure threefold.[200] This finding provided a logical reason for the initial trials of combined or staged carotid endarterectomy in these patients in an attempt to reduce peri-operative mortality. The contribution of the carotid disease in direct causation of perioperative stroke has been difficult to prove. It has been hypothesised that significant carotid disease exacerbated the effects of periods of intra operative hypotension during cardiac bypass, resulting in relative cerebral hypoperfusion causing intra-operative stroke. However, it is also conceivable that the presence of high grade carotid disease is purely an indicator of higher cardiovascular morbidity in these patients. Intra operative monitoring using Transcranial Doppler ultrasound has demonstrated that athero-embolism occurring as a result of aortic cross clamping is common and also contributes to the intra operative stroke risk.[201] It is conceivable, although unproven that stenotic carotids may have protected a sub group of patients from embolic neurological complications. What is now clear is that the aetiology of

stroke during cardiac surgery is multi-factorial and carotid stenosis is likely to play only a minor part.[202]

Despite this some cardiac surgeons remain reluctant to operate on patients with carotid disease and recommend combined or staged carotid endarterectomy in all patients with carotid artery disease to reduce the risks of perioperative stroke. Other centres reserve this procedure only for patients with recent neurological symptoms.[203] Supportive data in the form of a randomised controlled trial is lacking for either of these management strategies. The most recent systematic review of case series of carotid endarterectomy studies suggested that little or no benefit for stroke prevention appeared to be gained by performing staged or synchronous procedures in asymptomatic individuals undergoing on pump cardiac surgery.[204]

Carotid angioplasty as an alternative approach to managing carotid disease in these patients was originally proposed in 1997 with two published case series of balloon deployed carotid stents and a further retrospective series in 2002.[93, 205, 206] These small case series highlighted the fact that although technically feasible neurological complications continued to occur both at time of stenting and during cardiac surgery. Our centre has performed a staged carotid stenting procedure for significant carotid artery stenosis in patients listed for coronary artery bypass since April 1998. Stenting techniques have developed significantly since 1998 with reductions in morbidity and mortality. However, the effect the stenting procedure has on the overall morbidity and mortality following coronary artery bypass surgery remains unclear. We reviewed our recent experience of this two staged approach in an attempt to improve outcomes from all our stenting procedures.

ii) Methods

All patients since 1998 found to have a significant carotid artery stenosis on duplex ultrasound criteria during pre assessment for cardiac surgery were referred to the combined Vascular Neuroradiology multi disciplinary team. It should be noted that routine carotid duplex ultrasound screening of all patients prior to cardiothoracic procedures is not performed by all surgeons at our institution, and therefore not all patients with carotid stenosis meeting our criteria for intervention would have been offered stenting and be included in this review. This sample population is therefore a non randomised partially selected group which will bias the outcome assessment to a degree. Patients referred to the multidisciplinary team were deemed appropriate for carotid intervention if they had a severe carotid stenosis of greater than 80% on the side of the dominant hemisphere and a combined stenosis of the carotid arteries of more than 150%; a recently symptomatic carotid of greater than 70%, or contra lateral occlusion and moderate stenosis >50% . All assessments were performed using the NASCET criteria.[26] These patients then underwent arch angiography to assess the origins of the carotid vessels and to confirm the degree of stenosis, additional images to confirm circle of Willis anatomy were not routine in pre assessment work up of these patients. A limited number of patients did undergo MRI scanning as part of a separate ongoing MRI cerebral perfusion study, but results from these investigations were not used to make treatment decisions.[207] Where treatment was being recommended for a combined stenosis of the carotid vessels of greater than 150% the artery supplying the dominant hemisphere was preferentially recommended for stenting. If a vessel was found to have been symptomatic within the preceding 6 months, on further detailed questioning, it was chosen for stenting. Since 1998 carotid stenting has been the only management strategy used in our institution to treat carotid artery stenosis meeting these criteria. If patients

were unsuitable for stenting a staged or combined carotid endarterectomy was not performed as an alternative. The timing of cardiac surgery following the stenting procedure was at the discretion of the cardiac surgeon. Following the introduction of routine use of clopidogrel in carotid stenting it was recommended that this was continued for at least 14 days. Statistical analysis was performed using SPSS 12.01 software.

Stenting technique

All procedures were performed by an interventional radiologist (Dr T. Cleveland, Prof P. Gaines). During the study period the endovascular technique has evolved to include the routine use of dual antiplatelet therapy with aspirin and clopidogrel prior to stent insertion as well as the use of filter cerebral protection devices. The carotid stenting technique used in our centre has been previously described in peer reviewed literature.[4] Procedures performed in our institution since November 2002 use glycopyrolate 600 micrograms in place of intra arterial atropine to prevent peri-procedural bradycardia and hypotension. The 8mm or 10mm Monorail Carotid Wallstent (*Boston Scientific Corps*) was used in 90% of these procedures.

Outcome assessment

All patients were assessed prior to treatment, at discharge, and 30 days following the procedure by an independent stroke neurologist (Dr M Randall, Dr F. McKevitt, and Prof G. Venables). Outcome events occurring prior to 30 days, because of an intervening cardiac procedure were also classified by a neurologist. Outcome measures including all neurological complications occurring within the first 30 days of the

procedure and those associated with the cardiac procedure were included in this study. Yearly follow up from the date of the procedure has been maintained wherever possible with direct clinical assessment, or by telephone interview and review of hospital notes and death certification. Consent for data collection and storage of information on a computerised database was obtained from each patient at the time of the procedural consent. This database is registered for audit purposes in accordance with the audit procedures of our institution.

Neurological complications

Minor stroke was defined as a new neurological deficit lasting more than 24 hours but less than 7 days. Major stroke was defined as a new neurological deficit that persists more than 7 days. This classification has then been subdivided at 30 days post event into non-disabling stroke, if the Oxford modified handicap score was 0-2, or disabling stroke, if the Oxford modified handicap score was equal or greater than 3.[191]

iii) Results

65 patients were referred to the multi disciplinary team for assessment of staged carotid stenting prior to coronary revascularisation between April 1998 and July 2005. 52 patients had significant carotid artery disease according to our intervention criteria that was treated prior to cardiac surgery. The 13 remaining patients were referred back to their cardiothoracic surgeon with a recommendation to proceed without carotid intervention as they were shown to have combined stenosis of less than 150% at arch angiography. Additional history obtained at the time of stenting revealed that 7.7% (4/52) of our patients had transient neurological symptoms within the preceding 6 months and therefore in 3 cases were treated despite the combined stenosis being less than 150%. All patients were due to undergo cardiac bypass surgery and two patients required aortic valve replacement as well. Patient demographics of the treated patients are summarised in **table 7**.

Table 7 Baseline Characteristics of patients undergoing stenting pre CABG

	n = 52
Age (Mean)	68
Male : Female	45 : 7
Carotid treated Left : Right	36 : 16
Delay between Carotid and Cardiac operation. (range)	2 – 60 days
Combined stenosis (Mean/Median/Range) %	165 / 170 / 105 – 195
Treated vessel stenosis (Mean/Median/Range) %	83 / 80 / 65-99
Contra lateral pre occlusive 99% or occluded 100%	20 (38.4%)
Symptoms within 6 months	4 (7.6%)
Hypertensive	40 (76.9%)
Hypercholesterolemia *	47 (90.4%)
Diabetes	16 (30.7%)
Smoker (Current or previous)	46 (88.4%)
Technical	
Carotid Wallstent	47 (90%)
Protection device used	36 (69.2%)
Combined Aspirin and Clopidogrel	43 (82.7%)

* Total cholesterol > 5mmol/l

30 day follow up was performed in all patients with 94.2% (49/52) undergoing the cardiac surgical procedure that had been proposed following stenting. Our long term follow up data ranges from 30 days to 5 years with a mean follow up of 2.1 years. All 52 patients remained event free during and immediately following the carotid stenting procedure. **Table 8** summarises outcome events from both the stenting and cardiac bypass procedure. At the time of 30 day follow up, excluding events related to any cardiac procedure performed within 30 days of stenting, one patient had died. This event occurred at 24 days during sleep at home, no post mortem was performed, and heart failure prior to her CABG and valve replacement procedure was the presumed cause of death. This was the only event occurring not related to a cardiac procedure occurring with 30 days of stenting. Two further cardiac deaths occurred prior to the cardiac surgery being performed at 56 days, and 59 days respectively. One patient experienced delay to cardiac surgery due to worsening cardiac function, and the other suffered a fatal myocardial infarction, at another institution, 3 days prior to his cardiac surgery, 59 days following the stenting procedure.

Combined 30 day outcomes for the carotid stenting procedure and cardiac procedure include 3 strokes (1 minor, and 2 major non-disabling) all ipsilateral to the stented vessel, 2 cardiac deaths and 2 stroke related deaths (one ischaemic and one haemorrhagic) occurring in the 30 day post operative period for the cardiac procedure. Only one of these procedural strokes occurred at the time of the cardiac operation, this patient was unrousable following anaesthetic and eventually died 28 days later. The one minor and 2 major strokes occurred between 24 hours and 48 hours after the bypass procedure. On an intention to treat basis and including all stroke, cardiac death, and deaths in those patients awaiting treatment the overall event rate for stroke and all death is 19.2% (10/52) with an ipsilateral stroke and stroke death rate of 7.7% (4/52). The

stroke and all cause mortality for patients that underwent both procedures is 14.3% (7/49) with ipsilateral stroke and stroke death rate being 8.2% (4/49).

Table 8 Outcomes from combined carotid stent and CABG procedures

	Events within 30 days of stenting prior to CABG		Events greater than 30 days post stent prior to CABG		Peri-operative events within 30 days of CABG		Combined total outcome all events	
	% (n)	95% CI	% (n)	95% CI	% (n)	95% CI	% (n)	95% CI
Minor Stroke	0	0 - 5.7	0	0 – 5.7	1.9 (1)	0.3 – 10.1	1.9 (1)	0.3 – 10.1
Major Stroke	0	0 – 5.7	0	0 – 5.7	3.8 (2)	1.0 – 12.9	3.8 (2)	1.0 – 12.9
Stroke Death	0	0 – 5.7	0	0 – 5.7	3.8 (2)	1.0 – 12.9	3.8 (2)	1.0 – 12.9
Other Death	1.9 (1)	0.3 – 10.1	3.8 (2)	1.0 – 12.9	3.8 (2)	1.0 – 12.9	9.6 (5)	4.1 – 20.6
Stroke and Death	**1.9 (1)**	0.3 – 10.1	**3.8 (2)**	1.0 – 12.9	**13.5 (7)**	6.6 – 25.2	**19.2 (10)**	10.7 – 31.8

iv) Discussion

The benefits of carotid intervention in symptomatic carotid disease are well documented and accepted.[26, 27] The recently published asymptomatic carotid surgery trial has now highlighted the benefits that endarterectomy may offer in a selected group of patients diagnosed with asymptomatic carotid stenosis.[208] The place for carotid intervention in asymptomatic patients routinely prior to coronary artery bypass grafting and cardiac valve replacement remains controversial particularly as it is still unclear what additional workload carotid intervention prior to coronary artery bypass surgery would place on overstretched vascular services. The most recent statistics compiled by the Society of Cardiothoracic Surgeons of Great Britain and Ireland show that 25,277 CABG procedures were performed in the UK in the year 2003. Limited angiographic data is available on the frequency of concurrent carotid disease in those patients undergoing CABG so direct estimates of the number of these patients requiring intervention cannot be made. However, it is known that the prevalence of significant carotid disease (>50%) in patients with triple vessel coronary disease is approximately 25% [209] which suggest that up to 6000 procedures may be required per year in the UK were benefit to be shown from carotid intervention.

The most robust data set available in this regard is the recently published systematic review of case series and trials of endarterectomy and coronary surgery performed by Naylor et al.[204, 210] This work confirmed the historical data in Brener's paper from 1987.[200] They demonstrated that stroke risk is less than 1.8 % in patients with no significant stenosis (< 50%) increasing to 5.2% in patients with bilateral carotid disease and 11.2% in patients with a unilateral carotid occlusion. This historical outcome data can be compared with studies assessing patients that have undergone combined and

staged carotid endarterectomy or stenting. Published case series and systematic reviews report complication rates of stroke and death with combined and staged procedures ranging from 2.4% to 17.7%.[211-216] Naylor's systematic review suggests that the 30 day stroke risk, including both operative procedures, is 5.4% (CI 3.4–7.2) for a combined and 3.7% (CI 1.8-5.5) for a staged procedure.[204] If we are to include stroke and death from any cause the respective rates become 9.5% (CI 7.2-11.8) for synchronous and 6.6% (CI 4.4-8.8) for a staged procedure.[204] These figures were derived from the selected and limited data set available in the literature, and are therefore inherently affected by publication bias, but provided little justification for pre-operative carotid intervention with endarterectomy in patients with moderate bilateral carotid disease as the complication rates for procedures are not statistically different from the natural history data available.

The use of carotid angioplasty or stenting as an alternative to carotid endarterectomy prior to cardiac surgery has been proposed as a less risky carotid revascularisation strategy. The literature on carotid stenting contains numerous case series where stenting is being performed as an alternative to carotid endarterectomy in, "high risk," patients outside of randomised trials. The recently published SAPPHIRE trial of stenting in high risk patients included more than 80% of its patients from those with ischaemic heart disease.[179] The more specific literature highlighting those patients in whom the, "high risk," indication for stenting was imminent cardiac surgery is limited to three published case series with a combined patient population of 109 patients.[93, 205, 206]

Outcome events occurred in 8.25% (9/109) of these patients of which 4 were strokes, one resulting in death. The strokes in these reports all occurred in relation to the stenting procedure with 6 additional deaths following the cardiac procedure. Our

combined minor stroke, major stroke and death rate for our study group of 19.2% (10/52) on an intention to treat basis is higher than previously documented in the literature. What is not clear from previous literature is whether the 3 deaths that occurred prior to the cardiac procedure in our series would have been included in the analysis of these previous studies. The ipsilateral stroke and stroke death rate of 7.7% (4/52) compares favourably (Fishers exact p=0.0192) with the previously published results of the systematic review for carotid endarterectomy. However, the event rate of 11.5% (6/52) for all stroke mortality events, excluding cardiac events not related to the procedures, is not statistically comparable with the results of the systematic review for carotid endarterectomy (Chi square p=0.64).

Our case series of 52 patients undergoing carotid stenting concurs with previous findings from endarterectomy studies prior to cardiac surgery but fails to show any significant benefit in prevention of stroke or death when compared to the historical untreated data set. This is despite the fact that no complications occurred around the time of stent insertion. However, a small data set reporting zero event rates means very little, and we must remind ourselves that complication rates from stenting could be as high as 5.7 %.[217, 218] It is possible that the carotid procedure by adding delay to the bypass procedure in at least 3 of our patients may have contributed to their deaths of cardiac cause.

What this dataset fails to tell us is if there is a subset of patients who are more likely to benefit from pre operative intervention, and whether we can predict those most likely to have complications. Peri-operative strokes at the time of cardiac surgery may be embolic cholesterol particles from manipulation and clamping of the atheramotous aorta, air and platelet emboli from bypass or hemodynamic. The brains inbuilt ability to

regulate its cerebral blood flow and perfusion pressure during this period to prevent hypo-perfusion and clear away any emboli is key. However, the brain's auto regulatory function can be significantly impaired if a high grade stenosis has been present for some time.[219] The impact of this reduced reactivity in patients undergoing coronary bypass without prior carotid surgery has been proven with pre operative transcranial doppler assessment of patients undergoing cardiac surgery. Those who suffered strokes were more likely to have a pre operative low or exhausted cerebrovascular reserve.[220] It has been hypothesised, and subjected to computer simulation,[221] that some of this risk is due to the inability of a hypo-perfused brain to wash out emboli that lodge in the circulation and this can contribute to hypo-perfusion and watershed type infarction.[222, 223] However, it may be that this cerebrovascular reserve improves in some patients and adjusting the timing of the second surgery may have positive impact on outcome in these patients.[224]

To prevent peri-operative stroke during CABG we may therefore require routine preoperative assessment of the brains ability to regulate its own blood supply and maintain perfusion pressure within strict parameters. Techniques including MRI, transcranial doppler, and CT perfusion have been used to evaluate risk in patients with high grade carotid disease, and those undergoing carotid endarterectomy and carotid stenting.[225, 226] These techniques have successfully demonstrated that a lower cerebrovascular reserve in these patients is more likely to result in an adverse clinical outcome at the time of the carotid intervention.[225] Using one of these methods to identify high risk patients and then waiting to see if reserve has recovered my be the next step in assessing this process. Our centre is already using MRI scanning and diamox reactivity testing to assess the cerebrovascular reserve, and collateral circulation via the circle of Willis, in patients undergoing carotid intervention, and this technique

could be used in future patients in our centre to highlight the group of patients at higher risk for peri-operative hypo-perfusion during the cardiac procedure, thereby allowing better targeted carotid intervention at our centre in the future.[207]

What should not be forgotten is that there is a clear need for a randomised controlled trial of patients with significant carotid disease undergoing cardiac surgery with no prior carotid intervention in comparison to those undergoing either stenting or endarterectomy as a staged procedure. The current practice based on non randomised data appears to have very little impact on eventual outcomes, and may result in unnecessary procedures in up to 25% of the cardiac surgery population. However, as some cardiac surgeons refuse to perform cardiac bypass procedures unless the carotid arteries have been treated a randomised trial seems the only way to answer this question. At present because of the reluctance of the cardiac surgeons the decision making process at the multi-disciplinary team meeting is usually biased towards intervention without evidence to support it. We accept that a randomised study will require a considerable shift in attitudes away from the present management strategy employed in many centres worldwide and may prove problematic in its first stages. Our centres management policy with regard to stenting outside of such a trial is currently under review. The limitations of our non randomised observational study are recognised but this data adds to the limited world literature on stenting prior to cardiac surgery. However, with the lack of evidence the continued use of carotid intervention in these patients with carotid stenting is difficult to support.

4 The Effective Platelet Inhibition in Carotid Artery Stenting (EPICAS) study methodology.

Summary of Methods

The study was designed as a prospective evaluation into the clinical relevance of variations in an individual patient's response to the anti-platelet regime of aspirin and clopidogrel when undergoing carotid artery stenting using transcranial Doppler-detected micro embolic signals as a surrogate marker for possible stroke and transient ischaemic attack (TIA) risk after stenting. The study aim was to determine if variations in the level of platelet inhibition obtained from a standardised dosing regimen may be relevant in clinical practice. Transcranial Doppler monitoring of patients undergoing carotid stenting was performed before, during, and after the procedure and recorded onto digital audiotape (DAT) to allow off line single blind analysis. Blood samples obtained before (when possible) and after clopidogrel administration were used to assess each patient's response to this drug using an ADP dependant platelet function assay. Blood samples were also obtained to assess the inhibition of thromboxane synthesis by aspirin. Each patient underwent follow up six weeks after the procedure having completed the course of dual anti-platelet therapy, at which point they were expected to be taking only aspirin as antiplatelet therapy.

i) Study Population

Sequential patients undergoing planned elective carotid artery stenting procedures at the Sheffield Teaching Hospitals were approached for participation in this study. The study was designed to make no changes to routine practice currently undertaken in the selection of patients suitable to undergo a carotid stenting procedure. Patients were consented for the additional transcranial Doppler recordings and additional blood samples to be drawn for the platelet function assessments according to the ethically approved protocol. Selection of the design and manufacturer of the stent and protection device used during the procedure remained at the discretion of the individual radiologist performing the procedure. This study was not designed to be an assessment of the stenting procedure or technology, but to explore the role of platelet inhibition against outcome. Patients interested in taking part in the study were screened for a suitable ipsilateral bone window for monitoring of the middle cerebral artery.

Patients were excluded from enrolment based on the following criteria:

1) Patients undergoing a repeat procedure
2) Patients already taking dual anti platelet therapy with dipyridamole and aspirin. (later modified in view of antiplatelet trials in stroke *)
3) Patients on anticoagulant therapy.
4) Patients unable to tolerate clopidogrel or aspirin.
5) Patients unable to tolerate wearing headband monitoring device.
6) Patients with a possible cardiac source of emboli e.g. atrial fibrillation, mechanical heart valves.

* A protocol modification was made towards the end of the study to allow patients on aspirin and dipyridamole therapy to be included in the study after the National Institute for Clinical Excellence (NICE) technology assessment published in May 2005 (www.nice.org.uk/TA090) recommended dual antiplatelet therapy with aspirin and modified release dipyridamole in all Stroke patients.

ii) Study Methodology

A full history, general medical examination and baseline neurological examination was performed at recruitment in accordance with usual neurological practice. Clinical details were recorded on study data collection proforma (Appendix 1).

A one hour transcranial Doppler (TCD) ultrasound recording from the ipsilateral middle cerebral artery was recorded onto coded digital audio tape (DAT) as detailed in the TCD methods section. All recordings were monitored in real time with the Nicolet Companion III Doppler emboli detection software in addition to being stored for blinded off line analysis as part of the study protocol. This was a requirement of the ethics submission for all patients and all recordings in the study. If a significantly high embolic load were to be shown occurring at any point, suggesting a high risk of imminent stroke, it was expected that the investigators should use this information to guide clinical decision making. Transcranial Doppler had been shown to be a useful clinical tool during routine carotid stenting and endarterectomy and to deny access to this information should a problem arise was felt to be unethical.[111] This approach was in common with other studies using transcranial Doppler assessment of carotid artery stenting at the time. The benefits of this approach in preventing harm to patients have since been published.[113]

All patients were provided with the pre-treatment clopidogrel tablets and either loaded with two days 300 mg or given a minimum one week course prior to stenting in line with current practice. Patients entering this study were already receiving aspirin 75mg following their initial TIA or Stroke.

Immediately prior to, during, and following each stenting procedure further one hour recordings from the ipsilateral middle cerebral artery were made onto digital audio tape (DAT) for later blinded off line analysis. Post procedure this was performed for the first hour immediately the patient had been transferred to high dependency and therefore this recording began 10 minutes after completion of the procedure. Further blood samples as detailed in the protocol were obtained during the procedure from the arterial sheath prior to heparin being administered intra operatively and 10 minutes after the heparin bolus.

Patients were reviewed at between four and six weeks post stenting in the clinic when a post procedure carotid duplex examination of the stent was performed. In addition to the carotid duplex being performed a further TCD recording was obtained from the ipsilateral middle cerebral artery to assess what effect stopping clopidogrel at 4 weeks post procedure had on embolic signal detection rates. A planned additional interim visit at 7 days post procedure for further blood testing and TCD recording was available for any patients willing to travel for this additional examination. Unfortunately no patients volunteered for this examination and this was later removed from the protocol.

iii) Transcranial Doppler recording protocol

Standardised recordings were obtained using a commercially available EME Nicolet Companion III transcranial Doppler machine with a 2mhz transducer. Recordings were made from the ipsilateral middle cerebral artery, and the transducer was fixed in place using the manufacturers commercially available head set. A sample volume of 2mm, sweep speed of 5 seconds and a mean (SD) recording depth of 56 (2) mm was used. Recordings were undertaken for 60 minutes at each of the protocol time points. It was recognised that signal loss or a patient's inability to tolerate the head set may result in less than optimal recording times and this was to be adjusted for in the analysis. Recordings were made in accordance with the international consensus on TCD monitoring.[132]

A randomly allocated numbered tape was used for each recording. Patient details were recorded separately and no formal patient identification or episode number was visible on the tape. The log of patient name and recording episode remained blinded to the tape reviewer until the code was broken at the end of the analysis. Tapes were reviewed with at least a three month interval from initial recording following a data integrity check at the time of the recording to allow unbiased analysis of the data.

Offline analysis of TCD emboli data.

Raw Doppler signal was analysed by playback through the same transcranial Doppler ultrasound equipment for spectral analysis of embolic signals. Each instance of probable embolic signal was assessed against consensus criteria for embolic detection. Decibel thresholds of ≥5 dB and ≥3 bB above background were used to classify embolic

signals decided on by the sensitivity assessments performed on our machine. Each suspect embolic signal was saved digitally on the TCD machine for re review later and confirmation of the classification. These two threshold levels were then used for comparison with the platelet function tests. One hour of recording required approximately 2 hours of offline analysis.

iv) Platelet function testing

Platelet function testing was performed at the baseline visit (prior to initiation of clopidogrel whenever clinically possible), immediately prior to carotid intervention before administration of heparin, 10 minutes after administration of heparin (since heparin potentiates ADP-induced platelet aggregation)[227], 7 days after carotid intervention in select patients agree to the additional visit, and at 30 day review. Unless patients had been forced to continue taking clopidogrel for a specified indication this time point was between 5 - 7 days after termination of clopidogrel therapy to allow sufficient wash out time.

Platelet Aggregation

Platelet aggregation was assessed by whole blood single-platelet counting using hirudin anti-coagulated blood (6 ml total) and ADP 0.3-100 μmol/L as an agonist using techniques that have been previously described by members of the EPICAS trial group.[228]

Hirudin anti coagulant tubes containing 60 μl of Hirudin solution were used to collect 6mls of whole blood at each time point for platelet analysis. ADP antagonist solutions were prepared to the following stock concentrations 100 μM, 30 μM , 10 μM, 3 μM, 1 μM, and 0.3 μM and maintained on ice till required. Seven numbered test tubes were prepared and 20 μl of each ADP solution was added to the appropriately numbered tube according to the following set up and 20 μl of EDTA 100 mmol stock solution was added to tube number 7 as control.

Tube number	ADP Concentration (μM)
1	100
2	30
3	10
4	3
5	1
6	0.3
7	EDTA

A magnetic stirrer set at 37 ˚C was used to agitate 480 μl of huiridin anticoagulated whole blood with the ADP solutions for 4 minutes. After 4 minutes 1ml (1000 μl) of fixative was added to each tube to stop aggregation. Residual single platelet counting was then undertaken using a Sysmex KX21 haematology analyser.

Dr R. Storey, a member of the EPICAS study group, and supervisor for platelet analysis in this study has undertaken extensive studies on the effects of clopidogrel using this method to assess platelet aggregation. [228-230] This method has been shown to be particularly discriminating and reliable for assessing the effects of clopidogrel in an individual subject **(figure 3)**. This assay is not affected by aspirin, which does not inhibit ADP-induced aggregation in blood anti-coagulated with a direct thrombin inhibitor (such as hirudin) rather than citrate [231, 232], so this assay primarily assessed the effect of clopidogrel (as well as the potentiating effect of heparin). These values were then used with several classification criteria to select patients as "responders," "semi responders," or, "non-responders." The Sheffield Cardiovascular Research group has previously used cut offs for post exposure response classification of <40% aggregation, 40% – 60 % aggregation and > 60% for this classification when examining the response

to clopidogrel in patients undergoing coronary stenting procedures.[233] Analysis of published criteria from other investigators, who have suggested criteria for assessment of responder / non responder status using similar techniques, provided a combined criterion to improve classification. These criteria were also applied to our data set during analysis and then used in combination to classify individuals as non responders.[159, 161, 234-236]

Muller's group has proposed a classification based on absolute inhibition.[159]

Absolute Inhibition = (Base line aggregation – Post clopidogrel admin aggregation)

Samara et al [235] used the same cut off points as Muller's group [159] classifying patients into responder, non responder and semi responder groups but this classification was calculated by using relative platelet inhibition values.

Relative Inhibition = [(pre-treat agg – post-treat agg) / pre-treat agg] x 100 %

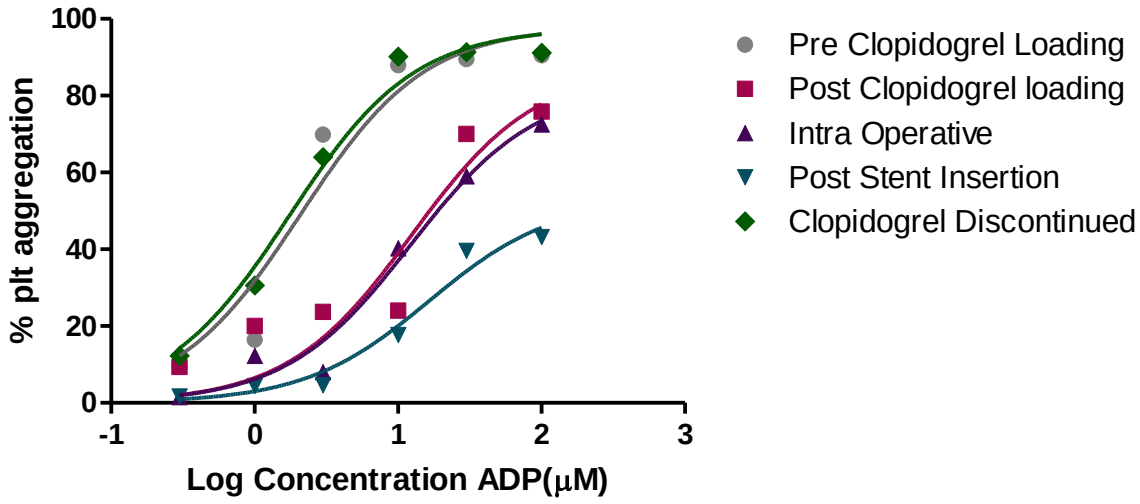
Patients have also been classified as normal responders and low responders by calculation of the percentage inhibition of baseline aggregation according to the following equation as previously described.[234]

$$\% \text{ inhibition} = (PA_{\text{baseline}} - PA_{\text{after clopidogrel}} / PA_{\text{baseline}}) \times 100$$

The patients were then classified as sub optimal or low response if the inhibition was less than 40%.

We applied all these criteria to our patients for values generated at 10μM ADP aggregation. Comparison of the demographic and patient characteristics of the three groups was undertaken using ANOVA for continuous variables and Chi squared and Fisher's exact test for discrete variables. Calculations were undertaken using SPSS v 15.01 for Windows and McKay's software tool for MSDOS for applying Fisher's exact test to any sized two dimensional contingency table where expected values were less than 5 negating the assumptions of the chi squared analysis **www.discourses.org.uk/statistics/fisher**.[237, 238]

Figure 3 Example of Clopidogrel Dose response. Effect of Clopidogrel Administration on ADP induced platelet aggregation when assessed by whole blood single platelet counting in a patient from the EPICAS trial.



Plasma sCD40L measurements.

Soluble CD40L released from activated platelets has been used previously to measure platelet activation. Platelet-derived sCD40L has been shown to constitute the majority of sCD40L found in circulating plasma.[239] It has been suggested that sCD40L may play an important role in the development of restenosis following arterial intervention and future cardiovascular risk has previously been associated with higher circulating levels of plasma sCD40L.[240, 241] The degree of inhibition of platelet activation achieved using antiplatelet agents can be related to the levels of circulating SCD40L.[242] The methods we used in the EPICAS study protocol have been previously described by other authors investigating patients with cardiac disease undergoing heart bypass.[243, 244] The measurement of sCD40L, at time points through out the procedure, was used as a possible indicator of any change in platelet activity in vitro to assess the degree of inhibition achieved by clopidogrel.

5 ml blood was collected into a tube containing EDTA and centrifuged at 15,000g for 10 minutes at 4ºC. The supernatant platelet-poor plasma was removed and stored at -20ºC prior to determination of plasma sCD40L levels. Plasma sCD40L levels were determined using the commercial available Bender MedSystems high sensitivity Human sCD40L ELISA. Specimen collection into EDTA was undertaken to prevent activation of the samples platelets. These samples are appropriate for the Bender Medisystem analysis as detailed in their accompanying instructions for use. The validity of using EDTA samples has been confirmed by other authors.[245, 246] A spectrophotometer with micro plate reader set to 450nm absorbance with 620nm as a reference wavelength was used to calculate the concentration of substrate. Labsystems genesis v3.05 software calculated a standard curve from control concentrations and then computed the

corresponding sCD40L concentration from the mean absorbance from each patient's paired samples. Concentrations of sCD40L were assessed for normal Gaussian distributions and where appropriate non parametric testing with Wolcoxon methods was applied, logarithmic transformation to normalise the data was also applied to allow parametric testing to be performed.[247-249] Results were analysed in comparison with the ADP platelet aggregation tests and transcranial Doppler embolic signal counts.

Serum thromboxane B_2 measurement

Aspirin exhibits mild antiplatelet activity through inhibition of the cyclo-oxygenase (COX-1) pathway causing a reduction in thromboxane A_2 (TXA_2) synthesis in platelets and consequently a reduction in platelet aggregation. The inhibition of the COX-1 pathway is rapid, saturable and at low doses of aspirin irreversible. The effects of aspirin variability was not a primary end point for the EPICAS study and therefore detailed analysis of thromboxane dependant platelet aggregation, with multiple agonist-induced platelet aggregation tests was not undertaken in this pilot study.

An alternative assay using the indirect measurement of thromboxane A_2 (TXA_2) production and inhibition was undertaken as a limited assessment of this pathway. This technique has previously been used extensively to characterise the clinical pharmacology of aspirin as an antiplatelet agent, and was performed as simplified indicator of aspirin effectiveness in our patient population. Thromboxane A_2 has a half life of 37 seconds and cannot be measured physiologically. However, as it decomposes into the stable metabolite Thromboxane B_2 (TXB_2) which can be measured physiologically this can be assumed to directly reflect TXA_2 production.[250] The

methods have been previously described for plasma and urine samples in patients with known cardiovascular and cerebrovascular disease.[251-254]

All our patients were taking aspirin prior to enrolment in the study and therefore pre aspirin plasma levels of TXB_2 could not be assessed in these patients. Baseline TXB_2 levels can vary in patients not treated with aspirin between 200 and 600 ng/ml.[255] It has been shown that functional relevance in vivo occurs when the maximum capacity to generate thromboxane ex vivo has been inhibited by at least 95%. Therefore levels of less than 10 ng/ml and 30 ng/ml were chosen as levels indicating sufficient suppression of the TXA_2 pathway in our patients.

A 5 ml sample of whole blood was collected in a plain glass tube incubated at 37ºC for 60 minutes prior to centrifugation at 2500 G for 10 minutes. The serum was separated and stored at -20ºC. The samples were then analysed using R&D systems, Inc Thromboxane B_2 (TXB_2) antibody mediated immunosorbent assay (www.rndsystems.com cat no DE0700). The assay is based on the competitive binding technique in which the TXB_2 present in a sample competes with a fixed amount of alkaline phosphatase-labeled TXB_2 for sites on rabbit polyclonal antibody. A spectrophotometer with micro plate reader set to 405nm absorbance with wavelength correction of 570 nm and 590 nm was used to calculate the concentration of substrate. Labsystems genesis v3.05 software calculated a standard curve from control concentrations and then computed the corresponding TXB_2 concentration from the mean absorbance from each patient's paired samples.

v) Serum C-reactive protein measurement.

Increased CRP following coronary intervention has been associated with an increased risk of restenosis. The relationship between inhibition of platelet function and CRP levels following carotid arterial interventions has not yet been defined by previous investigators. These levels were monitored in the study protocol to see if any inflammatory reaction noted during stenting was related to any impact on platelet inhibition.

A 5 ml sample of whole blood was collected in a plain glass tube incubated at 37ºC for 60 minutes prior to centrifugation at 2500*g* for 10 minutes. The serum was separated and stored at -20ºC. The commercially available Kalon Biological Ltd High Sensitivity C-reactive protein (HSCRP) enzyme linked immune assay was used to detect ultra low levels of CRP in the patients. This assay is based on the double antibody sandwich format. A spectrophotometer with micro plate reader set to optical densities of 405nm absorbance was used to calculate the concentration of substrate. Labsystems genesis v3.05 software calculated a standard curve from control concentrations and then computed the corresponding HSCRP concentration (μg/L) from the mean absorbance from each patient's paired samples.

vi) Study size calculation.

Previous work by other investigators allowed initial power calculations to be made before undertaking this study. Transcranial Doppler detection of micro embolic signals had previously been used to assess the benefits of adding a novel antiplatelet medication to low dose aspirin during carotid endovascular intervention. [114] This suggested that when appropriate platelet inhibition had been achieved with antiplatelet medication the number of embolic signals detected in the ipsilateral middle cerebral artery were significantly reduced or abolished.[114] In patients with insufficient platelet inhibition the number of embolic signals detected immediately post procedure could be as high as 76 signals per hour with a mean of 25. Our study was not investigating the impact of clopidogrel or placebo on emboli counts but by assuming that patients who are, "non responders," in our study are equal to patients only taking aspirin this previous data set could be used to calculate an initial sample size to demonstrate a statistically significant difference between our patient groups. It had already been shown that between 10 – 20% of patients may not respond or respond poorly to clopidogrel and this information allows us to assume a ratio of 1 in 5 or 1 in 10 for the power calculation of responders to non responders in the sample population.[108, 158-161]

Several assumptions were required to perform this calculation:

1) Individual patients will respond variably to the inhibitory effects of the antiplatelet agent clopidogrel and can be classified as responders, non responders or semi responders.

2) This variability directly affects the number of platelet rich embolic particles produced after stent insertion and consequently the production of detectable micro embolic signals.

3) A good responder will reduce or abolish embolic signal production post stenting. In contrast a poor responder will produce a significant number of embolic signals as shown in previous trials in patients treated only with Aspirin.

4) Between 10 – 20% of patients may not respond to clopidogrel.[108, 158-161] Ratios of 1 in 5 and 1in 10 can be used for the power calculations.

Calculations for sample size determination were performed using the using the open access PS power and sample size programme.[256] The data available from the previous investigators studies allowed us to conclude that responders to clopidogrel may produce up between 50 to 80% fewer embolic signals than patients who were non responders. Non responders were assumed to have a mean embolic signal count of 30 per hour with SD of 10.

The α type one error was chosen as 0.05 and the study was powered at 0.80. The difference in the mean emboli counts for the two groups was calculated to be between 15 or 24 based on a 50% - 80% reduction in counts from 30. An initial study size of between 10 patients and 44 patients was suggested to ensure sufficient power based on these preliminary calculations. An initial assessment point of 25 patients was chosen before preliminary review. External review of the protocol and power calculation was undertaken as part of an application for funding to the UK Stroke Association funding. Protocol modifications following this review were undertaken Dec 2003.

Statistical Analysis

The commercially available statistical analysis packages Microsoft Excel, LEADTOOLS Inc SPSS version 15.01 and Graph Pad Software Inc Graph pad Prism 5.01 were used for data analysis. The open access McKay analysis tool was also used to undertake some Fisher's exact analysis on r x c contingency tables that failed to satisfy requirements for Chi squared analysis with expected values smaller than 5. The methods used for data analysis is discussed in methods of analysis sections in the results.

5 Results of EPICAS Study

i) Platelet function analysis and classification of patients

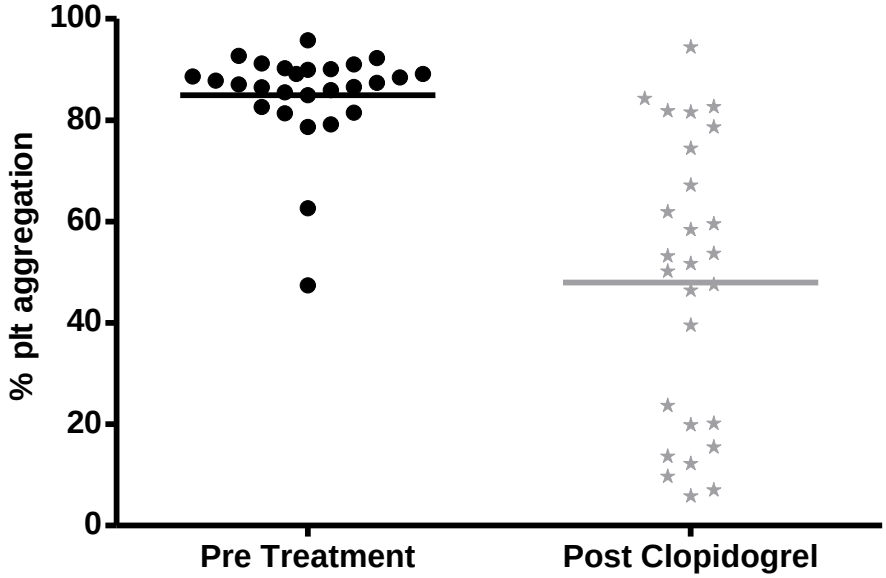
a) ADP dependant platelet aggregation tests

Standardised definitions to define an individual's responsiveness to clopidogrel are lacking. Some studies quote clopidogrel resistance based on differences between pre and post-treatment platelet reactivity.[161] Other studies have described it according to the degree of inhibition of platelet aggregation values obtained at baseline and after treatment.[159] These studies using arbitrary cut-off values to describe patients as clopidogrel resistant may not reflect the true pattern. It is likely that there is a spectrum between patients that have been classified, "resistant," and those patients who are, "super responders."

It has already been shown that an individual patient's response to a standard clopidogrel loading dose of 300mg followed by 5 days of clopidogrel 75 mg can be highly variable. This is easily demonstrated in the EPICAS patient group by comparing baseline aggregation with 10 uM ADP solution to post clopidogrel dosing regimes. This is illustrated in **figure 4**. When represented graphically it appears that before treatment most patients have a similar aggregation response to stimulation with 10 uM ADP solution but following administration of clopidogrel 3 clear groups of patients can be defined. The first group are those that respond extremely well to the standard regime of clopidogrel loading and have post exposure aggregation of less than 40%. The second group of patients have an intermediate level of response and aggregate between 40%

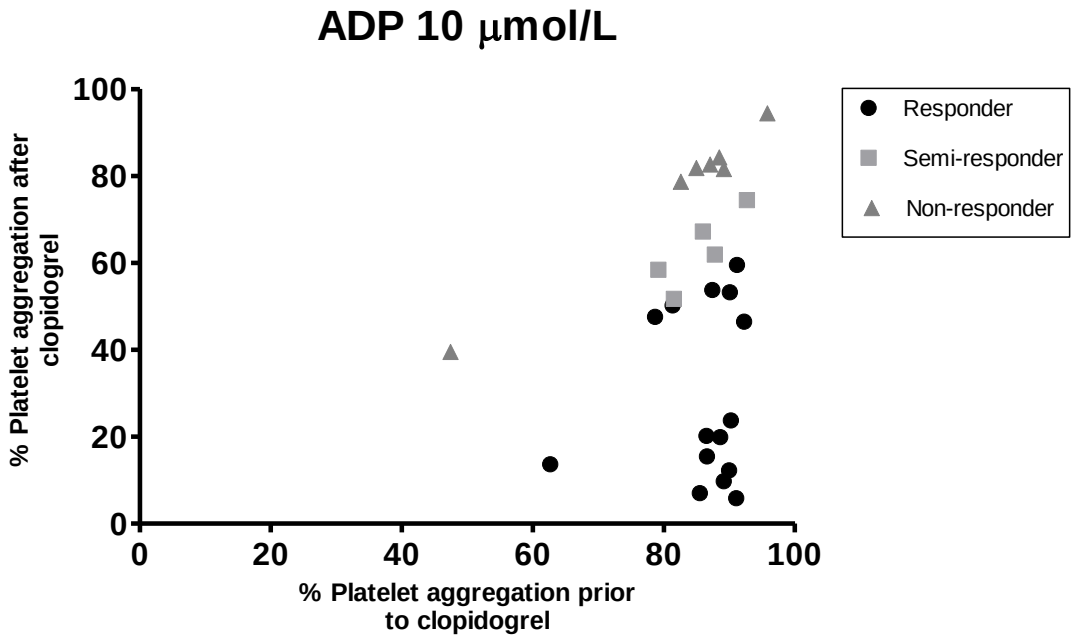
and 60 % of their platelets. A final group classified as the, “non-responders,” or, “resistant,” patients have post clopidogrel loading aggregation that remains >60%.[233]

Figure 4 Platelet aggregations before and after clopidogrel loading in the EPICAS study immediately prior to carotid stenting. Results of whole blood single platelet counting in response to a 10 uM ADP aggregation stimulus.



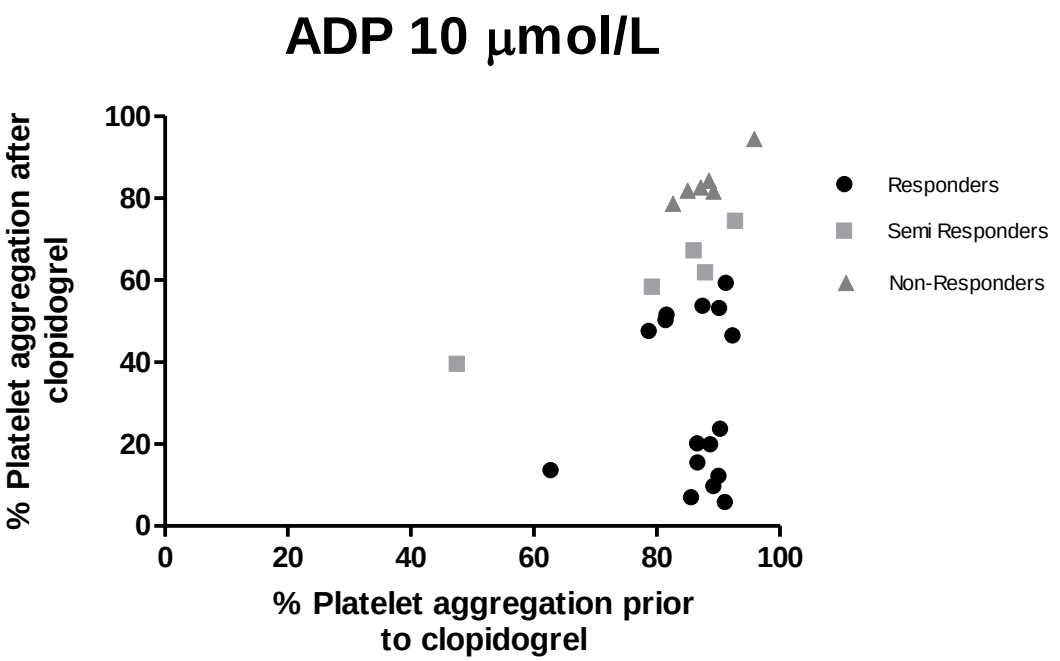
Classification of these patients as non-responders, semi responders, and responders based on Muller’s criteria as previously described in cardiac stent patients is shown graphically in **figure 5** and also in the comparison **table 9**.[159] Clopidogrel non-responders are defined by an inhibition of ADP (10 Mol/L) induced platelet aggregation that was less than 10% when compared to baseline values after clopidogrel intake, semi-responders were identified by an inhibition of 10 to 29% and patients with an inhibition over 30% were regarded as responders.

Figure 5 Effect of clopidogrel on ADP-induced platelet aggregation. Plots of individual patient values of maximal aggregation prior to and after clopidogrel administration. Response to 10 μM ADP and classification responder, non-responder and semi-responder based on absolute inhibition.



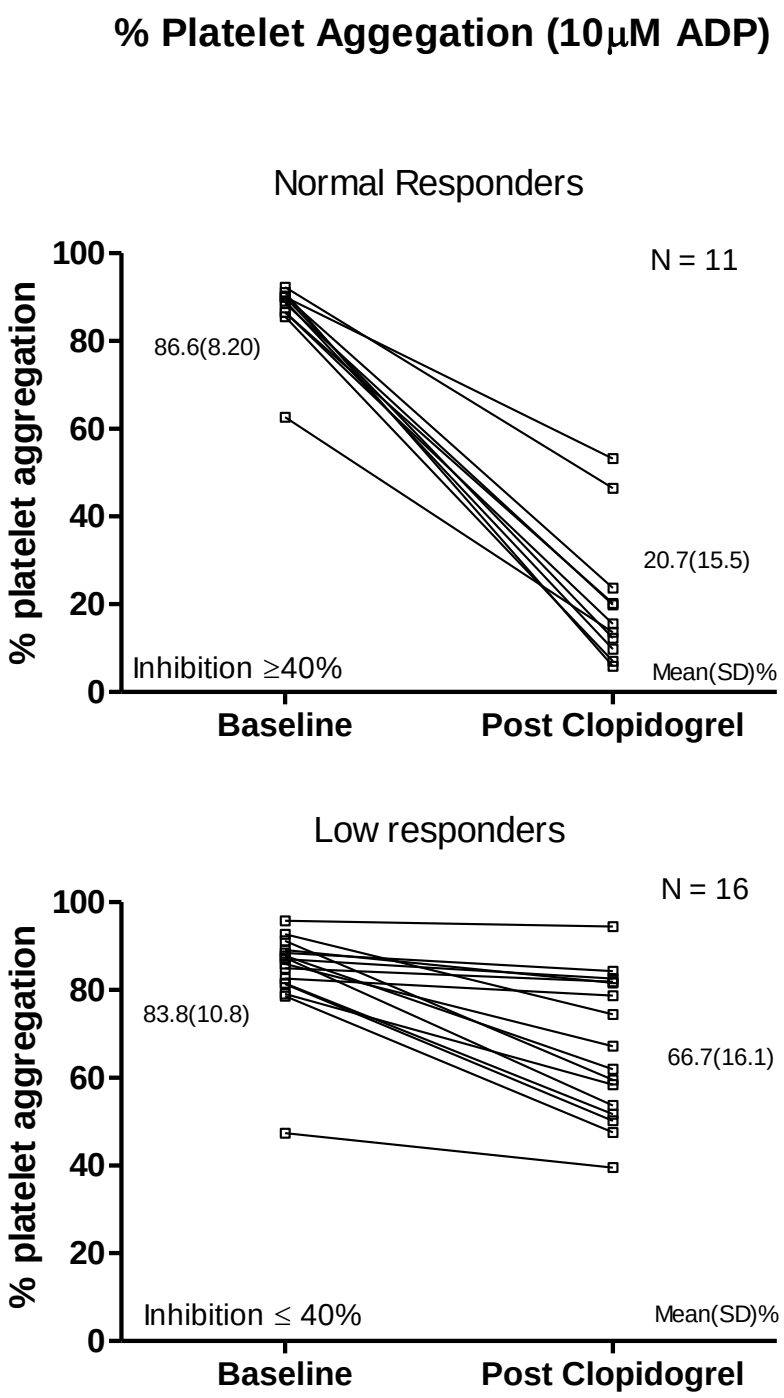
Samara et al [235] used the same cut off points as Muller's group [159] classifying patients into responder, non responder and semi responder groupings but this classification was calculated by using relative platelet inhibition values. The classification of these patients is graphically represented in **figure 6**. Using both relative and absolute reactivity has been shown to be more accurate at detecting non responders [233] and comparison of these methods on classification is shown in **table 9**.

Figure 6 Effect of clopidogrel on ADP-induced platelet aggregation. Plots of individual patient values of maximal aggregation prior to and after clopidogrel administration. Response to 10 μM ADP and classification as responder, non-responder and semi-responder based on relative inhibition.



Application of Angiolillo's technique for classification of platelet responsiveness [234] is demonstrated graphically in **figure 7** and compared with the other classifications used in **table 9**. Patients having ≥ 40% inhibition of platelet aggregation 24 h after clopidogrel administration were defined as normal responders, whereas those having <40% inhibition were classified as low responders.

Figure 7 Individual Values of 10μM ADP-induced platelet aggregation before and after administration of clopidogrel with normal and low responders classified. Mean and SD aggregation values are expressed for each time point.



Combining all 4 techniques on our data set makes it possible to identify patients who would be classified as non responders in all classifications. For the purposes of analysis patients 4, 10, 13, 20, 22 and 23 have been classified as non responders, patients 1, 7, 11, 14, 16, 17, 19, 24 and 27 are full responders and the remaining patients have been classified as semi responders. The EPICAS study group therefore had 6 non responders, 11 semi responders, and 9 full responders (Chi – Squared 1.462 p = 0.482). These results are shown in **table 9**.

Comparison of clinical characteristics of each of the patient groups is shown in **table 10**. It was expected that the distribution of patients exhibiting each characteristic would have been equally distributed between the groups and this was tested using one way ANOVA and Fisher's exact tests for any sized contingency table. The only statistically different variables between the groups were shown to be the initial clinical event (p = 0.030), a previous history of angina (p = 0.028) and previous history of stroke prior to the current index event (p = 0.005).

Table 9 Classification of individual patient response to clopidogrel loading comparison of 4 classification techniques and classification used for analysis.

Patient	Storey [233]			Muller			Samara			Angiolillo		EPICAS
	R	SR	NR	R	SR	NR	R	SR	NR	Norm	Low	classification
1	■			■			■			■		Responder
2		■			■		■				■	Semi
3		■		■			■				■	Semi
4			■			■			■		■	Non
5		■		■			■			■		Semi
6		■			■			■			■	Semi
7	■			■			■			■		Responder
8		■		■			■			■		Semi
9			■		■			■			■	Semi
10			■			■			■		■	Non
11	■			■			■			■		Responder
12		■		■			■				■	Semi
13			■			■			■		■	Non
14	■			■			■			■		Responder
15			■		■			■			■	Semi
16	■			■			■			■		Responder
17	■			■			■			■		Responder
18		■		■			■				■	Semi
19	■			■			■			■		Responder
20			■			■			■		■	Non
21												
22			■			■			■		■	Non
23			■			■			■		■	Non
24	■			■			■			■		Responder
25			■		■			■			■	Semi
26	■					■		■			■	Semi
27	■			■			■			■		Responder
28		■		■			■				■	Semi

Table 10 Patient demographics when classified based on response to clopidogrel.

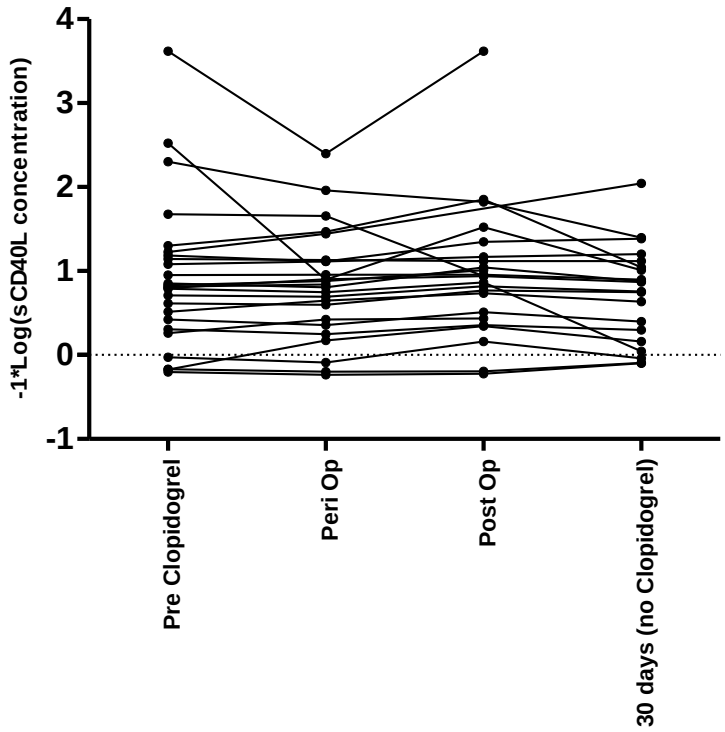
		Non Responder	Semi - Responder	Responder	p
Number patients in each group		6	11	9	0.482
Mean Age (yrs)		67	72.64	69.56	0.305
Male		5	9	7	0.909
Presentation	TIA	6	4	2	
	Stroke	0	6	5	**0.030**
	Asymptomatic	0	1	2	
Left Carotid treated		3	6	1	0.370
Previous medical history	Angina	0	3	6	**0.028**
	Prev TIA	1	1	1	0.885
	Prev. Stroke	4	0	0	**0.005**
	Hypertension	7	10	5	0.721
	Diabetes	0	1	2	0.155
	Hypercholesterolemia	7	10	5	0.721
	Statin	7	11	5	0.260
Smoker	Current	2	2	1	0.909
	Ex	6	7	4	0.933
Stent	Carotid Wall Stent	5	7	4	
	Nex Stent	2	2	1	0.843
	Other	1	2	1	
	Mean Diameter (mm)	8.00	8.09	8.33	0.836
	Mean Length (mm)	30	30	30	

ib) sCD40L analysis

Statistical Methods

The concentrations of sCD40L from the EPICAS patient population do not conform to a normal Gaussian distribution when analysed with the D'Agostino-Pearson test. Previous authors have used two methods for analysis of sCD40L data when it is not normally distributed. Both methods were applied to the EPICAS data. Logarithmic transformation of the sCD40L concentrations allowed for statistical analysis with student T tests [241, 247, 257]. In addition non parametric methods including the Wilcoxon signed rank were also applied to the data without the transformation.[249, 258, 259] Comparison between sampling points and between patient groups classified on the basis of platelet aggregation tests was undertaken. It was hypothesised that when compared to SCD40L levels at enrolment those patients with platelets not adequately inhibited by aspirin and clopidogrel would have higher levels of sCD40L. Individual patient data is displayed graphically in (**figure 8**).

Figure 8 Graphs of Individual patient data -1*Log(sCD40L concentration)

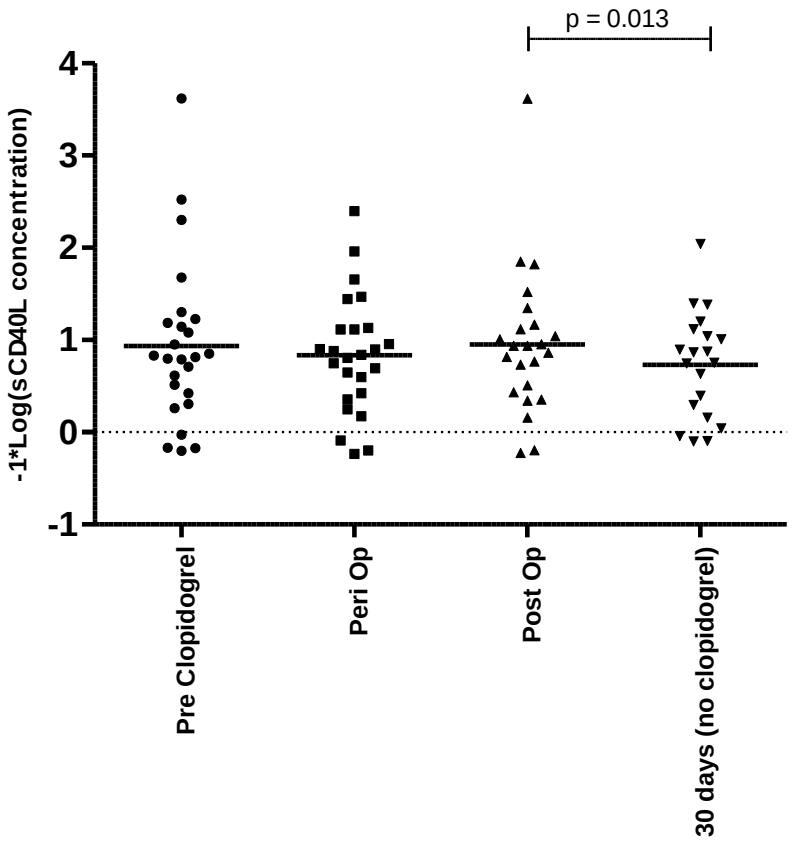


Results

No clear trends were identified from one time point to the next on graphical representation allowing identification of individual at risk patients. A one way ANOVA was applied to the data confirming this finding.[259] No significant difference in the means or variances between the 4 time points ($F = 0.4049$ $p = 0.7498$) were shown. **Figure 8** does suggest that there may be a downward trend in sCD40l levels from 2 hours after the operation to the 30 day follow up period, but this cannot be confirmed statistically.

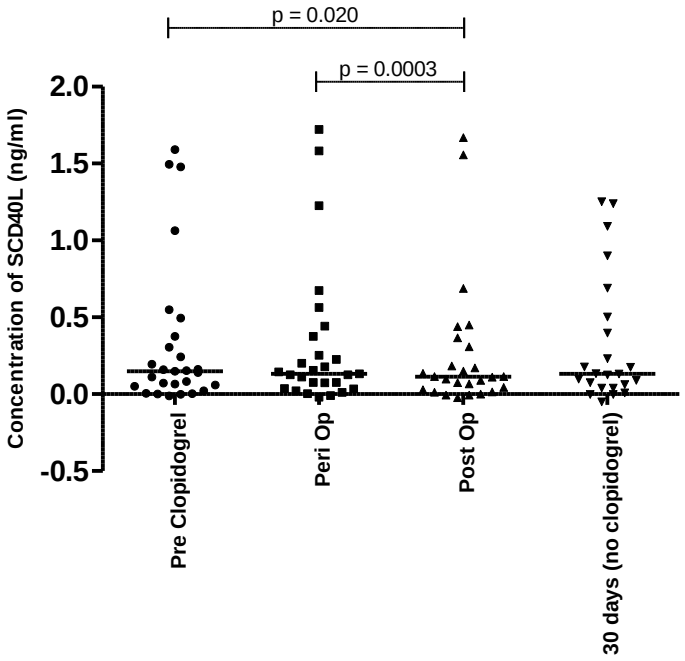
Paired T tests on the log transformed data were undertaken looking for significant changes between each of the sampling time points (**figure 9**).[241, 247] There was no statistically significant difference demonstrated between pre clopidogrel loading and 30 day post operative sCD40l levels ($p = 0.5027$), pre clopidogrel loading and peri operative samples ($p = 0.2620$), pre clopidogrel loading and post operative samples ($p = 0.9379$), or the perioperative and 2 hour post operative samples ($p = 0.0515$). The only significant difference was shown between the post operative and 30 day samples ($p = 0.0133$) as suggested in **figure 9**.

Figure 9 Dot plot of individual patient results for SCD40L concentrations with mean values shown. Significant differences shown by paired student T test.



These outcomes differed slightly when analysed with the paired Wilcoxon method on outcomes for the whole dataset with significant differences seen between the peri operative and 2 hour post operative periods (p = 0.0003), and the pre clopidogrel loading and 2 hour post operative periods (p= 0.020). (**figure 10**)

Figure 10 Comparison of median SCD40L concentrations at each time point with paired Wilcoxon test. Significant differences shown.

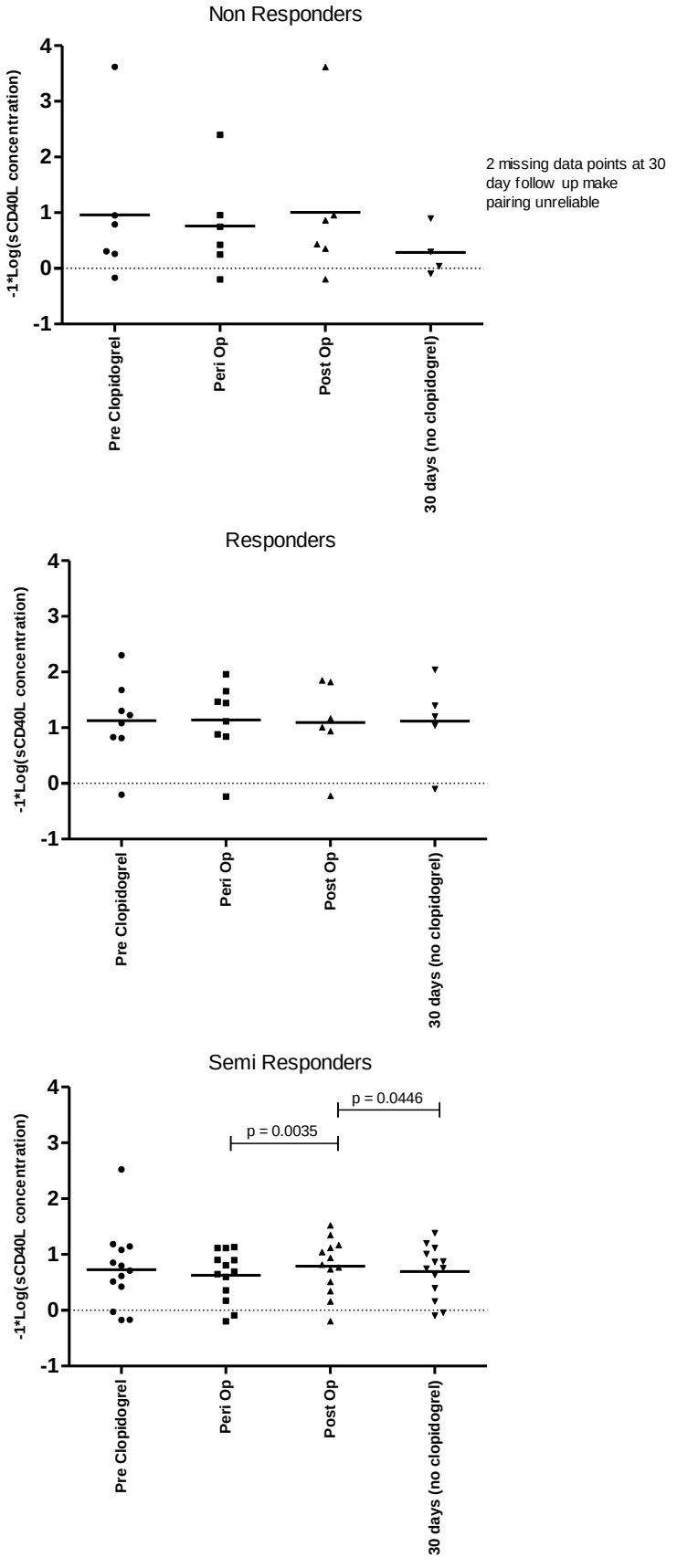


Data was then analysed for changes between the time points based on previous classification as responder, non responder or semi responder. ANOVA and Paired T tests comparing sampling time points were undertaken. This showed no significant difference in the means or variances across the 4 time points for non responder ($p = 0.76$) semi responder ($p = 0.89$) and responder ($p = 0.99$) groups. For the non responders no statistically significant differences were shown between pre operative and 30 day post operative sCD40l levels ($p = 0.41$), pre clopidogrel loading and peri operative samples ($p = 0.39$), peri-operative and 2 hour post operative samples ($p = 0.27$) or post operative and 30 day samples ($p = 0.39$). In the patients fully responsive to clopidogrel a similar pattern was seen between pre operative and 30 day post operative sCD40l levels ($p = 0.94$), pre clopidogrel loading and peri operative samples

(p = 0.86), peri-operative and 2 hour post operative samples (p = 0.78), and post operative and 30 day samples (p = 0.30).

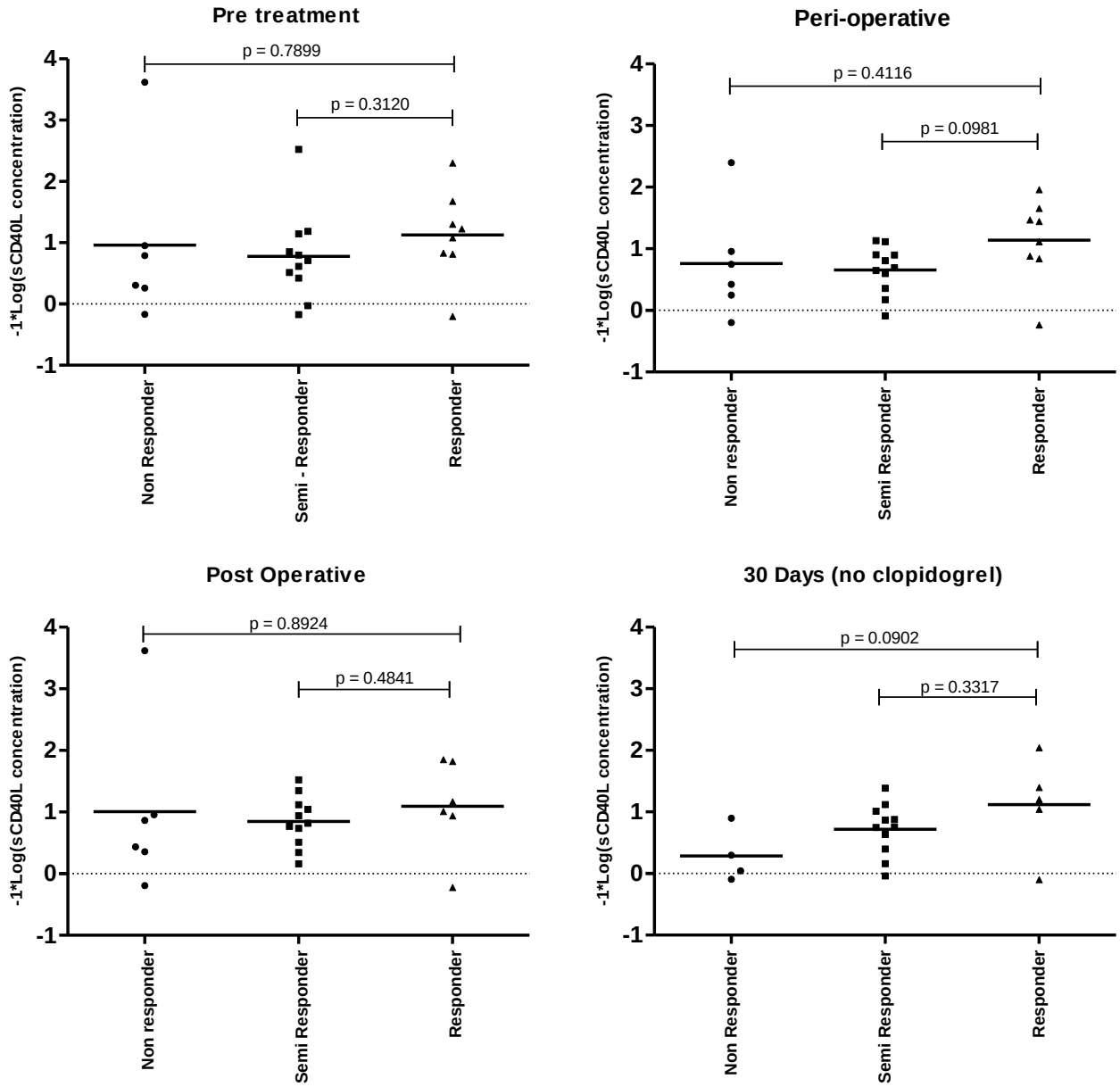
The semi responder group were different with no statistically significant differences demonstrated between pre operative and 30 day post operative sCD40l levels (p = 0.79), or pre clopidogrel loading and peri operative samples (p = 0.45). However, changes were seen between the peri-operative and 2 hour post operative samples (p = 0.004) and the post operative and 30 day samples (p = 0.045) (**Figure 11**). In these patients there was an increase in the mean level of sCD40L detected at the two hour point in patients who were not completely inhibited by clopidogrel. This transient increase returned to baseline values 30 days after the procedure.

Figure 11 Individual patient data highlighting change between time points for patients classified on the basis of platelet aggregation studies. Paired Student T test for significance differences shown.



Concentrations of sCD40L are expected to rise when platelets become activated. They have also been shown to increase after being suppressed when clopidogrel is withdrawn. Analysis at each of these time points between the three classifications with student's T test and Welch's correction failed to show any significant difference at any time point between responders and semi responders or non responders (**Figure 12**).

Figure 12 Time of sampling comparison with responder status for SCD40L concentrations. Comparison between groups by Student T test with Welch correction for unequal variance.

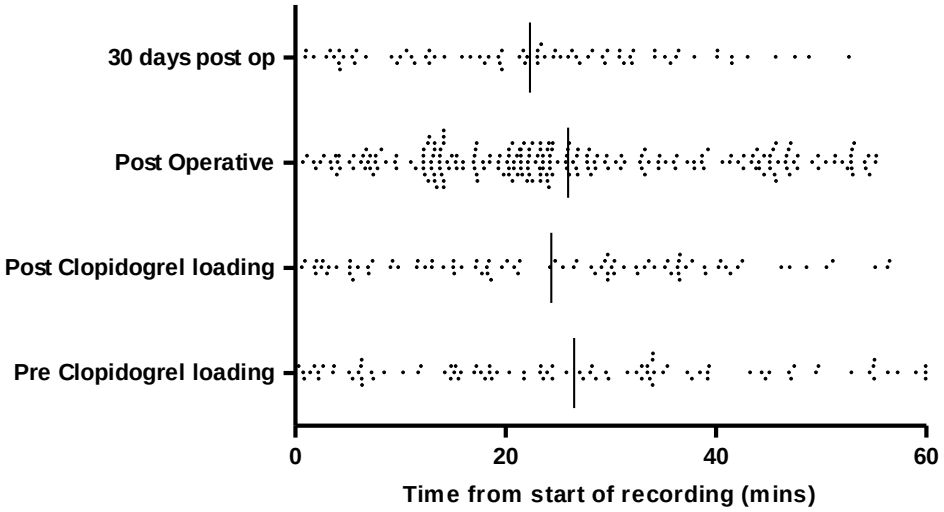


ii) Transcranial Doppler Emboli counts

Statistical methods

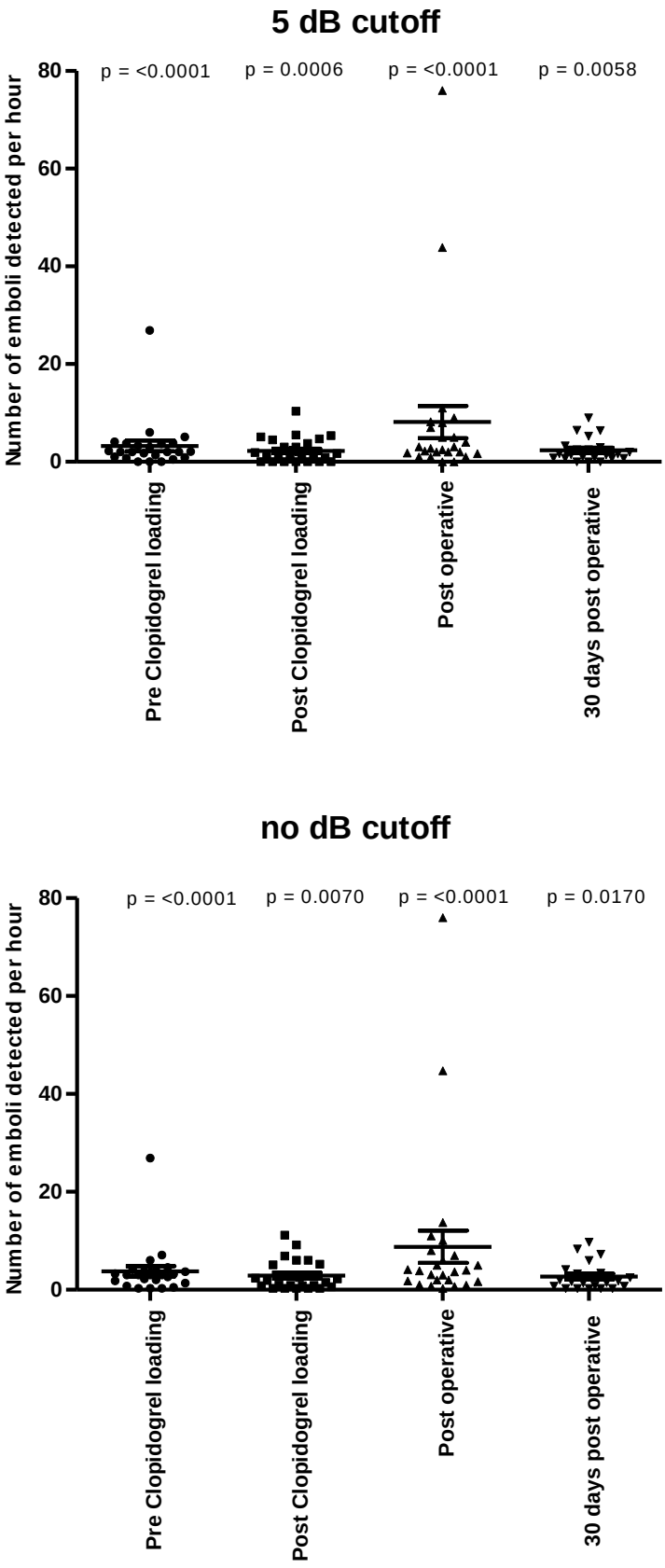
All analysis of transcranial Doppler emboli counts was performed off line by review of the coded DAT tapes. Recordings were performed for one hour at each time point in all patients. Emboli were classified according to the international consensus document tailored to our TCD equipment with a cut off of 5dB.[132, 260, 261] In addition we performed analysis based on no decibel cut-off as described by the CARESS investigators.[143] Due to signal degradation including movement of the headset on the patient some recordings had been degraded and the full hour was not available for analysis. The distribution of events detected was random throughout the hours recording time and therefore adjusted emboli counts were used to represent number of emboli per hour in the analysis. The spatial distribution of the emboli detected throughout the recordings have been plotted in **figure 13**.

Figure 13 Temporal distribution of embolic events throughout the hour recording period (5dB cut-off).



The data was assessed for normality with the D'Agostino & Pearson normality test. Small p values suggest a low probability that the sample comes from a Gaussian distribution and in all of the time periods assessed none had emboli counts that match a Gaussian distribution (**figure 14**). To facilitate analysis of embolisation rates a log transformation was performed to reduce the number of outliers and the degree of skew and thereby allowing assessment of the results with one way ANOVA and T tests performed between the time points. Patients with emboli counts of zero were given nominal counts of 0.3 per hour to assist in the transformation and analysis in line with previous practice.[143]

Figure 14 Transcranial Doppler emboli per hour per patient for each time period. D'Agostino & Pearson test for Gaussian distributions shown at each time point.



Results

A significant difference was seen between the emboli counts at each time point with the 5dB cut-off ($p = 0.035$) and when no decibel cut off was used ($p=0.043$) with ANOVA analysis performed across the time points.

Comparison between each of the time points for a cut off of 5dB showed no significant difference between pre clopidogrel loading and post clopidogrel loading emboli counts although the trend was for a reduction in the mean counts from 3.3 to 2.3 emboli per hour ($p = 0.24$). No significant differences could be demonstrated between pre loading and 30 days post operative ($p = 0.84$), or post loading and 30 days post operative ($p = 0.89$). Significant changes in emboli counts were seen between the pre clopidogrel loading and post operative ($p = 0.05$), post clopidogrel loading and post operative ($p = 0.03$) and post operative and 30 day ($p = 0.04$) time points. (**Figure 15 and Table 12**)

Including all emboli in the analysis ignoring a decibel level cut off using the CARESS investigators technique yielded similar results except in this analysis no statistically significant change was seen between the pre clopidogrel loading and post operative counts despite an increase in mean emboli detected from 3.8 to 8.8 per hour between the time points ($p = 0.08$).

Figure 15 Comparison between time points of emboli counts detected per hour for 5dB cut off and no specified dB cut off. Log (Emboli count/hour) normalisation of data before analysis with ANOVA and T test performed. Significant differences are shown.

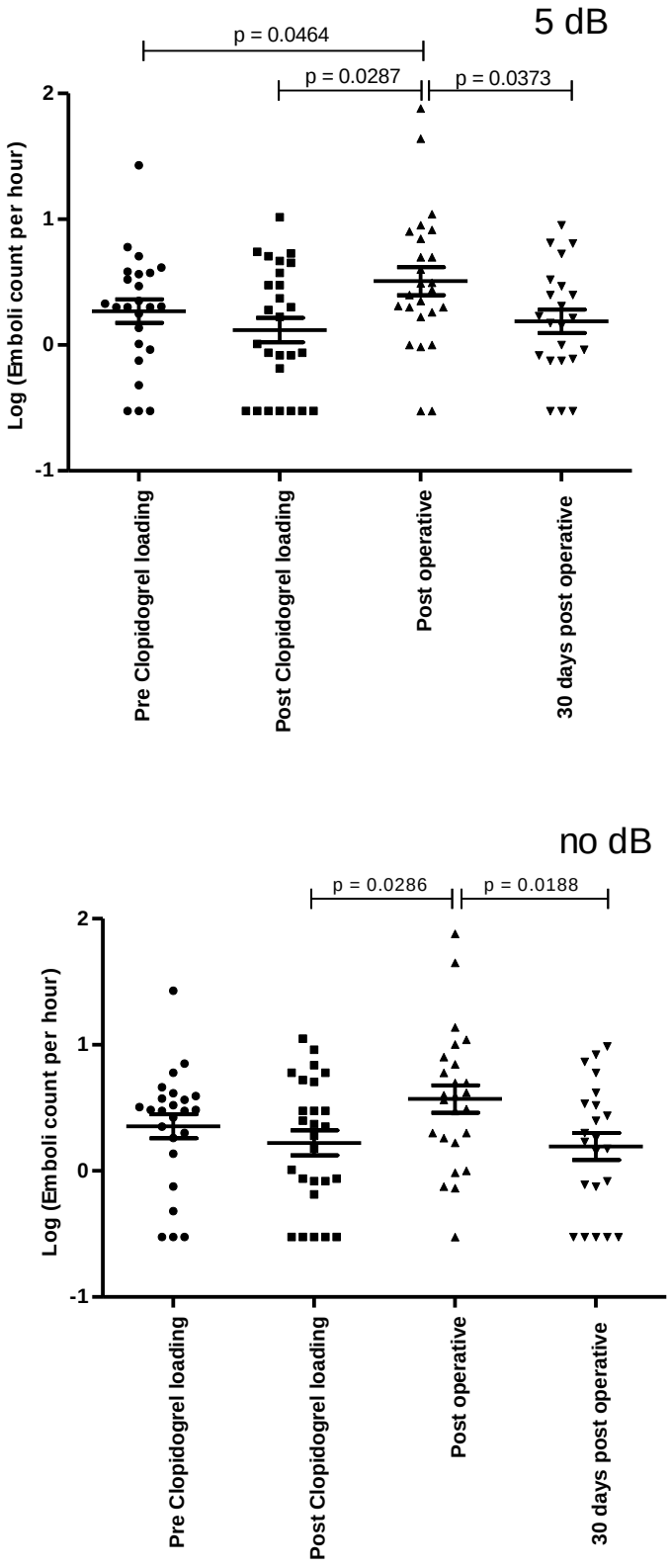


Table 12 Comparative Emboli counts per hour from each time period and statistical comparison between the groups using paired T tests. Significance level p = < 0.05.

Time	Emboli count per hour						Comparison	T test	
	5dB			no dB				5 dB	no dB
	Median	mean	SD	median	mean	SD		p	p
Pre clopidogrel	2.0	3.3	5.3	2.0	3.3	5.3	1 – 2	0.2390	0.2602
							1 – 3	**0.0464**	0.0759
Post Clopidogrel	1.3	2.3	2.4	1.3	2.3	2.4	2 – 3	**0.0287**	**0.0286**
							2 – 4	0.8921	0.2855
Post operative	2.8	8.2	16.5	2.8	8.2	16.5	3 – 4	**0.0373**	**0.0188**
30 days post	1.6	2.4	2.4	1.6	2.4	2.4	4 – 1	0.8411	0.3696

iii) Comparison of embolic load with platelet function tests.

Statistical Methods

Embolic signals were analysed as continuous and discrete variables when comparing the results of embolic counts with platelet function tests. Emboli counts at each time point were classified according to the patients responder status as previously described. Prior to each analysis the data was assessed for Gaussian distribution with the D'Agostino & Pearson test. Several groups failed to conform to a normal distribution and Logarithmic transformation was performed on the emboli count to improve normality and allow assessment with parametric techniques. The data was then analysed with ANOVA and T test's to compare between responders, non responders and semi responders, and within the groups at each time point. This procedure was repeated for patients using the 5 dB cut off and no decibel cut off to classify emboli. This analysis was then repeated using the methods described by Angiolillo [234] to classify platelet reactivity into normal and low responsiveness.

Previous studies investigating correlation between platelet function and Doppler embolic signals in carotid endarterectomy have split patients into high and low risk groups based on the number of embolic signals detected in the first 3 hours post operative. Patients with greater than 25 signals were classified as high risk and those less then 25 as low risk. A similar system of classification for analysis based on emboli per hour in the post operative period was used in this analysis.[262] Fishers exact test compared the groups using these methods.

A direct correlation analysis was performed comparing percentage platelet inhibition with 10 μM ADP solution and embolic counts at each time period to see if this impacted on embolic counts in a univariable analysis. This was performed for adjusted emboli counts. The data was analysed with Spearman's correlation for non parametric data as the results were not normally distributed.

Results

When the 5 dB cut off for emboli classification was used no statistically significant difference was seen at any of the time point for each of the patient response classifications. Analysis between the responder classifications at each time point also showed no difference in outcomes. Immediately post operatively patients classified as non responders had a mean emboli count of 9.4 emboli which was not statistically different from the semi responders count of 3.5 or the responders count of 13.05 ($p = 0.88$). Within the non responder group there was also no significant difference between pre procedure post clopidogrel loading counts and the immediate post procedure values. Mean counts increased from 2.6 to 9.4 but this was not statistically significant ($p = 0.32$). Complete results for the 5dB cut off are summarised in **table 13** and **figures 16 and 17**. The analysis was repeated using no dB cut off for emboli detection. The results were identical to the 5 dB cut off group and the results are shown in **table 14** and **figures 18 and 19**.

Table 13 Comparison of emboli counts classified with the 5 dB cut off compared between time points. Log transformation applied to data prior to T Test.

Time	Emboli count per hour									Compare	T test		
	Non Responders			Semi Responders			Responders				non	semi	resp
	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD		p	p	p
Pre clopidogrel (1)	0.92	1.2	0.78	3.5	3.1	1.81	2.0	4.6	8.40	1 – 2	0.36	0.18	0.40
										1 – 3	0.09	0.70	0.43
Post clopidogrel (2)	1.8	2.6	2.3	0.92	2.4	3.0	1.4	2.1	1.9	2 – 3	0.32	0.09	0.16
										2 – 4	0.79	0.80	0.41
Post operative (3)	2.2	9.4	17.0	3.1	4.0	2.9	2.9	13	25.7	3 – 4	0.25	0.14	0.40
30 days post operative (4)	1.6	1.7	1.2	1.7	2.4	2.7	1.5	2.8	2.6	4 – 1	0.48	0.27	0.93

Table 14 Comparison of emboli counts classified with no dB cut off compared between time points. Log transformation applied to data prior to T Test.

Time	Emboli count per hour									Compare	T test		
	Non Responders			Semi Responders			Responders				non	semi	Resp
	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD		p	p	p
Pre clopidogrel (1)	1.83	1.59	1.09	3.71	3.62	2.12	3.0	5.20	8.20	1 – 2	0.45	0.26	0.40
										1 – 3	0.23	0.63	0.37
Post clopidogrel (2)	2.20	3.60	3.59	1.63	2.80	3.26	2.25	2.55	2.12	2 – 3	0.59	0.11	0.12
										2 – 4	0.24	0.88	0.54
Post operative (3)	2.50	9.60	17.35	4.17	4.54	3.17	3.84	14.04	25.4	3 – 4	0.14	0.15	0.27
30 days post operative (4)	0.53	1.38	1.87	2.50	2.84	2.88	1.70	3.31	3.15	4 – 1	0.51	0.34	0.78

Figure 16 Emboli count per hour 5 dB cut off compared with ADP mediated platelet aggregation classification. Emboli counts on Y axis on log scale. Comparison on transformed data with ANOVA and T tests between 2 groups.

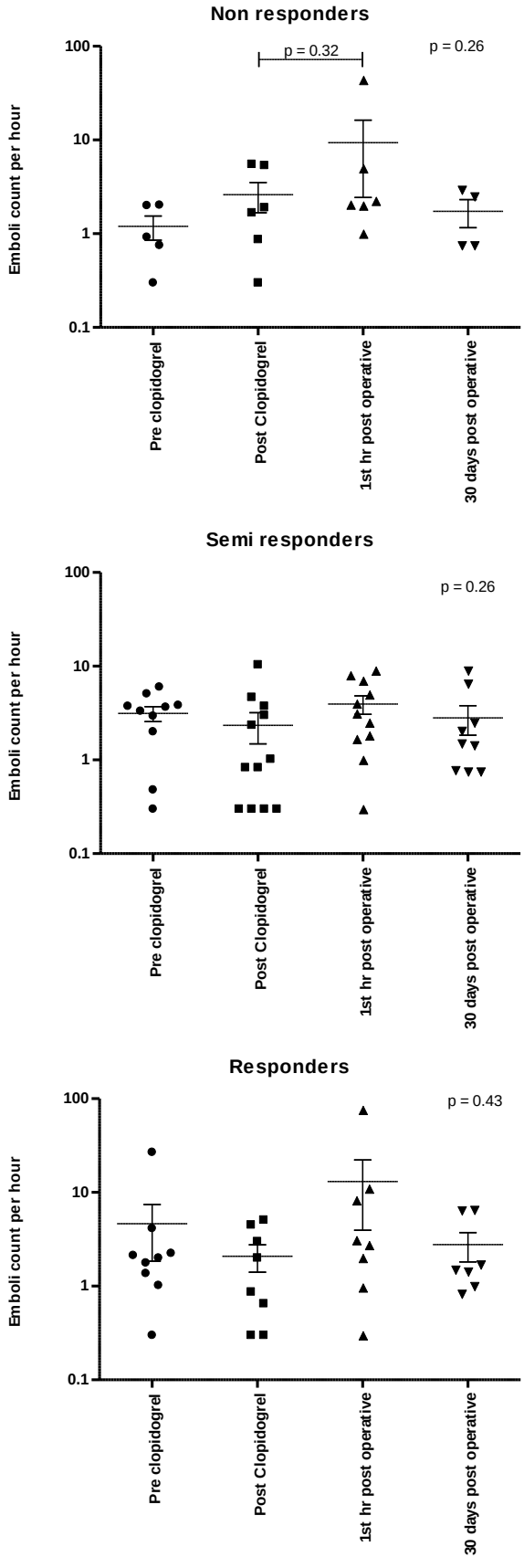


Figure 17 Comparison between each classification and each time point 5 dB cut off. Emboli counts plotted on Log scale. Statistical analysis performed with ANOVA on transformed data comparing between groups.

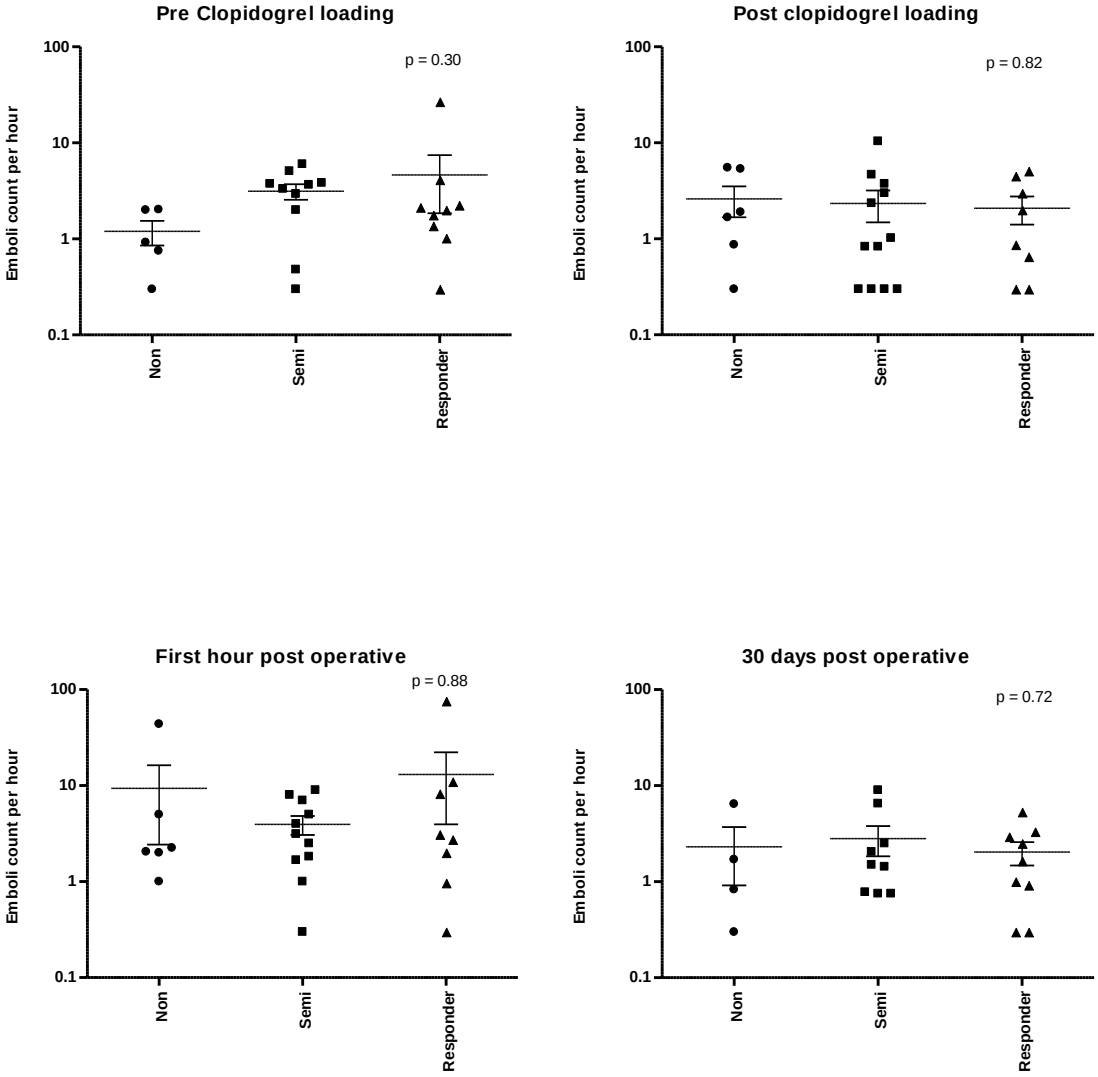


Figure 18 Emboli count per hour no dB cut off compared with ADP mediated platelet aggregation classification. Comparison on transformed data between groups with ANOVA and t test. Emboli counts on Y axis plotted on log scale.

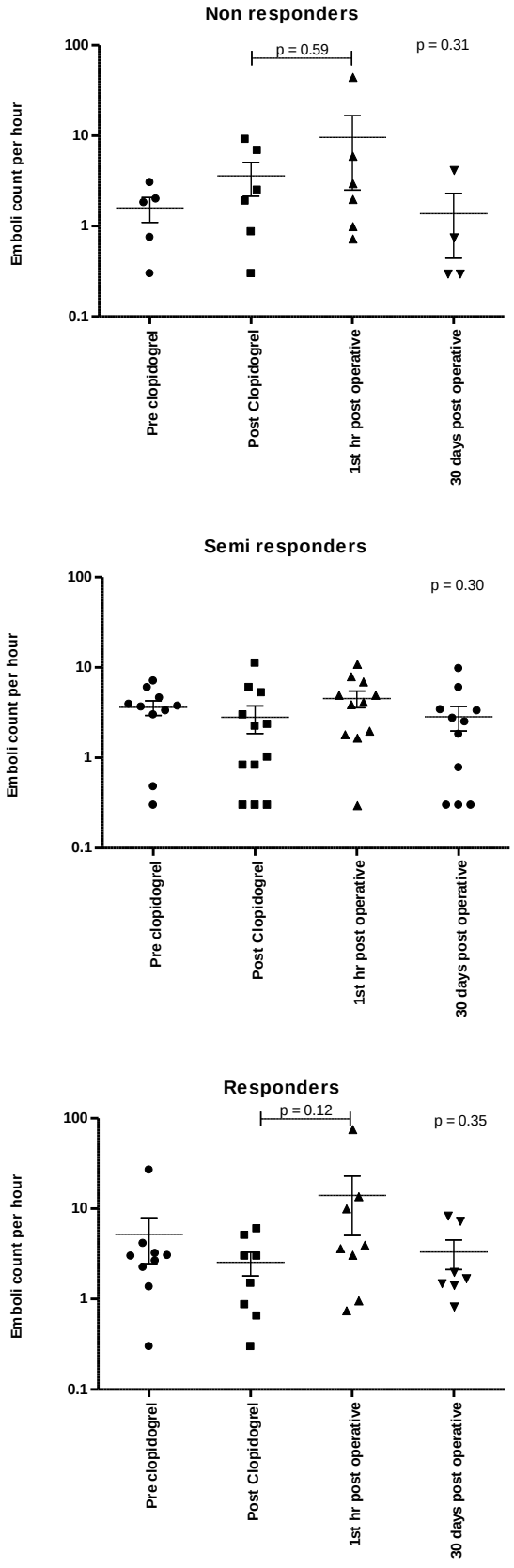
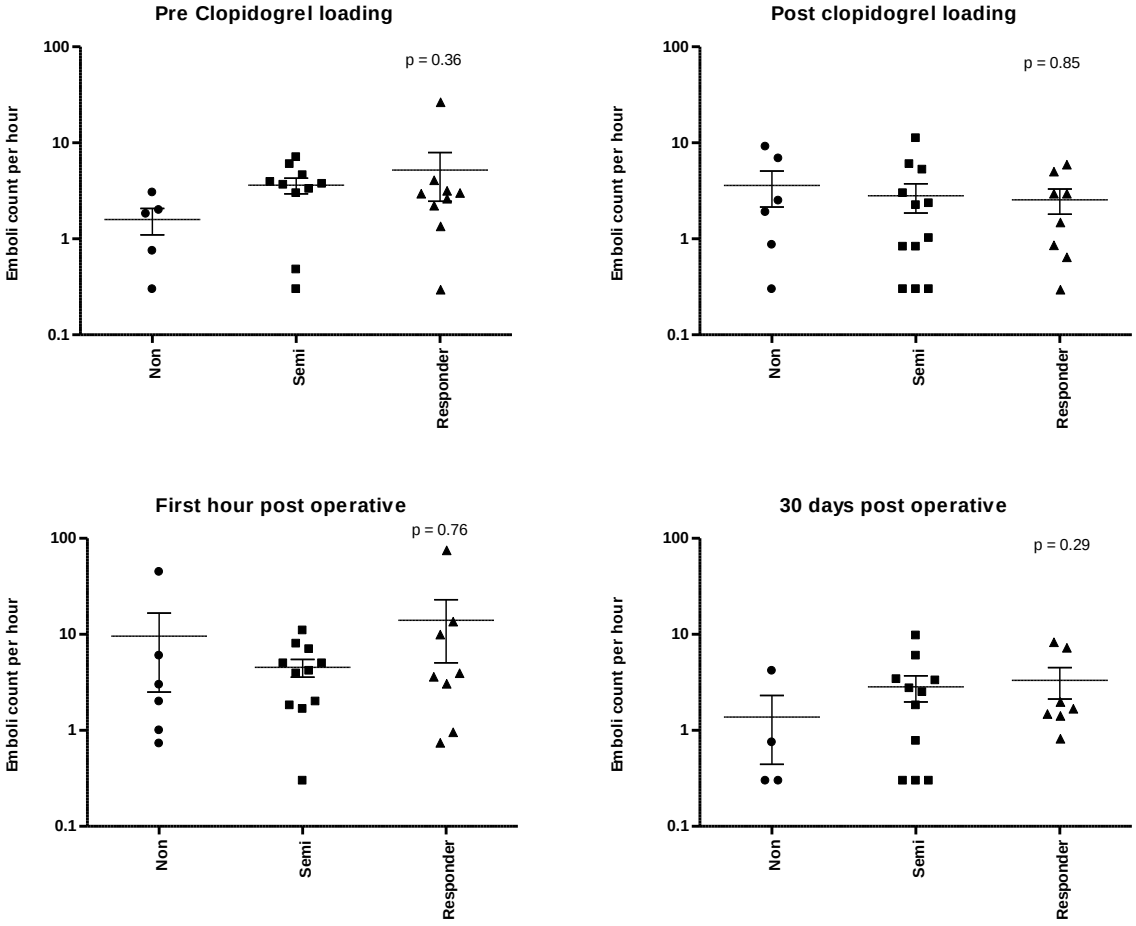


Figure 19 Comparison between each classification and each time point no dB cut off. Emboli counts plotted on log scale. ANOVA on transformed data comparing between groups.



The data was then re analysed using Angiolillo's criteria for classifying platelet responsiveness. At each time point there was no significant difference demonstrated between the mean emboli counts of patients classified as having a, "normal," response to clopidogrel and those having a, "low," response (**figure 20**). The post operative counts in particular showed no difference with mean and standard deviations of 11.63 (24.4) for normal responders and 6.20 (10.4) for low responders respectively ($p = 0.683$). Comparison within the group of normal responders showed no statistically significant difference in mean emboli counts between the post loading 1.935 (1.78) and post operative 11.63 (24.4) time points ($p = 0.217$). However, the difference in mean emboli count post loading 2.56 (2.79) to post operatively 6.21 (10.4) in the patients who were low responders was shown to be significant ($p = 0.024$) (**figure 21**).

The analysis was repeated with no decibel cut off for emboli. Similar results were obtained at each time point with no significant difference demonstrated between the mean emboli counts of patients classified as having a normal response to clopidogrel and those having a low response (**figure 22**). Comparison within the group of normal responders showed no statistically significant difference in mean emboli counts between the post loading 2.50 (1.86) and post operative 12.51 (24.2) time points ($p = 0.275$). As with the 5dB cut off the difference in mean emboli count post loading 3.17 (3.49) to post operatively 6.70 (10.5) in the patients who were low responders was the only difference shown to be statistically significant ($p = 0.042$) (**figure 22**).

Figure 20 Comparison between responders and low responders classified by Angiolillo's method using 5 dB emboli cut-off. T test performed on transformed data. Emboli count on Y axis plotted on log scale.

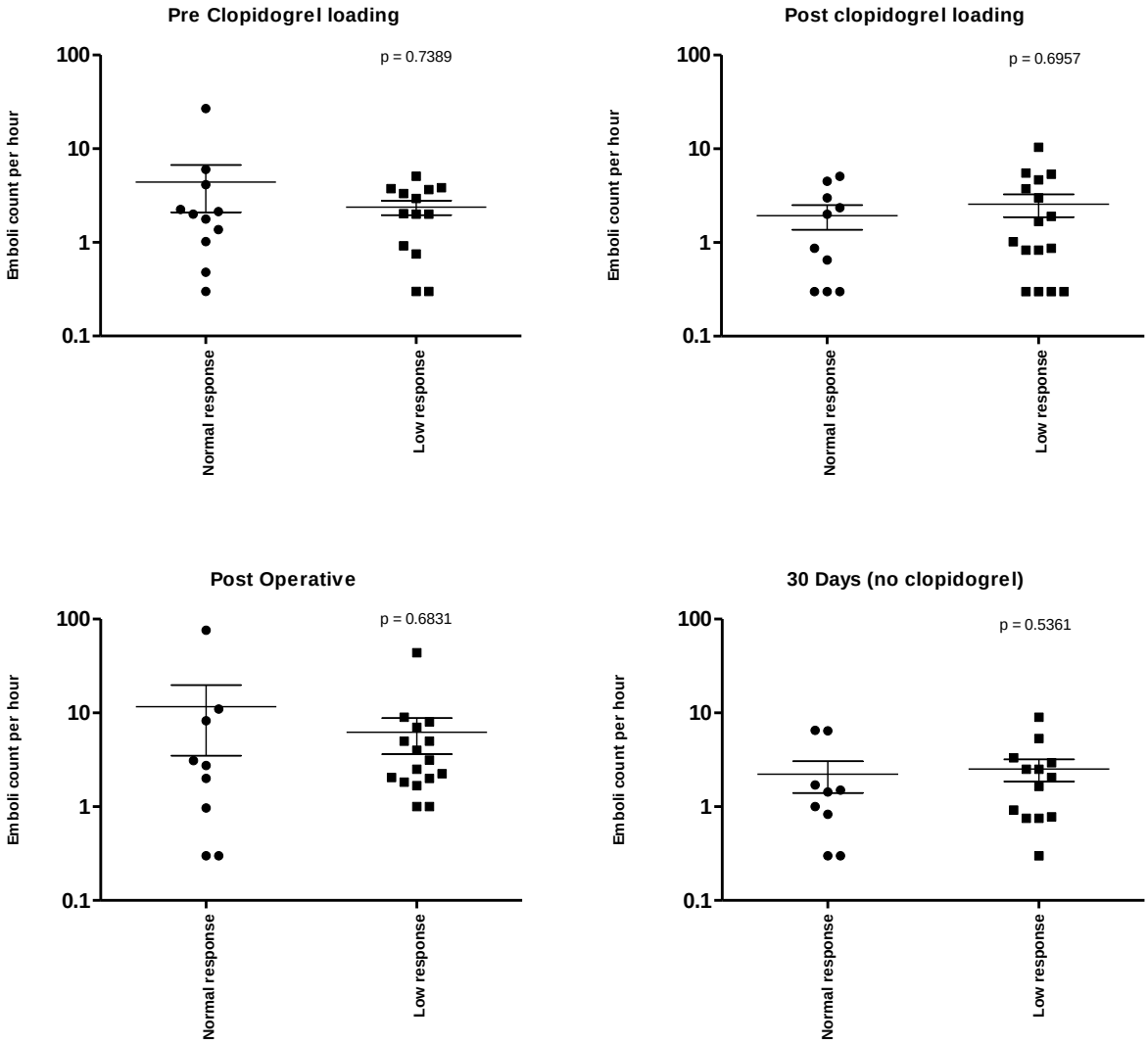


Figure 21 Comparison between time points within the groups of normal responders and low responders classified by Angiolillo's method for a 5 db cut off. Comparison between time points performed on transformed data with ANOVA and T test. Y axis plotted on log scale.

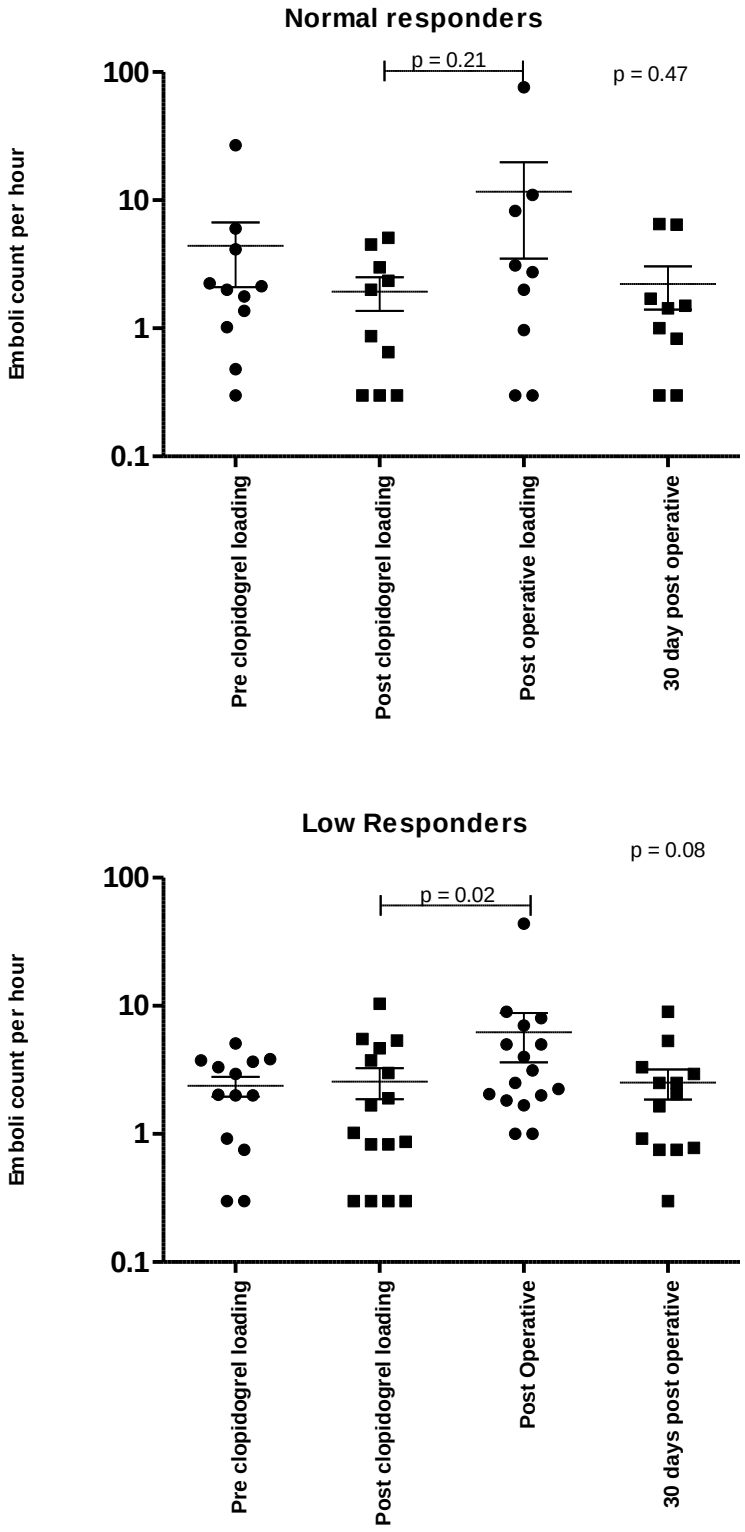


Figure 22 Comparison between responders and low responders classified by Angiolillo's method using no dB cut off. T test performed on transformed data. Emboli count on Y axis plotted on log scale.

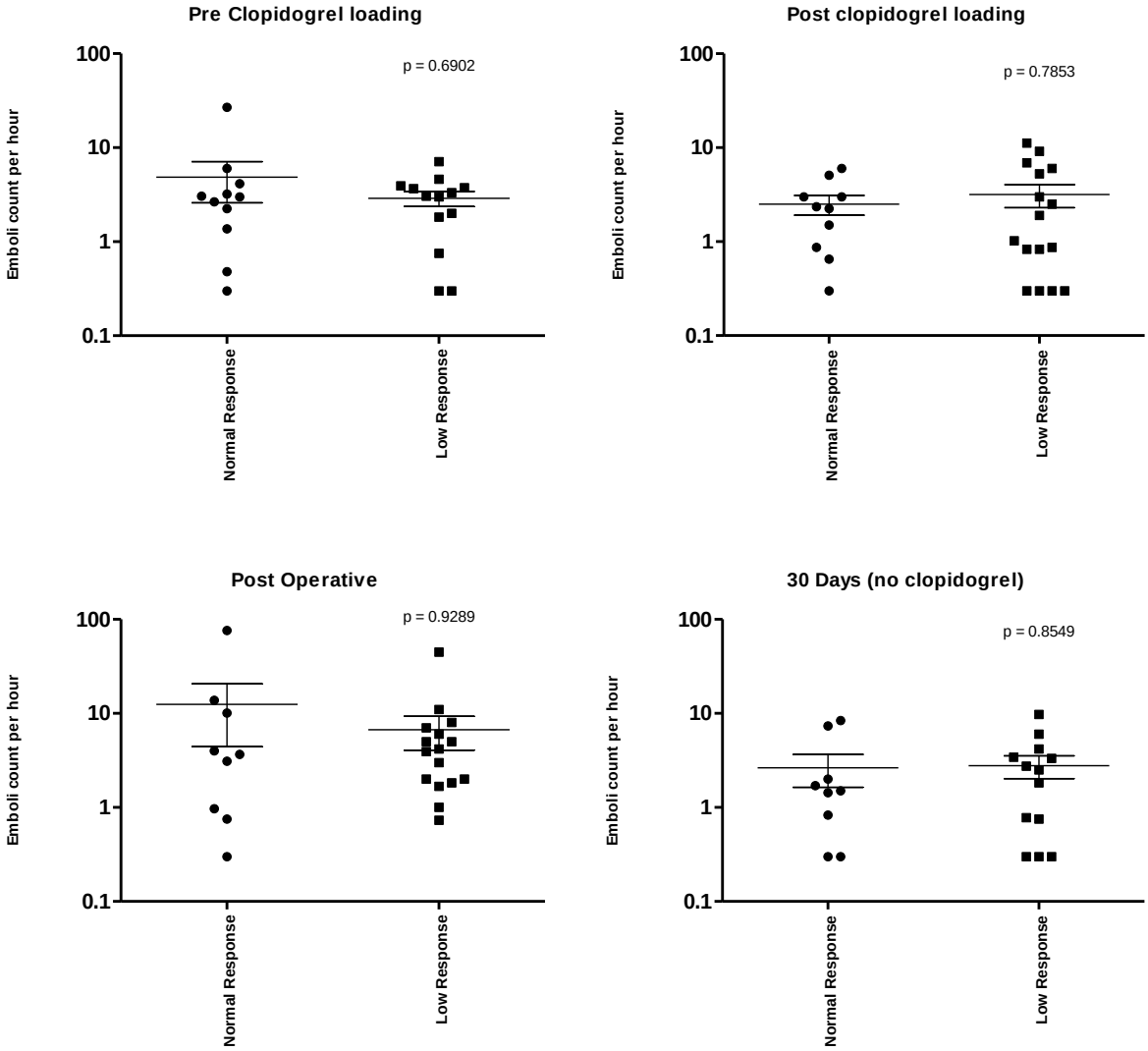
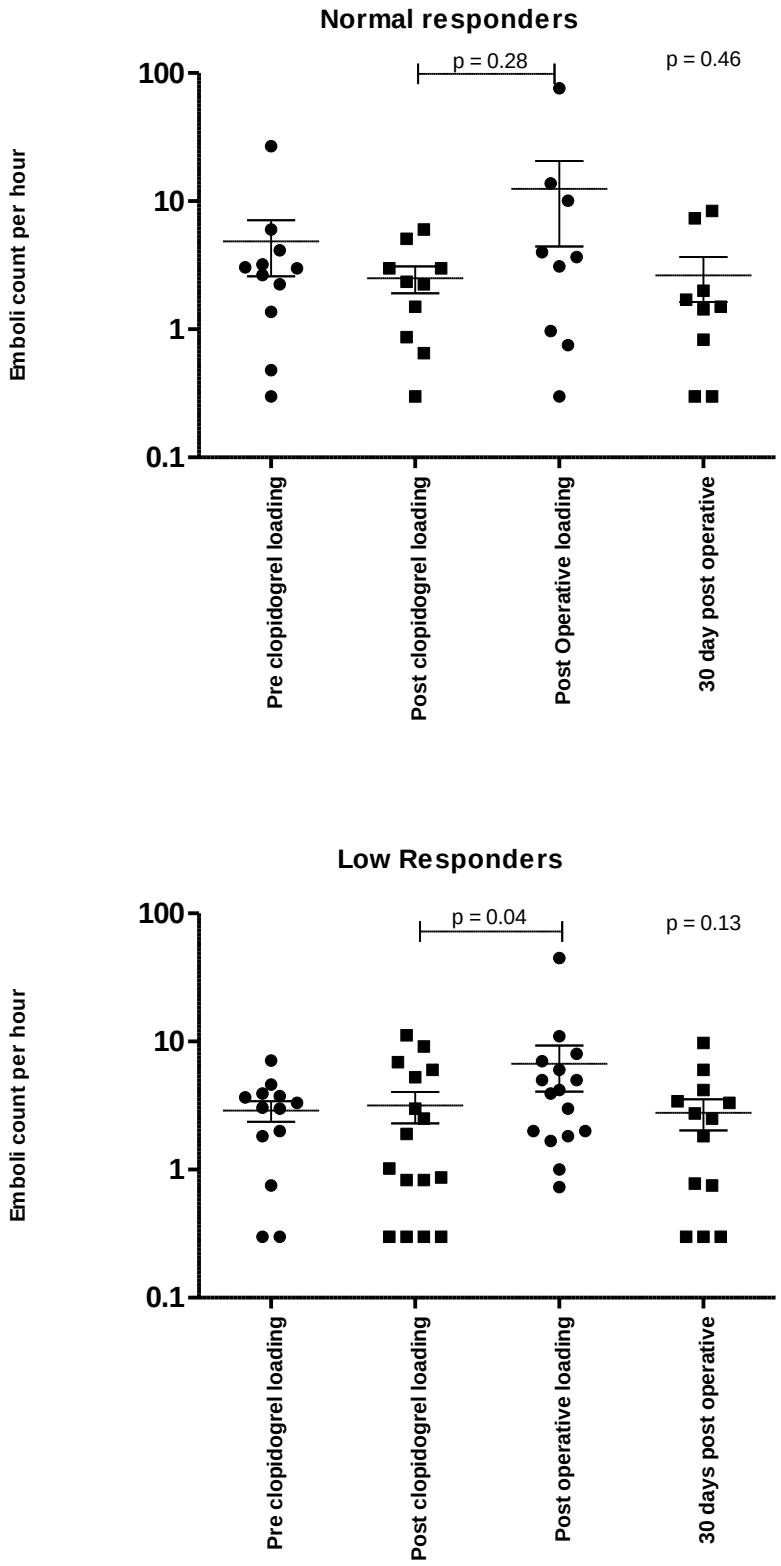
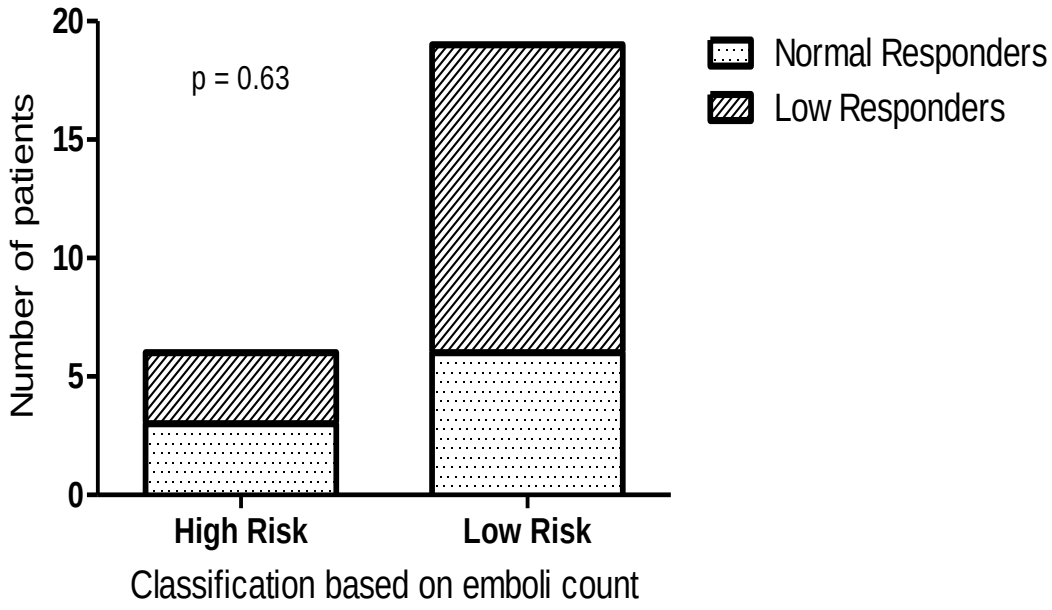


Figure 23 Comparison between time points within the groups of normal responders and low responders classified by Angiolillo's method for no db cut off. Comparison between time points performed on transformed data with ANOVA and T test. Y axis plotted on log scale.



Patients can also be classified by the number of emboli produced post procedure into high and low risk groups for future events as previously described by Naylor's group.[262] The EPICAS data was subjected to this classification to see if this classification reflected the degree of platelet inhibition achieved by the procedure when using Angiolillo's method of classification for low or high responders. No association using fishers exact test was shown between the future risk of recurrent events as classified by the emboli counts and the patients response to clopidogrel when assessed in our small number of patients (p = 0.63) (**figure 24)**.

Figure 24 Comparison between future risk of stroke based on post operative emboli count and response to clopidogrel as assessed by Angiolillo's method.



A direct comparison between embolic signal counts and the percentage of aggregation achieved with 10 μM ADP solution using Spearman correlation failed to show any significant correlation for all time points assessed (r = 0.0094 (-0.2936 - 0.1134), p = 0.36). The results were then separately analysed at each time point and a very weak statistical correlation was shown in the post stenting period, but this post hoc analysis should be viewed with caution as it is likely to have little clinical significance (**figure 25**). The analysis was then repeated with the sCD40L levels. No link had previously been demonstrated between sCD40L and ADP induced platelet aggregation and it was considered possible that plasma levels of sCD40L could be a more accurate reflection of platelet activity at each time point, and would therefore correlate more accurately with micro embolic signal counts. Direct comparison of sCD40L plasma levels and the number of embolic signals detected at each point was performed with Spearman correlation for non parametric data. These results are shown in **figure 26**. A significant correlation was shown between the levels of sCD40L detected post operatively and the number of micro embolic signals detected on TCD when assessed with spearman correlation co-efficient (p = 0.0023). A line of best fit has been plotted on the figure. For clarity the y axis has been plotted on a log scale but the line reflects the original data points and therefore plots as a curve. At no other time point was a correlation seen.

Figure 25 Correlation between 10 µM ADP induced platelet aggregation and embolic signal count per hour (5dB cut off). Spearman correlation co –efficient. Y axis plotted on log scale.

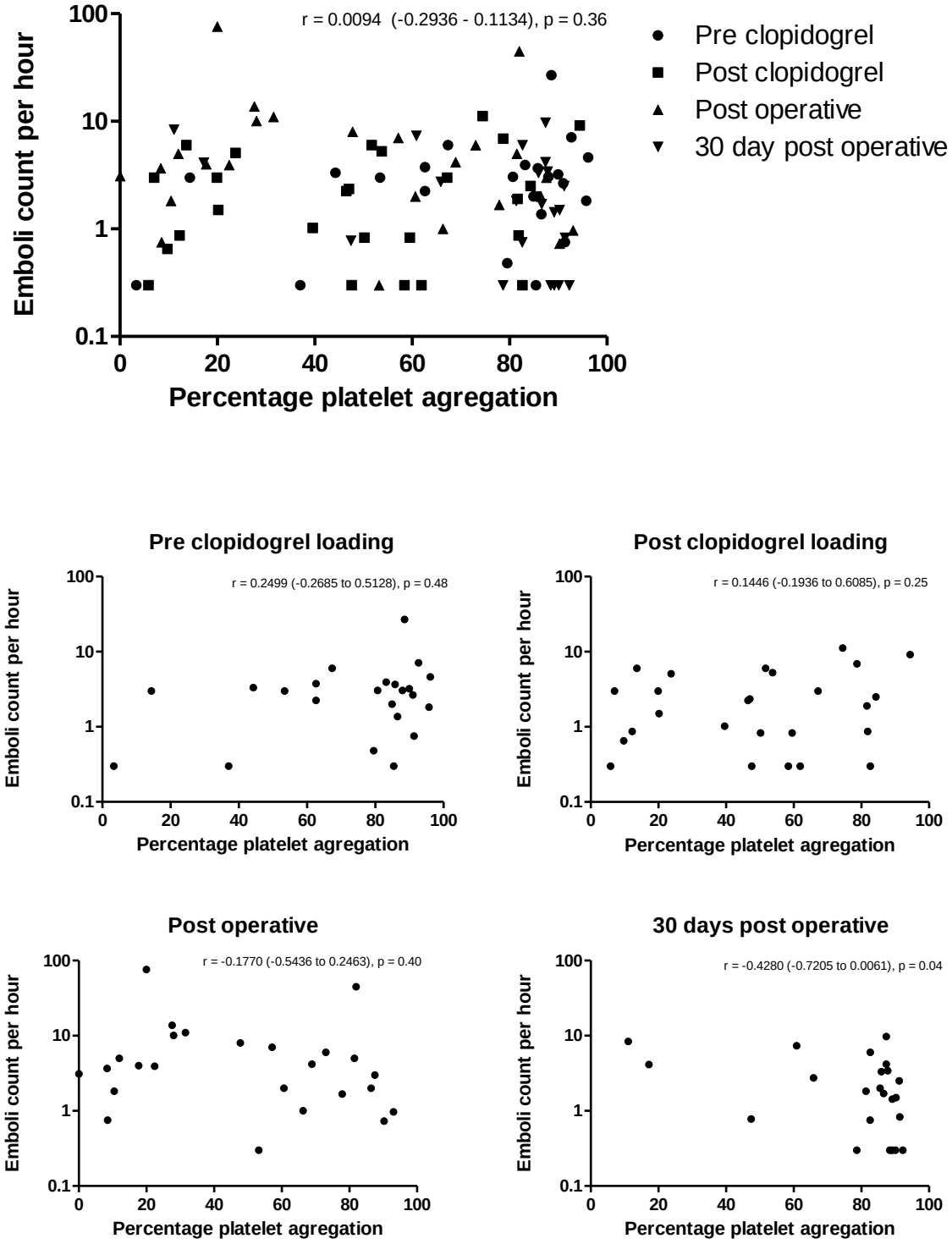
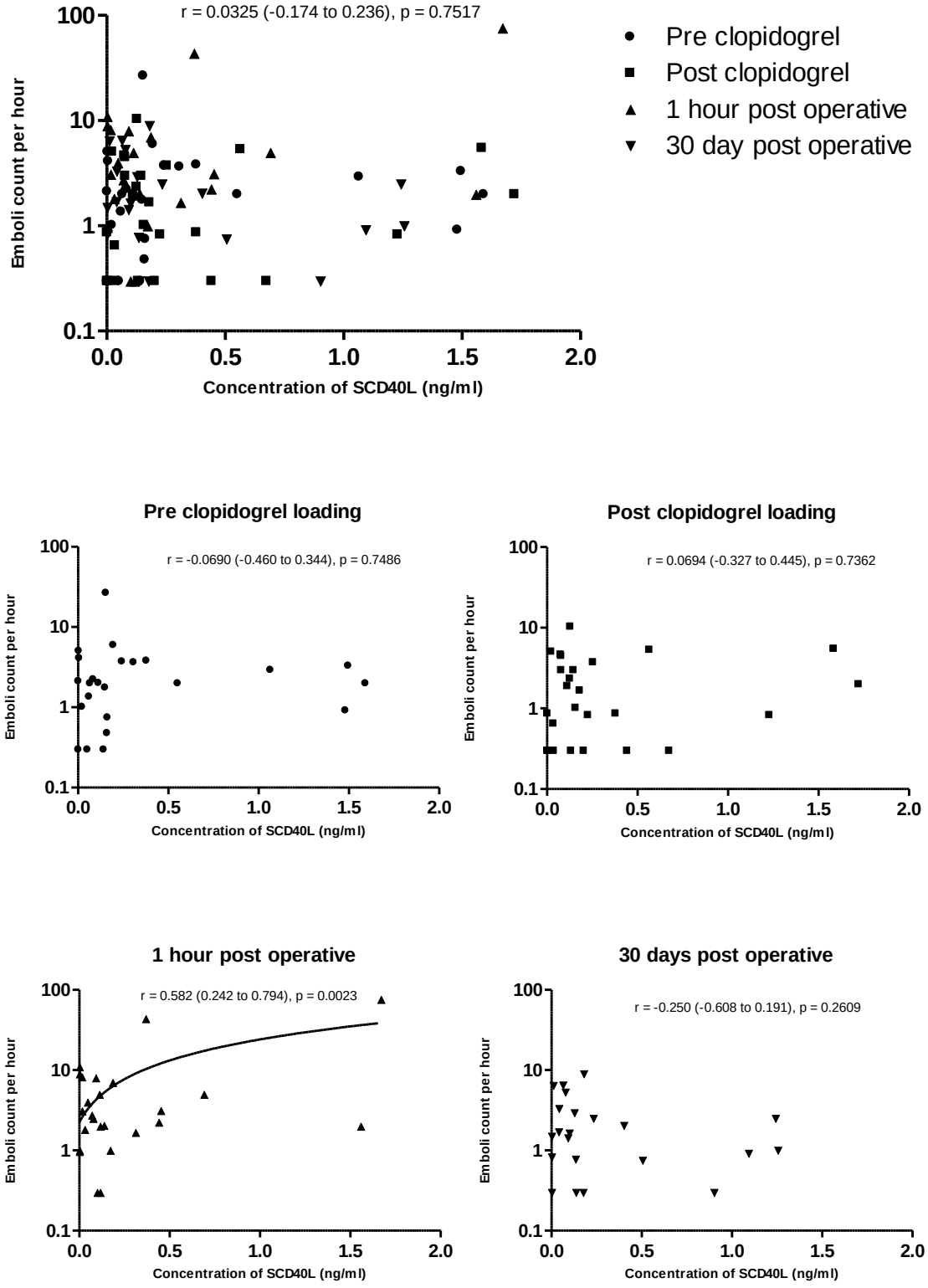


Figure 26 Correlation between plasma levels of SCD40L and Micro embolic signal count (5dB cut off). Spearman correlation co–efficient.



iv) High Sensitivity C Reactive Protein (HSCRP) analysis

Statistical methods

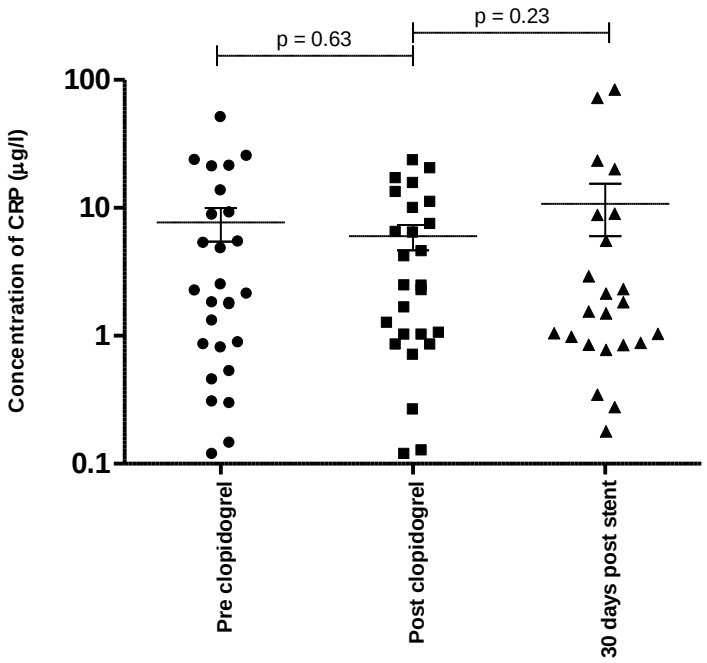
High sensitivity C reactive protein (HSCRP) samples were collected from the patients prior to loading with clopidogrel, immediately prior to the stenting procedure being performed and 30 days after stent insertion. Comparison between time points pre clopidogrel loading and post clopidogrel loading were performed with paired student T tests on log transformed data.

Analysis was then performed to see if a direct relationship could be observed between the emboli count at each time point and the measured level of HSCRP. This analysis was performed with spearman correlation for non Gaussian distributed data. The results have been plotted on log scales for clarity.

Results

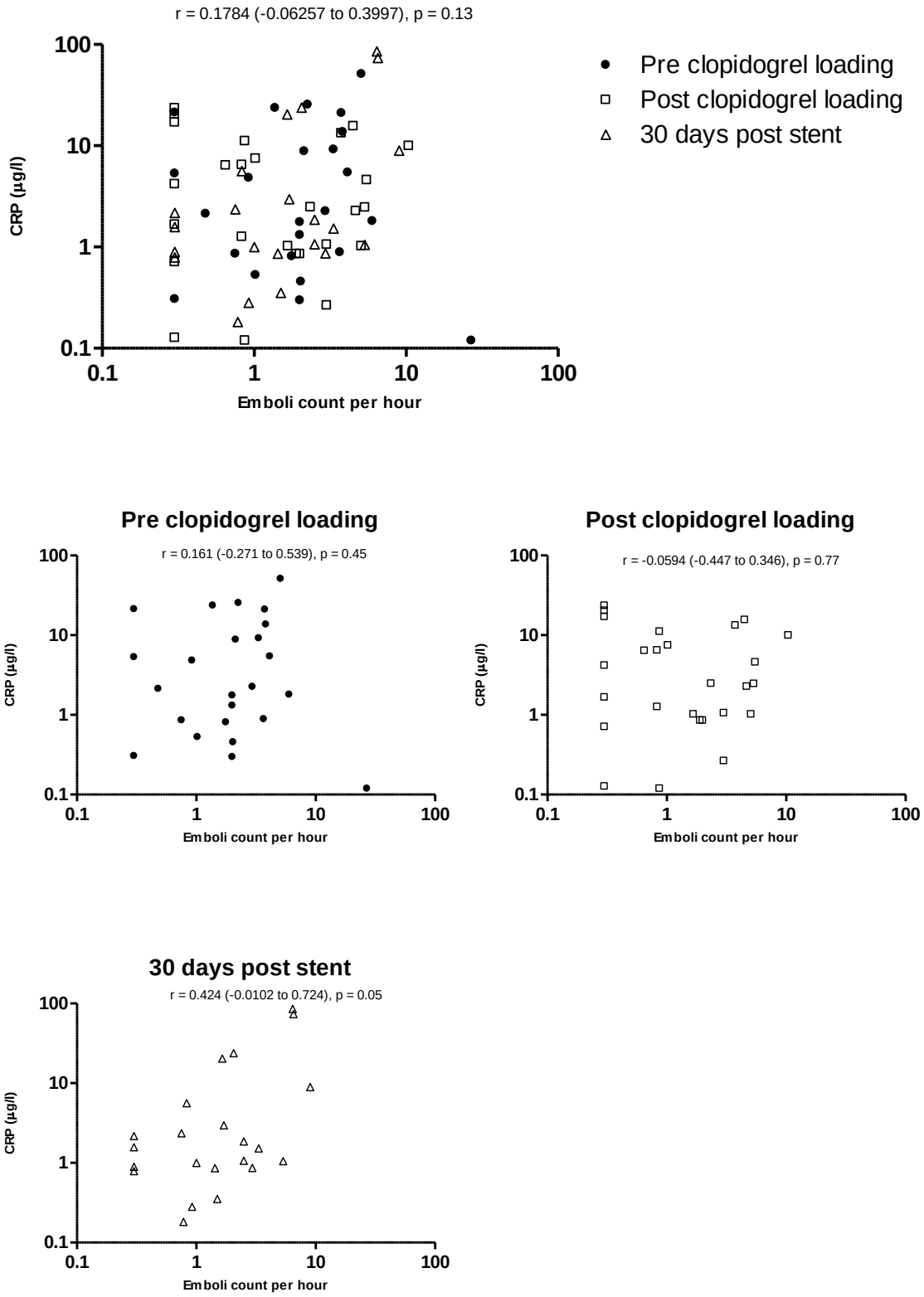
Comparing overall levels of HSCRP across the three measured time periods using the ANOVA method showed no statistically significant difference in levels between the groups ($p = 0.98$). Using t tests to compare between two time periods also failed to show the expected reduction on inflammation and overall levels of circulating CRP when clopidogrel was administered **(figure 27)**.

Figure 27 Levels of CRP measured at each individual time point. Comparison on log normalised CRP concentrations with paired T test.



Directly comparing the number of embolic signals detected using the 5dB cut off at all of the time points under consideration with CRP levels failed to show any correlation between HSCRP levels and emboli p = 0.13 **(figure 28).** When individual time periods were analysed no correlation was seen between the pre or post clopidogrel loading time periods, but was seen with the 30 day post procedure results. The correlation was statistically significant r = 0.424 (-0.0102 to 0.724), p = 0.05 at this time point and may reflect continued inflammatory activity in the stented carotid.

Figure 28 Comparison between HSCRP levels and mirco embolic signals detected per hour using the 5dB cut off. Analysis with Spearman correlation.



vii) Thromboxane

Serum thromboxane samples were collected from all patients at the time of entry into the study, during the operative procedure and post procedure at the 6 week follow up visit when clopidogrel had been discontinued. Thromboxane levels were measured in pg/ml by the assay used. Therefore the 10 ng/ml cut off for insufficient inhibition in our patients is equivalent to 10,000 pg/ml for analysis.

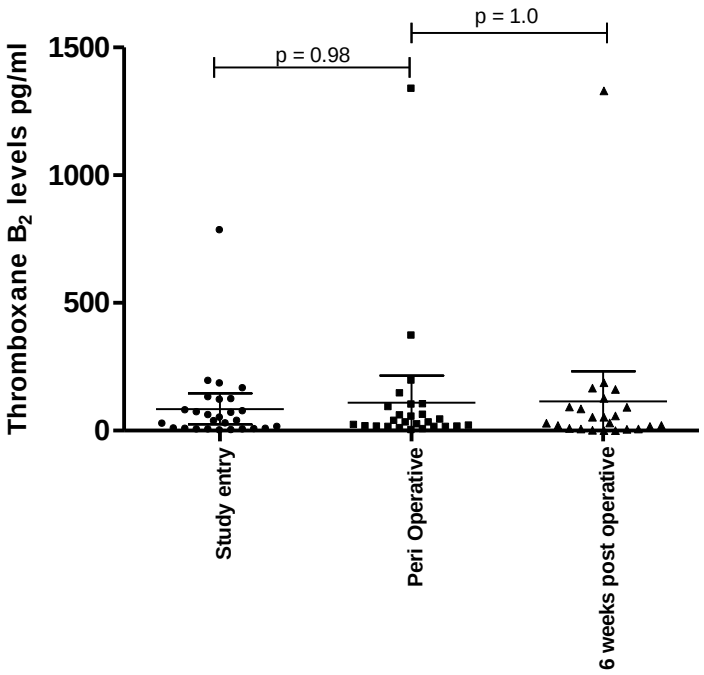
A comparison between each of the time periods was undertaken using the Wolcoxon signed rank test for non-parametric paired data.

Results

No patient sample was detected that had a level of serum thromboxane level of 10,000 pg/ml prior to, during or 6 weeks after the procedure. Assuming the assay to be accurate, all of the patients in our small sample are "aspirin responders." Applying a more stringent cut off used by some authors of 2.2 ng/ml or 2200 pg/ml [253] fails to indicate that any of the patients tested in our small sample have deficient inhibition of thromboxane synthesis. Using these criteria there appeared to be no patients that satisfied the previously described cut off's for reduced responsiveness to aspirin and therefore no further comparative analysis with emboli counts could be undertaken.

No statistically significant differences were seen between any of the time points tested (**figure 28**).

Figure 28 Plasma thromboxane B_2 levels in EPICAS study subjects at each time point. Wilcoxon signed rank test for significance.



6 Discussion of EPICAS study results

The primary end point of the EPICAS study compared the rate of transcranial Doppler detected micro embolic signals with an individual patient's responses to the antiplatelet agent clopidogrel. Prior to the study power calculations were undertaken to detect differences between patients classified as clopidogrel resistant and clopidogrel responsive. Patients classified as clopidogrel resistant were assumed to be likely to have a higher frequency of embolic events. The calculations were based on figures in published studies and abstracts that had used a similar technique for investigating novel antiplatelet agents during carotid endarterectomy and stenting procedures.[114, 263-266]

The primary assessment of the patient's platelet response showed the expected pattern of intra individual variation. Patient classification based on their response to clopidogrel was in the expected distribution in our patients when using accepted criteria in the published literature to determine whether a patient was a responder or non responder. When classification was compared across each patient it was seen on several occasions that the classifications disagreed. This variation in classification could have impacted on the outcomes when comparing directly the number of emboli produced at each time point, and therefore multiple analyses using the previously described techniques were employed in our analysis. In this study and the majority of classification schemes failed to demonstrate a significant cause and effect in univariate analysis between clopidogrel responsiveness and post operative rates of micro emboli.

The immediate message from this result is that clopidogrel responsiveness and clopidogrel variability have no effect on the clinically silent embolic signals seen during carotid stenting procedures. This may be because the changes we expected to see in the

numbers of clinically silent emboli are not clinically significant, although this seems unlikely in view of the outcomes now available from the CARESS study as previously discussed. It is more likely that the effects of variation in clopidogrel responsiveness are so small that even using a more prevalent surrogate end point such as Doppler detected emboli to increase detection sensitivity fails to show the difference in the small number of patients we have studied. It could also be hypothesised that even a poor clopidogrel response is sufficient to reduce cerebral emboli and provide a protective effect. What is clear is that the assessment of clopidogrel responsiveness in vitro has no clear standardised measurement and this makes the interpretation of study outcomes difficult.

The carotid stenting procedure is a complex process with multiple variables interacting to determine clinical outcomes and with patient physiology, operator experience, operator technique, and pre operative pharmacology all contributing. Within this complex process single variable analysis, unless it is a highly significant variable, is likely to have limitations and fail to detect minor trends. However, univariate analysis is an accepted technique when screening for significant variables for later multivariate analysis and in most cases when a variable is not shown to be positive in univariate assessment it has little or no effect on a multivariate model. It is therefore unlikely that a significant effect has been missed. In this study the limitation is likely to be that complete Clopidogrel resistance occurs at a relatively low frequency, and therefore its effects, if any are present, are likely to be small and undetectable at a clinically relevant rate based on the small numbers of patients studied due to these complex interactions.

Embolic signals were used in this study as surrogates for clinical endpoints and should have increased the likelihood of an index event occurring because they are often found

at higher frequencies than clinically detectable events during carotid stenting procedures. Previous publications have also suggested that the majority of the surrogate embolic signals detected by TCD are likely to have little clinical relevance as most would be incapable of causing a clinically relevant stroke event. Without applying additional prediction algorithms to separate out the embolic signals that could signify a higher clinical risk from those of lower risk it is possible that a significant trend in this trial data may remain hidden. The lack of significance found in the analysis may be shown as a stronger trend if larger trials using clinical endpoints of stroke and TIA were undertaken because only clinically significant embolic events would be included.

Patient selection should not have adversely affected the outcome from this study. All the patients recruited into the EPICAS study were representative of the population of patients undergoing intervention at the Sheffield teaching hospitals in 2003 – 2005. The majority of the patients studied underwent stenting for symptomatic carotid stenosis and asymptomatic procedures were only performed on a few patients awaiting coronary artery bypass surgery. The three asymptomatic patients included in the analysis could have had a minimal effect on the outcome. Other publications have suggested that asymptomatic patients have more stable plaque morphology than patients with a recently symptomatic stenosis.[267, 268] This could theoretically have affected the number of emboli detected post procedure by increasing the number of cholesterol based emboli released from symptomatic lesions. Symptomatic plaques have also been shown to generate higher baseline levels of platelet activation than asymptomatic plaques which could have increased the pre clopidogrel platelet responsiveness making any reduction detectable with clopidogrel use more significant in the symptomatic patients.[269] In the EPICAS study population this effect is likely to be undetectable and not impact significantly on the outcomes as only 3 patients were involved and are spread across the

classifications. Future studies should consider this in the planning stage and the patient population should be limited to a purely symptomatic cohort.

The EPICAS study design was intentionally observational not interventional. The aim was to investigate the true clinical impact of reduced clopidogrel responsiveness in real life situations, and therefore no prescription was placed on an operator's stenting technique, choice of stent or peri procedural protection device use. Stents used were of open or closed design as the operator felt appropriate for the individual patient being treated. Each design of stent has differing physical properties that make one more suitable than another for certain anatomical and operator variables. However, because they have a different anatomical profile and are constructed from different materials they may have a variable thrombo-embolic potential that could impact on emboli produced. The majority of stents used in routine practice by Sheffield vascular radiologists are of the closed stent design. In most situations it is possible for the operator to decide on the appropriate design and manufacturer of stent prior to the procedure which would have allowed us to limit the design of stent used in the trial. However, because unexpected procedural issues can mean that a different design is eventually inserted than what was planned, and it was therefore decided not to be prescriptive in this pilot study and determine the impact of clopidogrel resistance most reflective of real practice.

Recent authors have now confirmed that to some degree differences in clinical outcome may be related to stent design,[71] and therefore this may have had an impact on the embolic counts in the post operative period in our patients. The differing stent designs were distributed evenly between the three classifications of platelet response in our patients, and so the impact of this effect is likely to have been minimised in our dataset.

If a convincing effect of clopidogrel resistance had been demonstrated in the EPICAS study the effect of stent design would have warranted more detailed assessment in future larger cohorts, and prescription of a single design of stent for the procedure when carrying out follow on studies.

The variability in response to clopidogrel in patients recruited in the EPICAS study closely resembles that expected distribution described by previous authors. Combing classification systems used by previous authors to obtain an overall classification of responsiveness enabled us to be certain that patients we classified as non responsive were those that would have been classified non responders in all previous studies undertaken in patients undergoing cardiac procedures, despite variation between the analytical techniques. This classified 6 (22.2%) patients as non responders and is at the level expected for the higher estimates used in the original power calculation. This is also comparable with rates published by previous authors.[108, 158-161]

The creation of a semi responder group created a more clear definition between patients who definitely responded to clopidogrel and those that had little or no response. Direct comparison between the non responders and, "good," or, "normal," responders becomes more meaningful as they now represent the extremes of the antiplatelet response spectrum. If differences in emboli counts cannot be demonstrated at the extremes of clopidogrel effectiveness then any hypothetical effect that this may have on clinical outcomes is unlikely to be significant. We were also interested in, "semi responsiveness," or a partial response as this hypothetical group has never been fully assessed by any of the previous authors working in the field of cardiology.

The transcranial Doppler detected end points used in the EPICAS study should have been more sensitive to minor effects in the semi responder group of patients that would have not been detectable in the clinical endpoints used previously in the cardiac patients. The techniques used in the EPICAS study should have allowed study of this group of patients and to classify more accurately if they respond in a manner closer to patients in the non responder or responder groups. This analysis was not powered for in the initial calculations, and therefore any secondary analysis made based on this data needs to be treated with caution. Our complete analysis of this group has shown that no significant differences or trends were shown and we must therefore assume that either the study was under powered to detect any change or there was no true effect.

Whilst it is likely that the correct finding is that no true effect exists is should be remembered that the assumptions to power the study were based on previous authors published work. The mean embolic count of non responders had to be assumed to be close to those seen in patients who were previously treated solely with aspirin. It was also assumed that full responders would abolish any embolic events in a similar manner to the other agents studied previously assuming clopidogrel to be equally effective. It is clear from the results from the EPICAS study that this effect has not been seen in the patients we have studied. In our small number of patients no clear and repeatable differences between the responders and non responders could be demonstrated, although some interesting trends have been uncovered that may warrant future study.

Clear benefits of the addition of Clopidogrel to the antiplatelet regimes of patients undergoing stenting have been demonstrated based on clinical outcomes and are clearly documented in our analysis of our database data and in the literature to date.[9] The effects of clopidogrel on the number of embolic signals detected by the transcranial

Doppler over and above single agent antiplatelet regimes is not in question.[143] It is therefore assumed that the findings in this study are most likely to reflect the fact that the original assumptions leading to the hypothesis are incorrect. Therefore, differences in the level of clopidogrel inhibition between patients are not as clearly defined, and therefore emboli detection in such a small number of patients is unlikely to detect any significant variation. To attempt to perform a post hoc power calculations using adjusted assumptions based on these results is only likely to reflect what has already been determined by the primary analysis.[270] Further critical analysis is required to identify any flaws in the methodology and any sub group analysis that raises questions for future study.

If each stage of the experiment is reviewed in turn then a couple of modifications to the protocol could improve future studies. The majority of the transcranial Doppler recordings were of good quality and detection of embolic events using the two proposed criteria was easily undertaken. Degradation of some sections of the recordings and patient intolerance to the head band device limited the true length of recordings on occasions to less than the full hour. To make comparison of emboli counts meaningful between the groups it was necessary to express the figures as events per hour, and this introduces assumptions into the experiment that are difficult to adjust for in post hoc analysis which could conceivably influence the outcome. The first assumption was that embolic events, if they are going to occur, do so at a constant rate throughout the time period under analysis and in a truly random manner. Therefore, if a segment of recording was damaged, lost, or unusable at the beginning of the time period under analysis it would be the same as losing a similar section at the end of the recording. Therefore, consequent adjustments for rates can be made in a linear fashion. This assumption was made after assessing the timing of emboli and their distribution

throughout the recordings undertaken. It is conceivable that this assumption is wrong as clinically relevant embolic events have been shown to be more common closer to the time of immediate stent deployment when compared to those occurring 24 hours later.[271] The second linked assumption is that patients who have no emboli detected in the useable recording will continue to have no emboli detected should a full untainted hour recording have occurred. To a degree this was overcome by assuming a nominally low embolic rate of 0.3 emboli / hour for all emboli free recordings to allow for log transformation of non parametric data in analysis, but it is not perfect.[143]

Recordings were reviewed blinded to the patient data to reduce bias on the assessment and each embolic event selected from the original DAT tape recording was stored digitally and re-reviewed on one further occasion before being formally included as a positively detected event. This technique was chosen to increase the reliability of the classification of each event and such sequential double reading even by a single observer when comparing against a check list of characteristics does in part limit selection bias.[272] The technique is however limited as it depends on a single observer, ideally the analysis would have had a second observer but in this pilot study timing and budgetary constraints prevented this. A single observer is more likely to underestimate events and when assessing other radiological interventions single observers have previously been shown to have lower sensitivity and specificity scores than dual observers either reading together or independently.[272]

Two cut off points for true embolic events were used in analysis to ensure that the results of this study were comparable with previous work in carotid endarterectomy and stenting. The 5 dB cut off was used as the lowest level that the software could reliable detect embolic signals with 100 % agreement with the observer and is in line with the

consensus criteria suggesting the use of the lowest most accurate level for an individual machine is used this was chosen for the analysis. The CARESS investigators have also published since the EPICAS study began. In this analysis they did not define decibel threshold above baseline as long as the observers were convinced of a true embolic event it could be included[143] and therefore this analysis was also performed on the EPICAS data after a protocol amendment. No embolic signal was detected in our cohort at less than 3.6 dB above the background that could be classified with certainty as embolic. It is now becoming clear that the threshold at which emboli can be reliably detected is part of a spectrum and is highly dependant on the observer and background noise and it is likely this has limited agreement on dB cut off values previously. In some cases 7dB has been taken as the ideal level above background that can be relied upon, but newer research has suggested it may be as high as 14 dB or as low as 3dB in some situations.[260] Our dual cut off analysis hopefully ensures that any possible statistically links between clopidogrel effect and emboli in the EPICAS study would have been detected and subjected to more detailed scrutiny.

The overall trends in the numbers of embolic detected at each time point matched that expected in patients undergoing carotid stent insertion. A minor non significant reduction in mean emboli counts was seen after loading patients with clopidogrel pre procedure. In a small number of patients a statistical significance was not to be expected but the trend is in line with the reduction in emboli seen in the CARESS study where clopidogrel was shown to reduce embolic events in patients awaiting carotid endarterectomy. At both decibel cut-offs a statistically significant increase in the number of embolic events occurred in the first hours recording immediately post stenting. This was to be expected, as the stenting procedure leaves the exposed metallic stent to act as a platelet stimulant as well as damaged carotid plaque material and

endothelial lining which also have the capacity to produce thrombo-embolic material without effecting platelet activation. Trials using clopidogrel in patients undergoing carotid endarterectomy confirm that clopidogrel use reduces but does not entirely block emboli production. It is possible that this is a reflection the production of cholesterol emboli rather than a failure of platelet inhibition.[266] The embolic potential of the carotid returns to baseline 30 days after the procedure as expected when the patients were reassessed without clopidogrel. At this time point implanted stents have begun to develop an endothelial covering reducing an effect on platelet activation.[166] Stents have a very low embolic potential when assessed with delayed follow up confirming what has been seen in histological specimens of removed stents.[142]

The primary end point for the study was the impact of variable clopidogrel response on the number of embolic signals detected in the immediate post operative period. We undertook recordings during the stenting procedure for off line analysis in case an intra operative event occurred but there were no plans to analyse peri procedure data in the initial protocol. These results have not been included in this study as the small effects attributable to the variable antiplatelet effects of clopidogrel were felt to be undetectable during the stenting procedure itself because of, "noise," in the signal generated by wire manipulation, stent deployment and contrast injections.[97, 137, 273] The type of protection system used has been shown to impact on the particle number and size which would have made an assessment during this period even more complex.[139, 274] The majority of solid particles generated during the stenting procedure are athero embolic plaque material rather than thrombo embolic platelet rich thrombus therefore clopidogrel would have had no affect on their production during the peri operative period and therefore it could acceptably be excluded from the analysis. It has been argued by previous authors

that conventional TCD monitoring during this period has little impact on predicting outcome and no clinical benefit.[275]

The post operative analysis is the most clinically relevant period when the exposed metallic stent acts as a significant stimulus to platelet activation, and therefore the greatest benefit of the antiplatelet agents should have been seen at this time and analysis was focussed on comparing with pre operative levels.[276] This analysis in the EPICAS study failed to show a statistical link between the increased number of embolic signals already seen at this time period and the degree of clopidogrel inhibition.

When the 5 dB cut off was applied to the embolic signal detection a non significant trend to increased rates of emboli post operatively was seen in the non responder group with mean rates increasing from 2.6 to 9.4 emboli per hour ($p = 0.09$). However, a similar non significant increase in mean emboli rates was seen in the responder group 2.1 to 13 emboli per hour. Comparison across the post operative time period also failed to show a statistically higher embolic rate in the non responder group when compared to full responsive patients. The premise of the study was therefore not confirmed with the data obtained. Subset and post hoc analysis has revealed some trends that may be worthy of further discussion.

Post hoc analysis of the patient in the non responder group with the highest embolisation rate revealed immediately post stenting new onset visual symptoms occurred. This patient also had a continued high embolisation rate on TCD detectable after the EPICAS analysis period had completed. This had required IV Dextran 40 as an additional antiplatelet agent to terminate his symptoms as previously described and permitted in the protocol on review of the automated emboli counter for the first post

operative hour analysis.[111, 113, 264] The IV dextran therapy was continued for 48 hours, but this patient later suffered a stroke when the Dextran infusion was terminated and he was discharged home. The platelet function tests for this patient after analysis suggest he had a degree of, "clopidogrel resistance," and therefore terminating the dextran therapy in effect left this patient on single agent aspirin therapy with highly active platelets post operatively. Despite no significant effect on emboli in the cohort of non responders being demonstrated by the EPICAS study it must be remembered that for this individual patient the effect was devastating.

Individual patient notes review was also performed on the patient group classified as responders. One of these individuals had very high emboli rates detected post procedure and the following morning he became blind ipsilateral to the carotid that had been treated. He was later shown to have had a cholesterol embolic event with a cholesterol embolus seen on fundoscopy in the retinal artery causing an acute pan ischemic retina. Clearly in this case the clopidogrel status of this patient had no effect on the rate of cholesterol embolisation that contributed to the high post operative emboli counts and the clinical visual loss. However, very high embolic rates resulted in a clinical event supporting the link between detection of a high embolic load and the likelihood of poor clinical outcome.

Review of the post stenting angiographic run prior to removal of the sheath showed radio opaque material apparently being squeezed between the mesh of the stent making pure cholesterol plaque embolisation the likely cause of the increased embolic signals in this patient, although exact aetiology could not be determined from the TCD analysis alone.[277, 278]

Of note the Nexstent that was used in this patient has now been subject to an FDA withdrawal due to problems with its delivery system detaching and causing strokes. Excluding this count from the dataset, assuming all emboli were cholesterol emboli in nature, had no effect on the statistical significance comparing non responders to responders in the first post operative hour analysis ($p = 0.59$).

It has been previously argued that platelet inhibition by clopidogrel is a continuum of variation, patients should more correctly be classified as patients with platelets that have a high and low responsiveness to stimulation with ADP and the attenuation of this response by clopidogrel can then be classified into normal and low response. Patients should then only be classified into normal responders or low responders on this basis. This analysis when undertaken in the EPICAS patients suggested that a link between a low response to clopidogrel and high embolic rates post stenting in comparison to the pre operative state. However, this post hoc re–analysis of the data was not provided for or powered for at the outset of the trial and it is therefore difficult to draw any clinically significant conclusions from this, but it acts as a pointer for future studies, looking at this method of classification compared with clinical not Doppler endpoints.

Soluble CD40 ligand (SCD40L) was chosen in the EPICAS study as an alternative indicator of platelet function and activation because platelet derived sCD40L makes up the majority of the sCD40L detectable in circulating plasma. Activation of platelets releases sCD40L and therefore it has been hypothesised that low levels of sCD40L indicate that an acceptable degree of platelet inhibition had been achieved. We expected to see an increase in detectable levels of sCD40L in the post operative period and for these levels to be higher in patients classified by the other methods as non responders. This was not shown in the EPICAS patients, and may reflect the small

numbers of patients in each classification, as changes were seen between time points in the larger semi responder group. However, as sCD40L is released by platelets when they are activated plasma levels may be more reflective of global platelet activation as a whole explaining the correlation seen between sCD40L plasma levels and emboli counts in the post operative period. This assumption only confirms using different techniques what is already known on the subject of platelet activation and transcranial Doppler detected emboli that when platelets become activated, the number of detectable micro embolic signals increases. As a result this cannot be used as evidence that failure to inhibit platelet activation with clopidogrel also increases embolic signals.

After stent insertion a number of inflammatory processes occur within the stented vessel, carotid plaque and vessel wall that are likely to impact on the number of micro embolic signals detected in the immediate and late stenting periods. Raised plasma levels of CRP have been shown to be associated with histological features consistent with plaque instability and these unstable plaques may pose a higher stenting risk.[279] Platelet activation triggers inflammatory cascades that clopidogrel and other platelet agents may have some impact on by reducing platelet activation, but it is unlikely that they will have an effect on the inflammatory status of the plaques. Even when adequate platelet inhibition is achieved by suppressing SCD40L release no clear impact on high sensitivity CRP has been shown.[249] In contrast with clopidogrel, statins have been shown to have a significant effect on the inflammation within the carotid plaque, and all patients in the EPICAS study were taking statins at the time of stenting. CRP was measured in these patients before clopidogrel loading, after loading and at the 30 day follow up period. This failed to show any statistical link between CRP and emboli counts in the pre and post clopidogrel loading groups. The weak but significant correlation shown between the CRP level and emboli count in the 30 day post operative

period could have been predicted from what is already known. We already know that any patient with ongoing active inflammation is pro-thrombotic, and we would therefore have expected these patients to produce more emboli. It is possible that the persistently raised CRP indicates a continuing inflammatory process in the recently disrupted plaque and endothelium that has failed to heal, and therefore could directly explain the higher emboli count. Even though it is likely that this is the case, the raised CRP and emboli counts alone are insufficient evidence to make such a direct assumption without additional histological evidence of an in plaque inflammatory infiltrate which is not available in stented patients. It has been suggested that the inflammatory response seen in other vessels is not as significant in the carotid artery and may explain the minimal changes seen in the EPICAS data.[280]

The assessment of Aspirin resistance and its effect on carotid emboli was not one of the primary goals of the EPICAS study and therefore multiple platelet function assays assessing this pathway were not undertaken. Plasma thromboxane levels were chosen as a simple indicator of the global effect of aspirin in an individual patient by its ability to inhibit thromboxane dependant pathways of platelet aggregation. Using the diagnostic cut-offs for aspirin resistance previously described no patients in our cohort were felt to have laboratory evidence of reduced responsiveness to aspirin. However, this does not correlate with population estimates of up to 20% resistance and brings the validity of this data set into question. Using different point of care testing methods to those used in EPICAS another group has shown clopidogrel resistance may occur in around 20% of patients with no significant detectable aspirin resistance in patients undergoing a selection of stenting procedures.[281] Whilst this is not validation of our methods it is reassuring that similar patterns of platelet function have been seen in other groups of patients studied. With no differences between patients to allow classification

into responder and non responder groups for aspirin further analysis of the impact of aspirin therapy on outcomes was not feasible.

The EPICAS study is the first study to have attempted to address the issues of clopidogrel resistance using several definitions of non responsiveness during carotid artery stenting procedures. It has shown that the methods chosen were valid, as it has confirmed that the pattern of distribution of clopidogrel responsiveness shown in previous groups of patients was also present in this cohort, and the rise in sCD40L levels confirmed that platelet activation was occurring post stenting. We were also able to show a significant increase in emboli rates post procedure was detected as expected.

What the study failed to demonstrate with any certainty was how variation in clopidogrel resistance impacts on individual emboli rates. The clinical impact of very high emboli rates post procedure and the significant stroke risk attached to these high rates confirmed in previous studies has been confirmed by the EPICAS patient group.[282, 283] High embolic rates are clearly a marker of instability in the arteriovascular system and it is possible that the addition of clopidogrel to aspirin in the majority of patients does not abolished emboli entirely but instead modifies the rate of embolisation to below a threshold rate at which clinically detectable events are more likely to occur. A shift to focusing attention on monitoring all patients in the post stenting period aiming to maintaining emboli rates of less than 10 per hour may be clinically more relevant for future study. A similar analysis in patients undergoing carotid endarterectomy has been performed showing an increase in platelet response to ADP stimulation and clinical risk if patients had more than 25 emboli in a 3 hour post operative period.[262] We applied some of this reasoning to our data and this post hoc

analysis is shown in **figure 24**, but because the study was not powered to detect this sort of a difference it is not surprising that no change was seen.

It is therefore only sensible to conclude that if an effect of clopidogrel response does exist it is likely to be too small to detect with our current methods and is more than likely to be of little clinical significance.

7 Critique of the EPICAS study methods and design.

i) Problems encountered and solutions.

The study was designed based on previous work performed in the Sheffield Vascular Institute, Neurosciences division and Sheffield Academic Cardiology unit as described in the methods section. The individual elements of the study had robust methodology demonstrated previously but the combination in this small pilot study had been untried in this institution and to our knowledge not previously published by any other group. Similar studies have since been published in patients undergoing carotid endarterectomy.[266]

Performing this pilot study in a small number of patients has highlighted some issues with the methodology employed and also helped to focus future studies. The transcranial Doppler recordings were performed in the majority of the patients studied to a relatively high standard. One hour of recording was obtained at the lowest power settings achievable for that bone window, and the majority of the recording was useable in most patients. However, problems were noted with some of the recordings being less than the full hour. Patients often complained that towards the end of the hour recording that the head band holding the probe in place would become uncomfortable and when adjusting the position they could accidentally move the probe during recording. It is now evident that the number of TCD recordings could have been reduced to obtain similar outcomes and therefore the length of the recordings could have been extended to one and half hours each, with the first hour of useful recording used for analysis. The recordings made before clopidogrel loading and during the procedure may have increased patient fatigue with the study process and have not been shown to add any

useful data in our analysis. A significant number of embolic events have also been seen to occur at 48 hours post stenting on both MRI [284] and TCD [133] studies and therefore an additional recording 24 to 48 hours after the procedure may detect changes not seen in the first hour post procedure as platelet micro aggregates may have delayed formation. Focussing on pre procedure, one hour post procedure 24 hours post procedure and 30 days post procedure recordings would probably have been more effective in producing stable outcome measures.

The methods used to assess aspirin response in the study can also be criticised as there were no clear internal controls. All the patients in the study were taking aspirin following their stroke event, and many of these patients had been taking the medication for a considerable time prior to that for other indications. It might have been worth while performing a baseline battery of antiplatelet tests to investigate the aspirin pathway at entry into the study before clopidogrel loading occurred to more accurately classify these patients degree of aspirin responsiveness. This was not in the original protocol as the study was designed to look in a univariate fashion at the clopidogrel pathway, but might be appropriate in future studies. From our data it is not clear if the patient with the very high embolic count in the non responder group was also aspirin resistant. The failure of the combination of drugs may be what makes the difference to outcomes but could not be tested with the results obtained from these patients.

Surrogate endpoints always have drawbacks and are easily criticised for being non specific for clinically relevant events during carotid intervention, especially when used on their own. Other studies have looked at MRI based endpoints as indicators of clinical damage, and have shown significantly higher numbers of post procedure lesions in one of the patient groups when comparing protection methods.[285] However, it is

likely a proportion of these lesions have no clinical significance as most have been shown to disappear 48 – 72 hours later.[286] This raises the issue of using surrogate end points in any of these studies. To improve on the sensitivities' of these surrogates it would have been useful to have performed both TCD and MRI studies in our patients.

ii) Future work

We have shown that it is unlikely that any significant effect of clopidogrel variability can demonstrated in such small numbers of patients and future studies will need larger numbers of patients. It might be worth repeating this study with larger numbers of patients splitting the patients into the low or normal responder groups and looking for a cut off level of embolic signals of greater than 10 per hour to indicate future high risk.

The benefits of additional antiplatelet agents in patients who fail to respond optimally are still under investigation by other groups. It may be worth repeating the experiment in larger numbers of patients selecting out the suboptimal responders and randomising them to additional therapy, including the intravenous agents to assess in more detail the impact this may have on outcome. The intravenous GPIIB/IIIA inhibitor, eptifibatide has been used in one study in over 400 cases, and only 1 intracranial bleed was shown to have occurred, which was later felt to be secondary to cerebral hyperperfusion, suggesting this may be a relatively safe medication to consider.[287] The intravenous agents are limited by their length of use and an alternative approach may be to look at the triple combination of Aspirin, Clopidogrel and Dipyridamole that has been suggested in a review of antiplatelet therapies previously.[288] Safety data for short term use of this combination has already been obtained from a trial in acute stroke using this combination.[289]

8 Summary : Can we optimise carotid intervention further ?

Ensuring safety for patients during the procedure is the over riding priority in any intervention. This thesis attempted to identify ways in which that risk could be reduced. Selecting patients with low risk is clearly preferable but not possible in real clinical situations. What we have been able to show in patients stented in Sheffield is the different risk of adverse outcome in those patients who present with retinal events and cerebral events, allowing patients to be appropriately informed. Patients undergoing stenting prior to coronary bypass may derive little stroke prevention benefit based on the patients selected for this procedure so far. This issue clearly warrants a randomised trial, and patients need to be aware that the benefits of the procedure are not a clear as had been previously reported and should make their decision to proceed on that basis.

The current optimised therapy for carotid stenting is clearly superior to the historical regimes and the stenting procedure is safer now than at any point in its development. The individual elements that make up this optimised therapy have a minor role to play on their own, but the combined effect on 30 day outcomes is significant. The long term durability of the procedure will have to be proven by the randomised trials but the Sheffield cohort analysis provides supportive evidence over a 5 year period of stent durability.

The EPICAS study tried to improve on safety by critically assessing one aspect of the optimised treatment regime in an attempt to optimise it further. It failed in its primary outcome measure to convincingly demonstrate any impact of minor variations in an individual's response to clopidogrel therapy. This finding is in line with trials

undertaken in patients undergoing carotid endarterectomy that have investigated different components of this procedure, and attempted to optimise the intervention using antiplatelet agents, patch materials, and procedural transcranial Doppler monitoring. The successful developments are published with statistically significant reductions in clinical or Doppler detected micro embolic signal events and these developments gradually become part of the recommended procedural technique. However, the post operative complication rates for carotid endarterectomy when subjected to randomised trials appear to be very similar to the original NASCET and ECST trials of between 6 and 8 %. The complication rates for 30 day any stroke or death in the recent carotid intervention trials EVA 3S (3.9%), SPACE (6.84%) and GALA (4.5%) when scrutinised suggest no clear developments in procedural safety with time and this may suggest that this is the inherently lowest risk for carotid interventions that can be achieved.[170, 173, 290]

One explanation for this is that the limit of technical developments has been reached. It may be impossible to make significant further impact on outcomes that will lower complication rates below 4% for any form of carotid intervention in symptomatic patients. This could be because having had a recently symptomatic stenosis delineates a group of patients that are, "high risk," for any procedure that is undertaken on them. The experimentation with technical adaptations and monitoring has only served to focus the attention of these operators on undertaking as technically perfect a procedure as possible.[266] The dominant factor contributing to the risk that still remains is the, "symptomatic," status of the patient. This is likely to be an independent risk factor for complications and has been suggested when reviewing stenting procedures performed in centres where stenting asymptomatic carotid arteries are routine in the same way as symptomatic procedures.[291] The characteristics of the lesion itself will also

independently affect outcome and cannot be modified prior to the procedure, and in symptomatic patients these lesions are typically more ulcerated and unstable.[292] The combination of these risk factors is now likely to make up a significant degree of the hazard ratio apportioned to each procedure in the multivariate analysis and may explain why minor adaptations to technique have limited impact on the clinical outcome. [291]

It is inconceivable that further adaptations to the techniques of carotid stenting and carotid endarterectomy are not going to be proposed and tested but it is likely that they will have little or no real significant impact on clinical outcomes. We have probably reached the lowest achievable complication rates for both procedures as the procedure itself is inherently risky no matter what adaptations are undertaken to the operative technique.

Appendix 1

Patient Identification Record
EPICAS study

Patient name: _____

Patient unit number: _____

Date of birth: _____

Age: _____

Sex M / F

Trial number allocation ☐

Event type Amaurosis fugax / TIA / Minor Stroke / Major Stroke / CABG

Angio date _____ Stenosis L _____ R _____

Previous Medical History	Yes	No	Drug History	
Angina	□	□	Aspirin	_____
IHD	□	□	Dipyridamole	_____
MI	□	□	Clopidogrel	_____
Previous TIA	□	□	Warfarin	_____
Previous Stroke	□	□	Statin	_____
Hypertension	□	□	ACE inhibitor	_____
Diabetes	□	□	AII blocker	_____
Hypercholesterolemia	□	□	β blocker	_____
Renal failure	□	□	Ca^{2+} blocker	_____
Peripheral Vasc Disease	□	□	Diruretic	_____
Smoker	□	□	Nitrate	_____
Ex smoker	□	□		
			Other meds.	_____
				_____
Screening Doppler	□	□		_____
				_____

Aspirin randomisation number ☐ or Aspirin dose _____

Clopidogrel Batch number 1 _____ Exp Date _____
Batch number 2 _____ Exp Date _____

Date of pre assessment: _____

Date of procedure: _____

Date of 1st follow up: _____

Date of second follow up: _____

Stenting procedure information

Carotid Left / Right

Make of stent used _____

Length of stent used _____

Diameter of stent used _____

Embolic protection device used _____

Blood and urine samples taken

(check box or enter result when samples obtained)

	PRE ASSESSMENT	MORNING OF PROC	STENT PRIOR TO HEPARIN	STENT POST HEPARIN	1 HR	7-14 DAYS	6 WEEKS
Full blood count							
Platelet aggregation test (6ml hirudin)							
Serum thromboxane (clotted sample)							
CRP (clotted sample)							
Plasma SCD40L (5ml EDTA)							

	Morning of Procedure	Morning after Procedure
Urine Thromboxane		

Doppler recordings made

(see separate key to identify numbered tapes relevant to this recording)

Pre assessment □

Prior to procedure (<12 hrs) □ Time _____

During procedure □ Time _____

Post procedure (<2 hrs) □ Time _____

7 – 14 days □

6 weeks □

Doppler recording details

Entry recording

Date	Depth
Tape code number	Sample volume
Tape start time	Power
Vessel recorded	Duration

Pre procedure recording

Date	Depth
Tape code number	Sample volume
Tape start time	Power
Vessel recorded	Duration

Procedure recording

Date	Depth
Tape code number	Sample volume
Tape start time	Power
Vessel recorded	Duration

Post procedure (2 hours) recording

Date	Depth
Tape code number	Sample volume
Tape start time	Power
Vessel recorded	Duration

7 – 14 days recording

Date	Depth
Tape code number	Sample volume
Tape start time	Power
Vessel recorded	Duration

6 weeks recording

Date	Depth
Tape code number	Sample volume
Tape start time	Power
Vessel recorded	Duration

Visit Details

Pre procedure

Medication changes since last visit: _____

_____

Clinical events since last visit: _____

_____

7 – 14 days post procedure

Medication changes since last visit: _____

_____

Clinical events since last visit: _____

_____

6 weeks post procedure

Medication changes since last visit: _____

_____

Clinical events since last visit: _____

_____

Adverse events record

1)

Date of event: _____

Time of event: _____

Type of event: _____

_____

Outcome: _____

2)

Date of event: _____

Time of event: _____

Type of event: _____

_____

Outcome: _____

Genetic sub study

	Yes	No
Patient enrolled in genetic sub study	□	□

10 Publications and presentations arising from this thesis at time of submission.

Chapter 2 Published in Circulation : Cardiovascular Interventions 2010 3:50-56

The long term results of carotid artery stents to manage symptomatic carotid artery stenosis and factors that affect outcome.

Abbreviated form published as lecture notes

Symptomatic Carotid Stents with 1700 patient years follow up – How can we reduce risk?

Book Chapter Charring Cross Vascular meeting 2008

Chapter 3 Published in Stroke 2006 37:435-9

Is there any benefit from staged carotid and coronary revascularisation using carotid stents? A single centre experience highlights the need for a randomised controlled trial.

Plus responses to letters to the editor x3

EPICAS Presented in poster presentation at Association British Neurologists meeting Bournemouth May 2010

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