

https://dspace.org/

To determine the prevalence of aspirin resistance and/or platelet hypersensitivity as determined by platelet aggregometry in Caucasian patients who have suffered one or more atherothrombotic cerebro-vascular accidents (CVAS) and/or transient ischaemic attacks (TIAS) as compared with control subjects

Bernstein, Penelope Lizetta

2010-04-14T13:33:11Z
http://hdl.handle.net/10539/7983

Downloaded from a DSpace repository. https://dspace.org/

1. INTRODUCTION AND LITERATURE REVIEW

The platelet is a very important component of cardiovascular and cerebrovascular disease. Recent platelet research has resulted in many drugs that have been found to improve health in patients. The platelet was previously seen only as an 'onlooker' in haemostasis. The platelet is now recognised to be an essential component of thrombosis and inflammation.[3]

1.1 The haemostatic response

The normal haemostatic response to vessel injury is reliant on intricately linked interaction between the blood vessel wall, blood coagulation factors and circulating platelets (Figure 1.1).[4]

1.1.1 Vasoconstriction

A preliminary decrease in blood flow to the injured area is due to vasoconstriction of the damaged vessel. This ensures contact activation of coagulation factors and platelets. Thromboxane A_2 is liberated from platelets and fibrinopeptides are released during fibrin formation, which also have vasoconstrictive properties.[5]

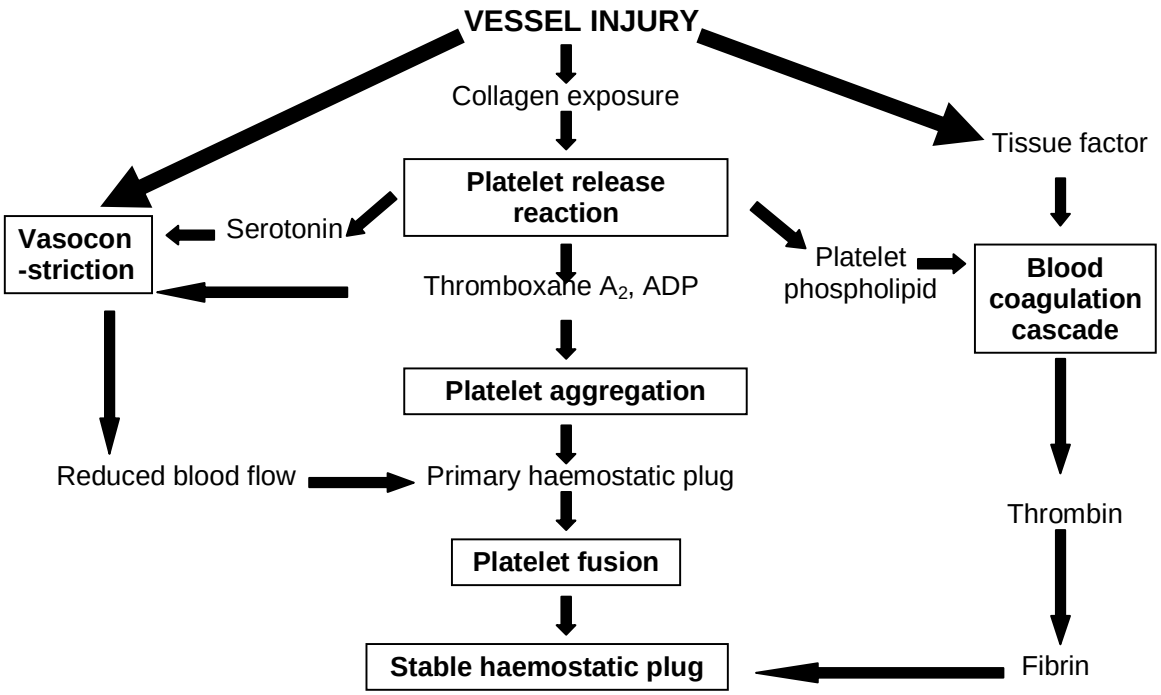


Figure 1.1 The interaction of blood vessels, blood coagulation and platelets in haemostasis.[4]

1.1.2 Primary haemostasis and platelet reactions

Primary haemostasis involves the development of a 'platelet plug' at the site of vessel damage (Figure 1.2).[5]

Single circulating platelets attach to the damaged endothelial surface. These are then activated via receptor-ligand interactions and release their granular contents (platelet secretion). The resulting platelet aggregate forms a physical barrier to further blood loss. Coagulation factor activity is enhanced and localised by platelet membrane phospholipids.[5]

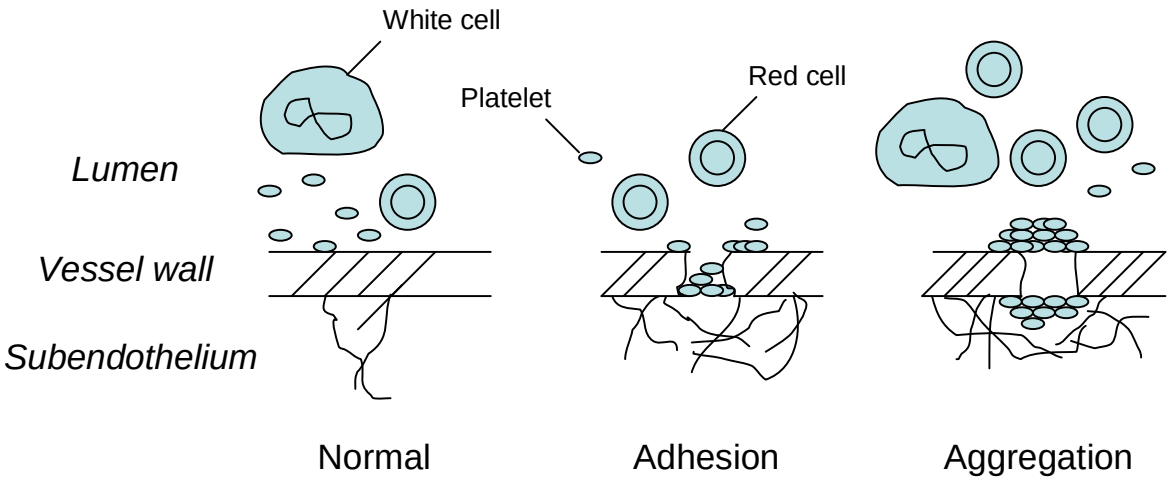


Figure 1.2 Primary haemostasis.[5]

Adhesion: VWF, collagen, fibronectin and other subendothelial molecules result in platelet adhesion to the exposed subendothelium at points of vascular injury. VWF adheres the platelet to the endothelium through interaction with its receptor, platelet GP1b, in arterioles which show high-shear conditions.[5]

Activation: Subendothelial collagen, thrombin and tissue factor all stimulate platelets by attaching to platelet surface receptors and a pathway of signal transduction events commences.[5]

Secretion: Agonists, such as adrenaline, collagen, thrombin and ADP attach to its' platelet membrane receptors and stimulate platelets through a number of biochemical events resulting in its' granule contents being released. The aforementioned process promotes further platelet activation and aggregation. Arachidonic acid is released from the cell membrane with the production of thromboxane A_2 which reduces cAMP levels and commences the release action. Substances such as $PGI_{2,}$ which is produced by vascular endothelial cells, increase the level of

platelet cAMP and prevent this release reaction. PGI_2 is a powerful platelet aggregation inhibitor (Figure 1.3).[4]

Aggregation: When agonists bind, they cause structural alterations in the platelet GPIIb/IIIa receptor, revealing its binding sites for VWF and fibrinogen. Single platelets at the site of vessel wall damage are then cross-linked by VWF and fibrinogen, thus promoting the creation and stabilisation of the platelet plug.[5]

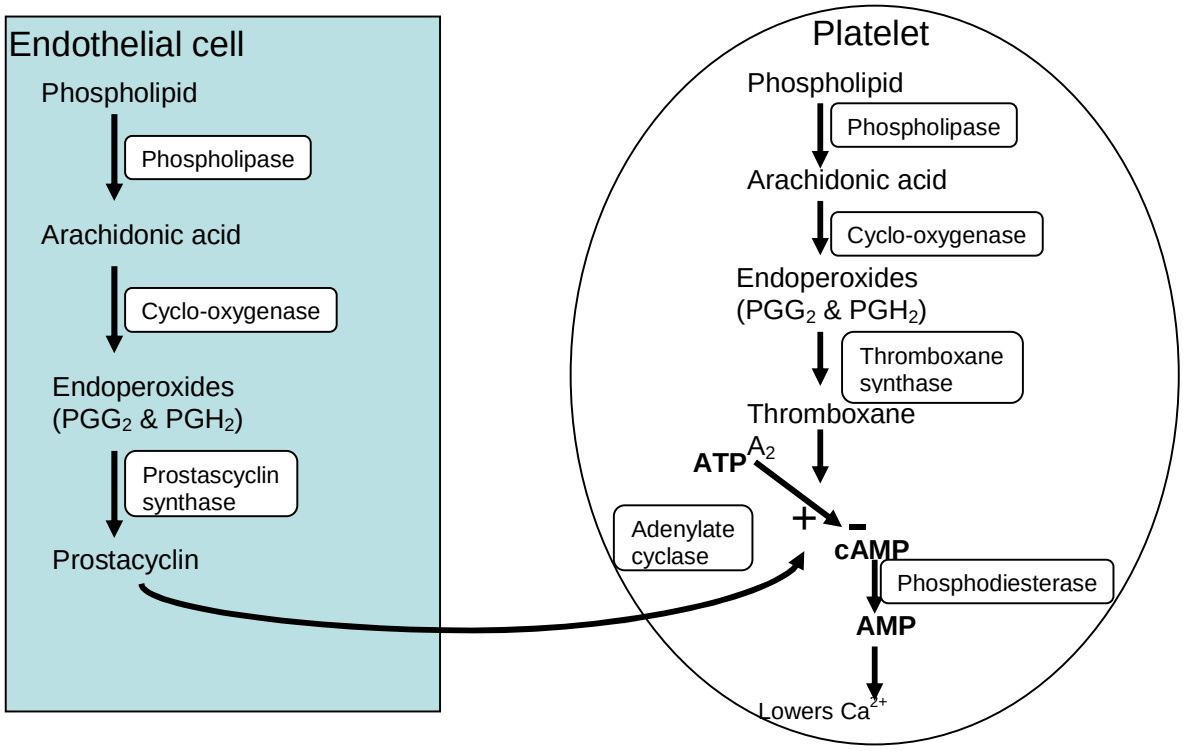


Figure 1.3 The production of prostacyclin and thromboxane.[4]

1.1.3 Stabilisation of the platelet plug by fibrin

Blood coagulation results in fibrin formation. When fibrin is added to the platelet complex and platelet-induced clot compaction occurs, definitive haemostasis results. Following vessel wall

damage, activation of tissue factor causes factor VII to initiate the coagulation cascade. By contributing further membrane phospholipid, platelet aggregation and release reactions speed up coagulation. Thrombin created at the point of damage transforms fibrinogen into fibrin and promotes platelet secretion and aggregation. Thrombin also activates factors XI and XIII as well as cofactors V and VIII. The fibrin component of the haemostatic plug enlarges as the mass of platelets autolyse. A few hours later the platelet plug is transformed into a strong complex of cross-linked fibrin., The platelet plug simultaneously autodigests as a result of plasminogen and tPA.[4]

1.2 Stroke

Stroke is the clinical term for acute loss of perfusion to a vascular region, that results in ischaemia and a consequent deficit of neurologic function. Strokes classically manifest with the rapid onset of focal neurologic deficits- such as weakness, aphasia or sensory abnormalities.[6]

Stroke is the second most common cause of death in most countries.[1] In South Africa, which has a population undergoing demographic and epidemiological transition, stroke is the third most common cause of death.[2] While HIV is changing the mortality statistics in sub-Saharan Africa, cardiovascular and cerebrovascular disease remains a leading cause of death in most population groups which has a severe impact on the South African economy.[7,8] HIV on its own is a cause for cerebrovascular disease having a myriad of complications.[9] Patients on antiretrovirals for HIV also suffer from atherothrombotic problems.[10]

A stroke can result in permanent neurological damage, complications and death. A stroke is therefore a medical emergency. The 24 hour limit separates stroke from TIA, which is a related set of stroke symptoms that completely improve within 24 hours. A cerebral infarct that lasts longer than 24 hours, but less than 72 hours, is term a Reversible Ischaemic Neurologic Deficit (RIND). Risk factors for stroke and/or TIAs include, *inter alia*, advanced age, hypertension, previous stroke or TIA, hypercholesterolaemia, diabetes mellitus, cigarette smoking and atrial fibrillation.[6]

A stroke is commonly treated with supportive care, namely physiotherapy, occupational and speech therapy in a 'stroke unit'. Thrombolysis is occasionally used to treat a stroke. Secondary prevention is accomplished with antiplatelet drugs, such as aspirin, as well as by lowering cholesterol levels with statins and treating hypertension with antihypertensives. Carotid endarterectomy and anticoagulation may be considered in certain patients.[6]

Strokes are separated into two major categories, these being ischaemic and haemorrhagic. Eighty percent of strokes are due to ischaemia. Ischaemia occurs as a result of disruption of the blood supply. The remaining twenty percent of strokes are secondary to haemorrhage. Bleeding occurs as a result of rupturing a blood vessel or an abnormal vascular structure. The blood supply to an area of the brain is reduced in an ischaemic stroke resulting in brain tissue dysfunction in that part of the brain. There are four reasons why this might occur: arterial thrombosis, embolism,[6] systemic hypoperfusion[11] and venous thrombosis.[12]

Numerous classification systems for acute ischaemic stroke are available. The Oxford Community Stroke Project classification relies mainly on the initial symptoms. Based on the

extent of the symptoms, the stroke is defined as total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), lacunar infarct (LACI) or posterior circulation infarct (POCI). These four entities predict the part of the brain involved, extent of the stroke, predisposing cause and the prognosis. [13,14]

A few techniques are used to diagnose stroke: a neurological examination, CT scans or MRI scans, Doppler ultrasound, and arteriography. The diagnosis of stroke is essentially clinical and is confirmed utilising imaging techniques.[15]

Notwithstanding the advances in acute stroke management, stroke prevention (both primary and secondary) remains the mainstay of management and is particularly important in low-income settings. The cornerstone in the prevention of ischaemic vascular events is aspirin (acetylsalicylic acid) therapy, which has been shown to cause a 25% reduction in death, myocardial infarction and stroke in high-risk vascular patients.[16]

1.3 Antiplatelet agents

Drugs that decrease platelet aggregation function are commonly used in patients with atherosclerosis who are at risk of arterial thrombosis. Four families of antiplatelet agents are used clinically: (i) Cyclooxygenase-1 (COX-1) inhibitors such as aspirin; (ii) Adenosine 5-diphosphate (ADP) receptor antagonists, for example, thienopyridine compounds such as clopidogrel and ticlopidine; (iii) Glycoprotein IIb/IIIa antagonists, such as Abciximab, Tirofiban and Eptifibatide; (iv) and Adenosine reuptake inhibitors, for example, Dipyridamole. All the above drugs are used for the medical management of acute coronary

syndromes and during coronary interventions. Aspirin, thienopyridines and Dipyridamole are, however only used in long-term prevention of cerebrovascular and cardiovascular events.[17] (Figure 1.4)[4]

Aspirin and thienopyridines selectively prevent a single pathway of platelet activation. Aspirin affects the thromboxane A_2 (TxA_2) pathway by irreversibly antagonising COX-1. Thienopyridines affect the ADP pathway by irreversibly inhibiting the platelet ADP receptor $P2Y_{12}$.[18] The excellent antithrombotic effect of these drugs is due to both the TxA_2 and the ADP pathways increasing platelet activation and these are important for platelet aggregation.

(i) *Cyclooxygenase-1 (COX-1) inhibitors, such as aspirin:* Please see Section 1.4.

(ii) *Adenosine 5-diphosphate (ADP) receptor antagonists, for example, thienopyridine compounds such as clopidogrel and ticlopidine (Figure 1.4)[4]:*

Clopidogrel is a pro-drug that is made active by cytochrome P450 enzymes in the liver, including CYP2C19. The active metabolite functions by creating a disulfide bridge with the platelet ADP receptor having a half-life of approximately 8 hours. Patients expressing a variant allele of CYP2C19 are 1.5 to 3.5 times more likely to have complications or demise than patients with the high-functioning allele.[19,20]

Significant adverse drug reactions related to clopidogrel therapy include severe neutropenia, Thrombotic Thrombocytopenic Purpura (TTP) and haemorrhage.[21]

Ticlopidine is utilised in patients who cannot tolerate aspirin or in those patients who require dual antiplatelet therapy. Side effects also include neutropenia and TTP.

(iii) *Glycoprotein IIb/IIIa antagonists, such as Abciximab, Tirofiban and Eptifibatide (Figure 1.4)*[4]*:*

These drugs inhibit the Glycoprotein IIb/IIIa receptor on the platelet surface, thus inhibiting platelet aggregation and thrombus generation.

Abciximab is a platelet aggregation inhibitor used during and after coronary artery procedures. Abciximab is linked to a lower incidence of ischaemic complications due to the aforementioned intervention and a reduced need for repeated coronary artery revascularisation in the first month after the procedure. Abciximab is made from the Fab fragments of an immunoglobulin that targets the GP IIb/IIIa receptor on the platelet membrane. Abciximab may occupy some receptors for weeks due to its strong affinity for its receptor on the platelets.[22]

Tirofiban is a synthetic, non-peptide inhibitor of GP IIb/IIIa receptors on platelets. In combination with heparin and aspirin, it is required in the treatment of patients with unstable angina or non-Q-wave myocardial infarctions (MI). This reduces the risk of refractory ischaemia, new MI and death.[23]

Eptifibatide is a cyclic heptapeptide originating from a protein in the venom of the southeastern pygmy rattlesnake (*Sistrurus miliarius barbouri*). It forms part of the class arginine-glycine-aspartate-mimetics and reversibly binds to the platelet GP IIb/IIIa receptor.

(iv) *Adenosine reuptake inhibitors, for example, Dipyridamole (Figure 1.4)*[4]*:*

Dipyridamole prevents the cellular reuptake of adenosine into platelets, red blood cells and endothelial cells, resulting in increased extracellular concentrations of adenosine. It also inhibits the enzyme adenosine deaminase as well as the phosphodiesterase enzymes which

usually degrade cAMP. Modified release dipyridamole is used together with aspirin in the secondary prevention of stroke and TIA. Absorption of dipyridamole is pH-dependent and simultaneous treatment with proton pump inhibitors (PPI) will prevent uptake as ratified by the ESPRIT trial.[24]

1.4 Aspirin

Aspirin is a salicylate medication used as an analgesic, antipyretic, antiplatelet and anti-inflammatory drug. Low dose (75 to 81 mg/day) coupled with long-term aspirin intake helps prevent MIs, CVAs and TIAs in patients with diagnosed cardiovascular disease.[25,26] Aspirin is not prescribed to children and adolescents due to the risk of Reye's syndrome.

Sir John Vane was awarded the Nobel prize for defining aspirin's mechanism of action. Aspirin inhibits the formation of thromboxane A_2 (an essential mediator of platelet aggregation) by activated platelets, via inactivating cyclooxygenase enzymes.[27] (Figure 1.4)[4] Aspirin irreversibly inhibits COX-1 and modifies the enzymatic activity of COX-2. Aspirin also uncouples oxidative phosphorylation in cartilaginous and hepatic mitochondria.[28] Recent data shows that salicylic acid and its derivatives modulate signalling through NF-κB. NF-κB, a transcription factor complex, plays a pivotal role in numerous biological processes, including inflammation.[29]

Salicylates are excreted mostly by the kidneys as salicyluric acid (75%). When small doses are taken, all pathways proceed by first order kinetics, with a half-life of approximately 2 to 4.5

hours.[30] Individuals with ibuprofen or naproxen allergy, salicylate intolerance, increased risk for both gastrointestinal bleeding[31] and asthma, or NSAID-precipitated bronchospasm should not ingest aspirin.

Aspirin can interact with other drugs, for example, ammonium chloride and acetazolamide which augment the stimulating salicylate effect. All these drugs imbibed with alcohol enhance the risk for gastrointestinal bleeding. Aspirin can displace certain drugs from their protein binding sites in the blood, for example, the anti-diabetic drugs, chlorpropamide and tolbutamide, the immunosuppressant drug methotrexate, valproic acid, phenytoin, probenecid and other NSAIDs.

Studies have indicated that there may be a role for aspirin in decreasing the incidence of certain types of cancer, including those of the colon[32] and lung[33]. By inhibiting COX-2 enzymes expressed in adenocarcinomas, aspirin may have a preventative effect against these two malignancies.[34]

1.5 Aspirin resistance

It appears that aspirin's antiplatelet effect is not uniform in all patients as some patients experience aspirin resistance.[18,35-37] Aspirin resistance is demonstrated by patients who experience secondary thrombotic or embolic vascular events while on therapeutic doses of aspirin.[38] Previous studies have estimated that between 8 and 45% of studied populations are

aspirin resistant.[39,40] The prevalence of aspirin resistance is very dependent on the type of assay utilised and the population studied.

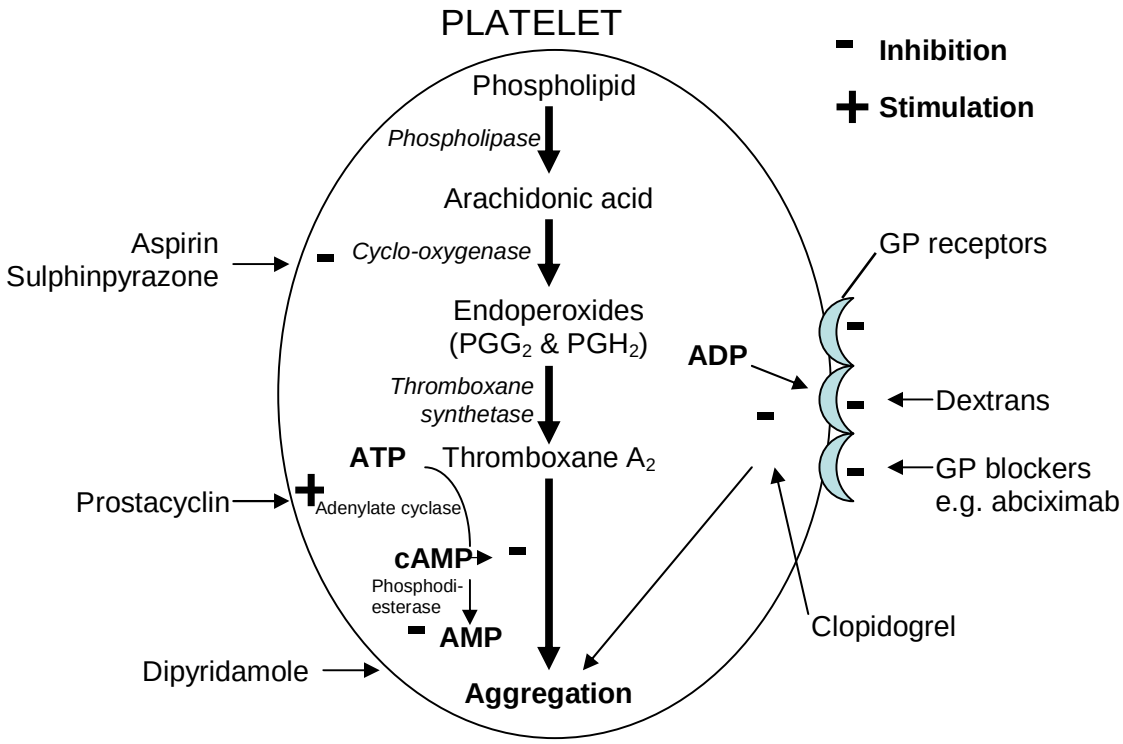


Figure 1.4 Sites of action of antiplatelet drugs.[4]

A laboratory test to diagnose aspirin resistance would be beneficial to the clinician in deciding on optimal antiplatelet therapy for a particular patient. Aspirin resistant patients should be offered newer antiplatelet agents, such as clopidogrel or a combination of aspirin with clopidogrel.[41] Platelet aggregation and sensitivity assays have been studied as indicators of aspirin resistance. Available studies have used varying definitions of the term "aspirin resistance"[42] . There is still a paucity of confirmatory data showing that those patients

diagnosed “aspirin resistant” by laboratory investigations are the ones who will be more likely to suffer recurrent strokes.

A clinical definition of aspirin resistance could be phrased as follows - the failure of the compound to protect the patient from an ischaemic event despite regular intake of appropriate doses[35]. Functional or biochemical *in vitro* tests would be best if these tests could be shown to correlate with clinical prevention, and thus guide management. Antiplatelet therapy could be individualised for those patients at risk of thrombotic events, for example, the secondary prevention of a Stroke or TIA.

1.6 Platelet aggregometry testing

Haematopathologists and clinicians have increased their interest in using various tests of platelet function to assess the efficacy of new platelet therapies because platelets play an essential role in haemorrhagic, thrombotic, and vascular ischaemic disorders. Antiplatelet therapy is seen as ‘the cornerstone of treatment’ for various cerebrovascular and cardiovascular thrombotic disorders[16] and this has resulted in an increase in new antiplatelet drugs.

Platelet function testing has been a component of the clinical laboratory since the early 20th century. Various methodologies have been used to determine normal and pathologic platelet activity. These include the *in vivo* bleeding time, platelet aggregometry techniques, measurement of platelet granular content and release, evaluation of platelet membrane surface markers, assessment of platelet signalling pathways, and *in vivo* determination of platelet survival.[43]

Platelet aggregometry is a well known technology which has been in use for more than 40 years. It is used to assess abnormal platelet function, to measure platelet response, and determine platelet inhibition by medications.[44]

Platelet aggregating agents (also known as agonists) include thrombin, collagen, ADP, arachidonic acid, antigen-antibody complexes, vasopressin and serotonin. It is important to assess the mode of action of these agonists. ADP, adrenaline, and vasopressin cause direct aggregation. Arachidonic acid, thrombin and collagen, by acting as stimulating chemicals, promote thromboxane A_2 and ADP release.[44]

Born detailed the first platelet aggregometry performed on a turbidometric aggregometer.[45] Once platelet rich plasma is stirred in a cuvette with an added agonist, the change in light transmission as a function of time is recorded. We utilised, for the purpose of this study, the technique of platelet aggregation and sensitivity testing/Light Transmission Aggregometry (LTA) as it is regarded as the 'gold standard test' for determining the prevalence of aspirin resistance.

The analytical performance parameters, accuracy, precision, positive and negative predictive values of the 'gold standard test' are as follows:

Accuracy: In platelet aggregation, accuracy is dependent on the test system and is a relative parameter.

Precision and reproducibility: The limitations of platelet aggregometry make it challenging to supply a typical precision or reproducibility ranges. However there is an experienced based consensus for these parameters.

Test to test reproducibility:	better than ± 7.5%
Instrument to instrument reproducibility:	better than ± 15%
Reagent lot to lot variation:	better than ± 10.5%
Laboratory to laboratory (same test system):	better than ± 12.5%

Positive and negative predictive values: The nature of the testing system predicts if there is aggregation or no aggregation.[46]

2. STUDY OBJECTIVE

The study objective was to assess the prevalence of aspirin resistance and/or platelet hypersensitivity as determined by platelet aggregometry in sixty Caucasian patients who had suffered one or more strokes and/or TIAs. These patients were compared with sixty controls having no history of ischaemic heart disease, peripheral vascular disease, connective tissue disease, familial hypercholesterolaemia or any previously diagnosed bleeding or clotting disorder.

3. STUDY POPULATIONS, MATERIALS AND METHODS

3.1 Case selection

Patients attending the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) (an urban quartenary care hospital) neurovascular clinic were consecutively selected. Patients were between 20 and 90 years of age. Sufficient funding was available to include sixty Caucasian patients who had previously suffered one or more strokes and/or TIAs, and who were on long-term Aspirin therapy (75-150mg po daily enterically-coated or otherwise) for the prevention of further atherothrombotic events.

3.2 Control selection

The patient group was compared to sixty controls, none of whom had suffered a stroke and/or TIA, and were not on long-term aspirin therapy. Patients were encouraged to bring their spouses, family and friends between the ages of 20 and 90 years for testing. The control subjects were not age and sex-matched with the patients due to the high number of older people who were already on prophylactic long-term aspirin therapy. Laboratory and hospital staff also volunteered to be control subjects.

The control group took enterically-coated aspirin (81mg po daily; Ecotrin-Goldshield Pharmaceuticals Ltd.) for one week prior to platelet aggregation and sensitivity testing.

General exclusion criteria for both cases and controls included non-caucasians, pregnancy, usage of any other antiplatelet drugs within a week of testing, and ingestion of onions or garlic within three days of testing.

3.3 Laboratory methods

3.3.1 Principle

LTA measures the increase in light transmission through a platelet suspension that occurs when platelets aggregate in response to an agonist.

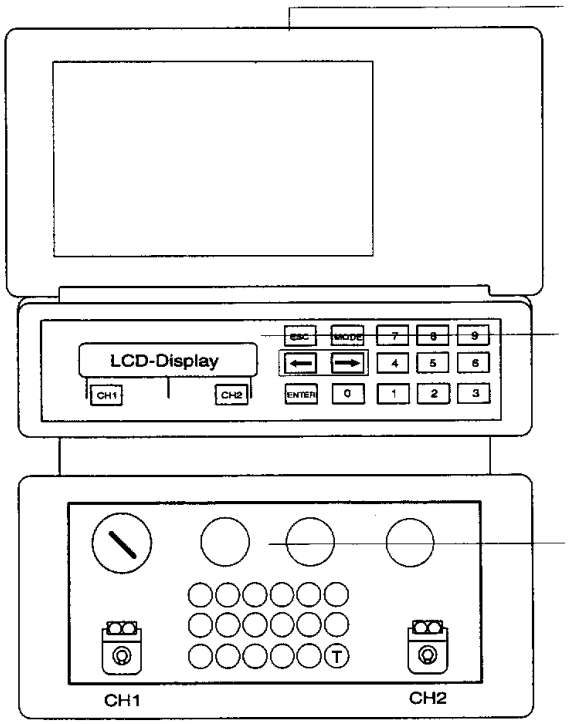
The absorbance of platelet-rich plasma decreases as platelets aggregate. The amount and rate of fall is dependent on platelet reactivity to the added agonist if other variables, such as platelet count, temperature and mixing speed, are controlled. The absorbance changes are monitored on a chart recorder on the attached computer.[47,49]

3.3.2 Instrument description

Platelet aggregometry was performed utilizing the APACT 2 ON Helena Aggregometer™ (Figure 3.1). The APACT 2 is a new development based on the Aggregometer APACT (Automated Platelet Aggregation and Coagulation Tracer) is manufactured by LAbor in Ahrensburg, Germany. The APACT 2 is built in modular units. The measuring channels are labelled with the keys CH1 and CH2. The measuring channels are integrated into a 37°C incubation block with four positions for cuvettes and four positions for reagent bottles per measuring channel (Figure 3.2).[48]



Figure 3.1. APACT 2 ON Helena Aggregometer ™.



Power switch ON I, OFF 0

Display 2 lines, 20 characters each
Membrane keypad with the keys
CH1, CH2,
0-9, Mode, Enter, ESC, ←, →

Incubation block 37°C:
- 18 positions for cuvettes
- T = position for temperature calibration
- 4 positions for reagent bottles
- 2 measuring channels

Figure 3.2. APACT 2 ON Helena Aggregometer ™ - Top View.[48]

3.3.3 Patient preparation and specimen collection

Patient preparation: Each patient and control subject had fasting (overnight) blood taken at the Johannesburg Hospital phlebotomy room.

Specimen collection: 30 ml of blood was collected into citrated tubes (1 vol 3.2% trisodium citrate = 9 vol blood). Accurate coagulation studies depend on the correct whole blood to anti-coagulant ratio. Only plastic or siliconised glass labware was used during all phases of collection and post-collection of the specimens.[49]

Handling conditions: Platelet aggregations and sensitivities were all performed within an hour of sample collection from the patient.

3.3.4 Preparation of platelets

Samples were placed in the centrifuge and spun at a low speed (750 rpm for 15 minutes). The top third of the plasma (Platelet Rich Plasma (PRP)) was removed. The pooled plasma from all the tubes was placed into a plastic tube. The rest of the plasma was spun at a high speed (3500 rpm for 10 minutes) to obtain Platelet Poor Plasma (PPP). The PPP was separated into a second plastic tube.

The platelet count was measured on the PRP using the FBC analyser
(Beckman Coulter - GENS).

The aforementioned value was adjusted to obtain a platelet count of 250 x 10^9/l using the following calculation: PPP (Platelet Poor Plasma) to be added µl = (Platelet count x 10^9/l – PRP (Platelet Rich Plasma/10)) x 10.[49]

3.3.5 Aggregating agents (agonists)

The most common agonists are:

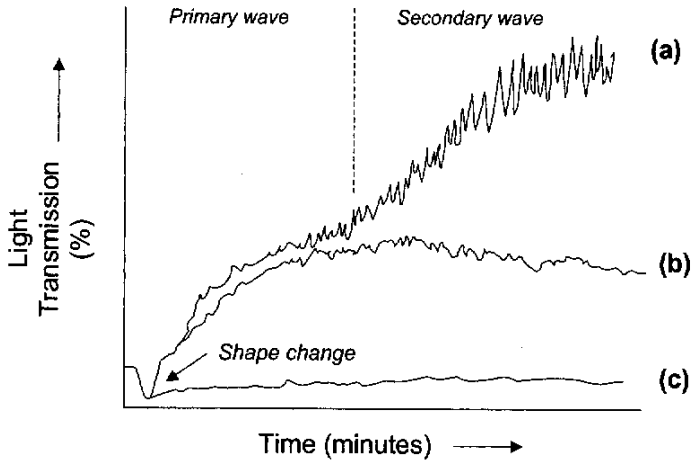
Arachidonic acid – determines the viability of the thromboxane pathway.

Adenosine 5-Diphosphate (ADP) – binds to a specific platelet membrane receptor and causes platelet activation and release of dense granule stored ADP. Biphasic aggregation is noted.

Adrenaline – adheres to a specific receptor and causes ADP secretion, but does not cause aggregation in release defects or storage pool disorders.

Collagen – no primary wave of aggregation seen and it relies on intact membrane receptors, normal cyclooxygenase and thromboxane pathway function as well as membrane phospholipase pathway integrity.

Aggregation tests using PRP require a photometer with a cuvette equipped with a stir bar and heated to 37°C. Agonists are included and alterations in optical density are plotted to visualise the aggregation curve (Figure 3.3).[47]



(a) Normal.

(b) Storage pool disease.

(c) Lack of GP IIb/IIIa expression.

Figure 3.3. Platelet aggregation studies.[5]

3.3.6 Stepwise procedure and interpretation of platelet aggregation curves

The procedure outlined in the Standard Operating Procedure (SOP) for platelet aggregation and sensitivities on the APACT 2 ON Helena Aggregometer™ was followed.[49]

The baseline optical density was set with PPP and then activated utilising the following agonists in two concentrations (Table 3.1). Cuvettes containing the new diluted plasma (PPP and PRP) were placed in the incubation block of the aggregometer according to the manufacturer's instructions.

Table 3.1 High and low concentrations of agonists.

Agonist: high concentration	**Agonist: low concentration**
Arachidonic acid high - 1.5 mM	Arachidonic acid low - 0.187 mM
ADP high - $2.0x10^{-5}$ m	ADP low - $0.1x10^{-5}$ m
Adrenaline high - 10.0 µg/ml	Adrenaline low - 0.05 µg/ml
Collagen high - 2.0 µg/ml	Collagen low - 0.2 µg/ml

Response to the reagents was measured as a percentage of platelet aggregation and reported as normal (>60%), reduced (>20%) or flat (<20%).

Aspirin resistance was determined by platelet aggregation (>20%) to one or more of the agonists, namely arachidonic acid high (1.5mM), ADP low ($0.1x10^{-5}$ m), adrenaline low (0.05 µg/ml) or collagen low (0.2 µg/ml).

3.3.7 Comments and limitations

3.3.7.1 Granulocytes and red cells should be excluded from PRP as these will cause a decreased response height by disturbing the light transmission and the result could be misinterpreted as abnormal aggregation. These can be eliminated by further centrifugation of PRP at 1500 rpm for two minutes, or after settling has occurred.

3.3.7.2 Alterations in transmission similar to spontaneous aggregation may occur if there are cryoglobulins. The PRP and PPP should be heated to 37°C for five minutes thus ensuring aggregation can be performed in the normal manner.

3.3.7.3 Lipaemic plasma may cause difficulties in changing the aggregometer. The response could be decreased due to the small difference in transmitted light between PRP and PPP,

3.3.7.4 Aggregation must ideally be carried out within 60 minutes. In the event that testing will be delayed, it is advisable to store the PRP under 5% carbon dioxide or to allow a very small air gap over the PRP and cap the tube. [47,49]

3.4 Statistical analysis

The basic collection and sorting of data was performed using Microsoft Excel. Prof. Piet Becker from the Biostatistics Unit of the South African Medical Research Council (SAMRC) reviewed the advanced data analysis. Prof. Piet Becker imported the data electronically into Stata Statistical Software version 10, i.e. software used for data analysis.

Groups were compared with respect to categorical variables using Fisher's exact test and Student's t-test was employed to test with respect to age. The odds ratio, along with its 95% confidence interval, was computed to further facilitate interpretation. Demographics were summarised using frequencies, percentages and cross-tabulation for categorical parameters i.e. sex, hypertension etc. Age was summarised using mean and standard deviation. Testing was done at the 0.05 level of significance.

3.5 Ethics

Ethics approval was obtained from the Human Research Ethics Committee

(R14/49 Jacobson Protocol number 03-09-53) of the Faculty of Health Sciences, University of the Witwatersrand.

Informed consent was obtained from both the patients and control subjects.

4. RESULTS

4.1 Patient results

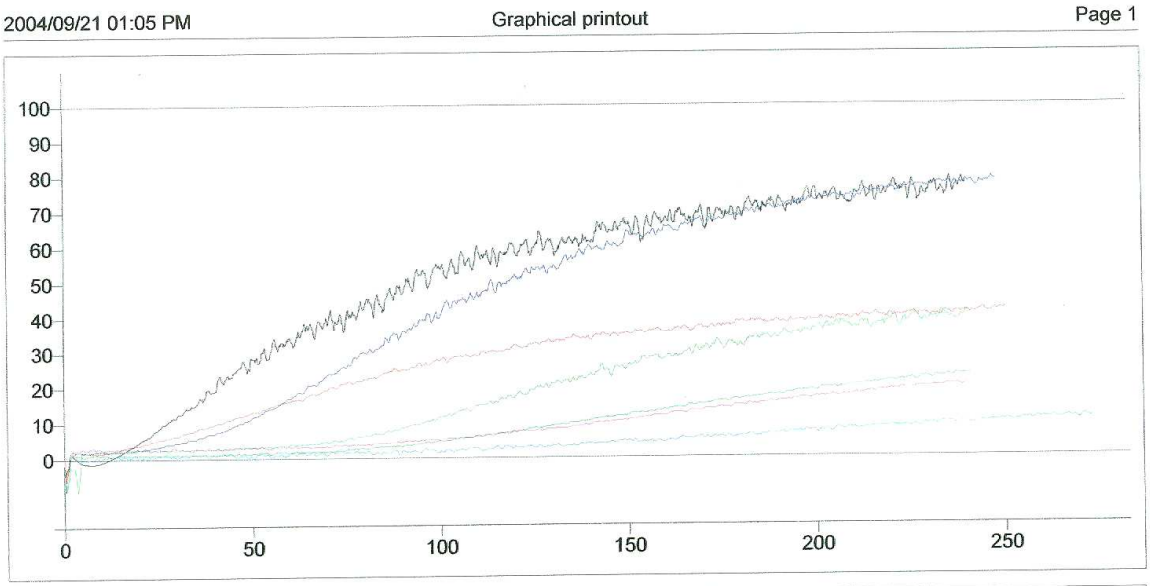
Sixty patients and sixty control subjects were investigated (Appendix B). The mean age of the patient group was 65 years. Two patients demonstrated resistance to arachidonic acid (high concentration) i.e. ‘complete aspirin resistance’. Of these two patients, one also showed resistance to collagen (high and low concentrations), ADP and Adrenaline (both high concentrations). The second patient also showed resistance to both ADP (high and low concentrations) and collagen (high concentration).

Three patients demonstrated ‘partial aspirin resistance’ with resistance noted to adrenaline, ADP and collagen (high and/or low concentrations) respectively (Figure 4.1).

Five of sixty patients showed ‘complete or partial aspirin resistance’. This was significantly more than the control group. The results showed an 8.3% (95% CI: 0.5%-16.2%) prevalence of aspirin resistance (complete and partial) in South African Caucasian patients with previous atherothrombotic cerebrovascular events.

A history of prior stroke or transient ischaemic attack was associated with an increased risk of aspirin resistance with an odds ratio of 5.36 (95% CI: 0.6-47.4). The wide confidence interval (CI) can be attributed to the very low prevalence of aspirin resistance in the control group.

Many more patients (58.3%) smoked than controls (5.0%). Fewer control subjects (16.7%) had hypertension compared with the patient group (76.7%). A higher percentage of patients (53.3%) had hypercholesterolaemia when compared to the control group (8.3%).



	Date	Time	Inductor	Sample ID	Aggregation	Remark
✓	2004/09/21	12:45:30 PM	ADP 2.0x10^5M	P050	77.0 %	STAR STUDY-AGGREGATION (PENNY)
✓	2004/09/21	12:46:40 PM	ADP 0.1x10^5M	P050	41.4 %	STAR STUDY-AGGREGATION (PENNY)
✓	2004/09/21	12:49:59 PM	ARACH ACID 1.5mM	P050	23.2 %	STAR STUDY-SLIGHT VISIBLE AGGREGATION (PENNY)
✓	2004/09/21	12:51:17 PM	ADRENALINE10ug/m	P050	78.1 %	STAR STUDY-AGGREGATION (PENNY)
✓	2004/09/21	12:54:39 PM	ADRENAL 0.05ug/ml	P050	20.4 %	STAR STUDY-SLIGHT VISIBLE AGGREGATION (PENNY)
✓	2004/09/21	12:56:09 PM	COLLAGEN 2.0ug/ml	P050	39.9 %	STAR STUDY-AGGREGATION (PENNY)
✓	2004/09/21	12:59:08 PM	COLLAGEN 0.2ug/ml	P050	10.8 %	STAR STUDY-NO AGGREGATION (PENNY)

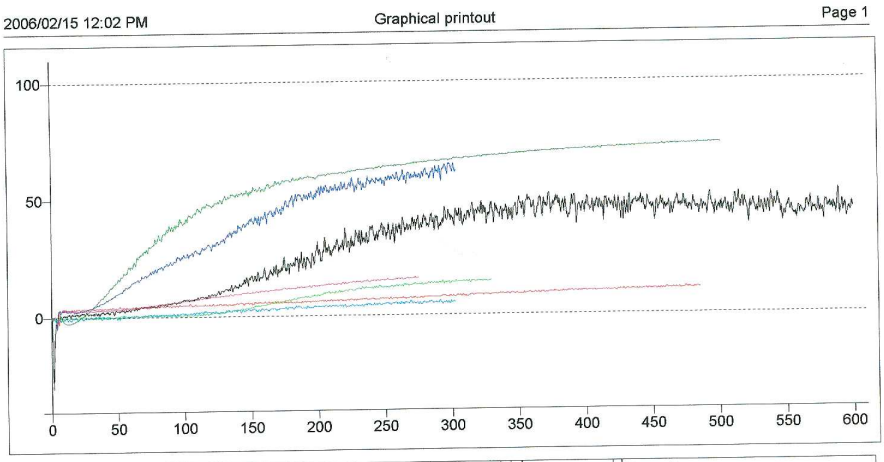
Figure 4.1 Trace from a patient with ‘partial aspirin resistance’.

4.2 Control results

The mean age of the control group was 50 years. One control subject showed 'complete aspirin resistance' with resistance to Arachidonic acid, ADP (high and low concentrations) and adrenaline (high concentration) (Figure 4.2).

None of the control subjects showed 'partial aspirin resistance'.

There was a 1.67% (95% CI: 0%-5.7%) chance of a control subject being resistant to aspirin in a general South African Caucasian population. This very low prevalence in the control group prevented testing for potential confounders, for example, sex, age, hypertension, hypercholesterolaemia etc.



	Date	Time	Inductor	Sample ID	Aggregati	Slope	I	Shape Change	Remark
✓	2006/02/1	11:35:10 AM	ARACH ACID 1.5mM	C 049A	45.9 %	42.8 %/min		43.1 %sec	STAR STUDY- AGGREGATION
✓	2006/02/1	10:56:19 AM	ADP 2.0x10^5M	C 049A	11.1 %	15.9 %/min			STAR STUDT (POST) - AGGREGATION
✓	2006/02/1	10:56:04 AM	ADP 0.1x10^5M	C 049A	72.6 %	38.8 %/min		22.5 %sec	STAR STUDY (POST) - NO AGGREGATION
✓	2006/02/1	11:06:14 AM	ADRENALINE	C 049A	62.9 %	27.1 %/min			STAR STUDY - AGGREGATION
✓	2006/02/1	11:06:43 AM	ADRENAL 0.05ug/ml	C 049A	15.9 %	15.1 %/min			STAR STUDY - SLIGHT AGGREGATION
✓	2006/02/1	11:13:23 AM	COLLAGEN 2.0ug/ml	C 049A	14.5 %	8.0 %/min		1.5 %sec	STAR STUDY - SLIGHT AGGREGATION
✓	2006/02/1	11:13:51 AM	COLLAGEN 0.2ug/ml	C 049A	5.3 %	4.0 %/min		7.9 %sec	STAR STUDY - NO AGGREGATION

Figure 4.2 Trace from a control subject with 'complete aspirin resistance'.

5. DISCUSSION

Cattaneo recently defined the term 'resistance to antiplatelet drugs'. Cattaneo states, "the term 'resistance' to a drug should be used when a drug is unable to hit its pharmacological target, because of inability to reach it (as a consequence of reduced bioavailability, *in vivo* inactivation, or negative interaction with other substances) or to alterations of the target."[17]

'Clinical aspirin resistance' appears to be the failure of the drug to protect the patient from an ischaemic event despite regular intake of appropriate doses. 'Functional or biochemical aspirin resistance' is ascertained by certain platelet assays and seems to be a real phenomenon which is clinically relevant. Increasing the dose of aspirin does not overcome the resistance.[3]

These results indicate a prevalence of 8.3% (5/60) biochemical aspirin resistance in Caucasian South Africans having a prior history of ischaemic stroke or transient ischaemic attack. This finding is in keeping with the reported prevalence of 5 to 40% in previous study populations which are aspirin resistant.[38-40] The wide range in prevalence is most likely due to a number of factors including (i) inconsistency in the definition of aspirin resistance, for example, variable number and types of agonists used and resistance to one agonist (partial aspirin resistance) or many agonists (complete aspirin resistance) (ii) variations in the type of assay used and (iii) differences in the population studied.

In vitro platelet function is measured by many methods. This is problematic as the tests do not recreate *in vivo* physiological states that result in platelet aggregation.

The ‘gold standard’ for testing of platelet aggregation is Light Transmission Aggregometry (LTA), which is extensively described in the materials and methods section. Unfortunately further limitations of this test are that it should be carried out in reference laboratories and is also time-consuming. The results from LTA are influenced by numerous pre-analytical and analytical variables. Unfortunately the reproducibility and accuracy of this method is also poor and there is minimal standardisation. The results are influenced by the type of anticoagulant used for venesection as well as the type and concentration of agonist used.[17] LTA may also exaggerate the incidence of aspirin resistance.[18]

For the purpose of this study ‘complete aspirin resistance’ was defined as platelet aggregation of greater than 20% to the agonist Arachidonic Acid (AA) high concentration alone or together with aggregation at a low concentration. Platelet aggregation to other agonists such as adrenaline (high concentration), and/or ADP (high concentration) and/or collagen (high concentration) may also be present, but are not definitive of ‘complete aspirin resistance’.

For the purpose of this study ‘partial aspirin resistance’ was defined as platelet aggregation of greater than 20% to two or more agonists other than Arachidonic acid i.e. to Adrenaline (high concentration) and/or ADP (high concentration) and/or collagen (high concentration).

Other assays that have been used to measure platelet function during antiplatelet treatment include:

Impedance aggregometry

This measures the alteration in electrical impedance which occurs when platelets aggregate on platinum electrodes which are placed in diluted whole blood after stimulation with a platelet agonist.[50]

Whole blood platelet aggregation measured by platelet counting

Individual platelets in whole blood activated with an agonist diminish as a result of platelet aggregation. This is a precise and simple method.[51]

The Platelet Function Analyser (PFA-100®) system - *in vitro* bleeding time.

It is an automated, rapid and easy system requiring a small amount of whole blood.[52] The PFA-100® system should not be utilised to assess platelet inhibition by aspirin or thienopyridines.[53,54]

The Ultegra Rapid Platelet Function Assay (RPFA): Verify Now

The agglutination of fibrinogen-coated beads by platelets activated by an agonist is determined in citrated whole blood. The RPFA has been slightly modified to become more sensitive and specific for the monitoring of aspirin treatment (*RPFA – Verify Now ASA*).[55]

Flow cytometry

A costly and complicated instrument is required. It permits the assessment of platelet sensitivity to agonists *in vitro*, platelet-leucocyte masses, circulating stimulated platelets and platelet-derived microparticles.[56]

Thromboelastography® (TEG)

The TEG assesses the viscoelastic alterations throughout clot development in whole blood.

Platelet Mapping®/Modified Thromboelastrography (mTEG)

This is a modification of the Thromboelastograph® (TEG) that can determine the extent of platelet inhibition by anti-platelet drugs. Samples are heparinised to inhibit the contact

activation of thrombin. Clot development commences with Factor XIIIa and reptilase. The resultant maximum amplitude of the clot relies on platelet agonists -Arachidonic Acid or Adenosine Diphosphate added to the blood. This is compared to a kaolin activated trace to determine the degree of inhibition.[57] The mTEG's sensitivity to monitoring the effect of aspirin and clopidogrel has been shown to be satisfactory in a new study.[57]

Serum thromboxane B_2 (TxB_2)

The most precise test to assess the pharmacological outcome of aspirin. It indicates the entire ability of platelets to produce thromboxane A_2, of which it is a permanent metabolite.[18]

Urinary levels of 11-dehydro-thromboxane B_2 (TxB_2 metabolite)

This characterises a time incorporated indicator of TxA_2 biosynthesis *in vivo*.[58] Eikelboom *et al*. showed that atherosclerotic patients on aspirin who had increased concentrations of urinary 11-dehydro-thromboxane B_2 were more likely to suffer future ischaemic episodes.[59]

Evaluation of various laboratory techniques for aspirin resistance in published studies frequently illustrate inadequate or no association signifying that they are susceptible to various parameters.[53,55,60,61]

The prevalence of 'aspirin resistance' differs extensively in published studies, depending on the laboratory assay used to determine the pharmacological outcome and the cut-off values selected to classify patients as 'complete or partially aspirin resistant'. The prevalence of aspirin resistance as reviewed by Campbell and Steinhubl varies between 5.5% and 61% in 11 studies. These studies utilised various tests of platelet aggregation to determine aspirin's effect.[62]

Laboratory monitoring of antiplatelet therapy using platelet aggregometry and sensitivity testing to aspirin may guide clinical decision making and prevent ischaemic vascular events, however, this approach needs to be tested against clinical outcomes. The cost effectiveness of this approach needs to be determined in the South African setting before monitoring of antiplatelet therapy can be recommended in clinical practice.

Findings should also be interpreted in conjunction with the platelet polymorphisms known to cause the phenomenon of aspirin resistance or an increased tendency to stroke. A polymorphism found to have a positive correlation with aspirin resistance is the polymorphism of the Human Platelet Antigen 1 (HPA1) (comprising the PLA1 and PLA2 alleles of the GP IIIa gene).[63]

Other polymorphisms suspected of increasing the tendency to stroke are:

- Polymorphisms involving platelet glycoprotein Ia/IIa, Ib/V/IX, and IIb/IIIa receptors[64], collagen and VWF receptors.[65]
- Polymorphisms of cyclooxygenase-1[66], cyclooxygenase-2[18], thromboxane A_2-synthase, or other arachidonate metabolism enzymes.
- Factor XIII Val34Leu polymorphism leading to variable inhibition of factor XIII activation by low dose aspirin.[67]

Other possible mechanisms of resistance to anti-platelet drugs such as aspirin include:

- Reduced bioavailability of aspirin.[68]
- Interference with other drugs. Other non-steroidal anti-inflammatory drugs (NSAIDS), such as, ibuprofen can compete with and prevent aspirin access at certain enzyme sites.[69]
- Recently produced non-aspirinated platelets which are able to create TxA_2 may be introduced into the blood due to increased platelet turnover .[18]
- In recently produced platelets TxA_2 formation by the aspirin-insensitive COX-2 isoform may occur.[18]
- The existence of COX-1 variants that may be less reactive to aspirin inhibition.[66]
- Possibly the most common and likely cause of aspirin resistance is lack of compliance.[56]

In patients who are found to be aspirin resistant (either complete or partial), other antiplatelet agents may be useful, for example, ADP receptor antagonists, such as thienopyridine compounds like Clopidogrel. The aforementioned drugs may be used alone or in combination with aspirin for the primary or secondary prevention of thrombotic cerebrovascular and/or myocardial events. Whilst it would appear logical to offer these aspirin resistant patients other antiplatelet agents such as Clopidogrel, confirmatory tests to show benefit are urgently required.

6. CONCLUSION

In conclusion, this study shows an increased prevalence of aspirin resistance in South African Caucasian patients who have had prior strokes / TIAs and raises the question whether people who have had these events are somehow predisposed to vascular events or recurrent vascular events. ‘Aspirin resistant’ patients or ‘poor responders’ to aspirin must be considered at heightened risk of atherothrombotic events. Laboratory monitoring of antiplatelet therapy may become clinically useful and a long term observational study of these patients would be extremely interesting.

The perfect test for laboratory monitoring of aspirin therapy should be quick, inexpensive, simple to perform, accurate, reproducible and well standardised. This would ensure that similar results would be obtained from a patient on aspirin therapy in any laboratory.

Further studies are needed to definitively conclude whether laboratory monitoring of aspirin therapy is clinically relevant and cost effective.

7. APPENDICES

Appendix A: Copy of the Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Jacobson

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER 03-09-53**
PROJECT	Stroke and Aspirin Resistance
INVESTIGATORS	Prof BF Jacobson
DEPARTMENT	School of Pathology
DATE CONSIDERED	03-09-26
DECISION OF THE COMMITTEE*	Approved unconditionally with modification

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 02:02:04AM **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof BF Jacobson
School of Pathology

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

This ethical clearance will expire on 1 February 2008
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B**:** Results on which statistical analysis was performed (raw data).

																		FBC					aa		adp		adr		coll	
No.	id	group	gender	age	resist	resist01	strdate	strtype	sbp	dbp	smoke	highbp	highchol	dm	af	wbc	rcc	hb	mcv	plt	hi	low	hi	low	hi	low	hi	low
P001	1	Patients	Females	35	0	0	2002	TIA	124	82	Yes	Yes	Yes	No	No	7.3	5.25	15.5	84	207	3.4		61.9	4.6	12.6		9	
P002	2	Patients	Males	33	0	0	2003	TIA	114	80	Yes	No	No	No	No	10.9	5.33	16.8	90.5	252	3.5		54.6	2.9	16.9		12.9	
P003	3	Patients	Males	53	0	0	2003	R-TACI	138	90	Yes	Yes	Yes	No	No	7.3	4.77	15.8	90.6	194	1.8		80	5.3	11		15.6	
P004	4	Patients	Males	65	0	0	1980	L-MCA	126	82	No	Yes	No	No	No	4.7	4.82	15.1	92.2	209	26.4	0	73.8	20.8	12.2		27.4	4.8
P005	5	Patients	Males	55	0	0	2000	L MCA	130	80	Yes	No	Yes	No	No	7.3	5.04	15.7	91.9	278	0		50.1	10.6	31.2	1.7	14.2	1.5
P006	6	Patients	Males	44	1	1	1995	L MCA	110	84	Yes	No	No	No	No	7.4	5.15	16.1	92.1	227	82.5	1.9	83	58.1	25.1	1.1	86.7	
P007	7	Patients	Males	63	0	0	2003	TIA	130	86	Yes	Yes	Yes	No	No	5.1	4.56	15.3	99	197	1.5		65.6	11.5	24	29.3	23.7	0.7
P008	8	Patients	Females	63	1	1	1993	Multiple	150	80	Yes	Yes	Yes	No	No	5.3	4.83	14.4	85.8	152	71.6	3.8	75.2	19	58.4	2.4	48.1	34.9
P009	9	Patients	Males	66	0	0	1995	R-MCA	120	80	Yes	Yes	Yes	No	No	9.4	5.33	16.3	91.1	215	1.4		69.4	9.7	36	36.7	4.4	
P010	10	Patients	Females	80	0	0	2000	L-LACI	130	82	No	Yes	Yes	No	No	6.5	4.54	14.2	91.9	286	4		57.6	17.2	37.1	24.7	55	11.6
P011	11	Patients	Females	75	0	0	2002	R-PACI	126	86	No	Yes	No	No	No	8.9	4.77	15	91.9	286	0		55.6	3.1	17.5		54.8	0
P012	12	Patients	Males	63	0	0	1991	L-MCA	120	70	Yes	Yes	No	Yes	No	5.8	4.56	14.6	91.4	371	4		59	17.8	33.9	2.2	12.1	0.9
P013	13	Patients	Females	76	0	0	1992	L-MCA	120	84	Yes	Yes	Yes	No	No	6.4	4.87	15.1	89.9	271	3.2		65.9	4	28.6	4.1	42.1	5.9
P014	14	Patients	Females	59	0	0	1997	TIA	110	78	Yes	Yes	Yes	No	No	5.8	4.84	14.1	84.7	307	2.6		79	18.6	26.2	11.8	9.6	1.2
P015	15	Patients	Males	67	0	0	2004	R-MCA	140	80		2	Yes	No	No	8.2	5.39	17	88.6	248	0		61.5	16.9	31.6	2.8	37.8	7.5
P016	16	Patients	Females	76	0	0	2003	TIA	132	80	Yes	Yes	Yes	No	No	7.3	4.39	16.2	103	333	1.9		54.7	5.2	12.9	2.4	6.8	
P017	17	Patients	Males	67	0	0	2003	TIA	130	72	Yes	Yes	No	No	No	7.6	3.85	14.8	106.2	336	1.1		50.4	0.2	0		3.4	
P018	18	Patients	Males	67	0	0	2003	TIA	180	100	Yes	Yes	Yes	No	No	7.6	4.79	12.7	77.2	269	0.7		52.1	11.6	31.9	4	14.8	
P019	19	Patients	Males	68	0	0	2004	TIA	150	90	No	Yes	No	No	No	8.1	4.76	14.8	91.9	203	0.7		60.1	5.4	15.8	1.1	19.3	2.5
P020	20	Patients	Males	76	0	0	1995	L-PACI	130	80	No	Yes	No	Yes	No	8.9	4.01	12.6	90.9	276	1.6		68.1	3.9	41.1	2.5	25	
P021	21	Patients	Females	75	0	0	2003	TIA	168	100	No	Yes	No	No	No	5.4	4.8	13.7	86.1	307	0.9		52.4	2.5	20.6	0.2	20.9	3.1
P022	22	Patients	Females	85	0	0	2000	MCA-PAC	130	86	Yes	No	No	No	No	3.9	4.2	13.6	96.7	179	2.9		57.9	1	28.9	7.1	18.7	
P023	23	Patients	Males	67	0	0	2003	MCA-PAC	140	88	Yes	Yes	No	No	No	7.7	4.58	15.2	95.9	281	3.8		74.6	2.6	26.9	3.2	16.7	0
P024	24	Patients	Males	91	0	0	2003	L-LMS	110	70	Yes	Yes	Yes	No	No	6.3	5.07	15.8	90.7	238	3.6		67.7	2.8	38.1	11.9	31	16.7
P025	25	Patients	Males	75	0	0	2003	POCI	120	82		2	Yes	No	No	4.9	4.42	15	98.2	431	1.1		56.8	7.4	20.5	2.1	5.8	
P026	26	Patients	Females	64	0	0	2003	LACI	130	80	No	Yes	Yes	No	No	5.6	4.18	13.6	93.8	239	0.9		52.5	8.5	9.5		5.1	
P027	27	Patients	Females	49	0	0	2002	LACI	120	70	Yes	Yes	Yes	No	No	10.1	4.93	15.1	90.5	377	4.4		84	8	21.6	1.9	32.7	14.2
P028	28	Patients	Males	61	0	0	2003	MLS	140	86		2	Yes	Yes	No	12.1	4.74	14	88.8	280	3		61.1	14.9	26.8	2.9	30.8	11
P029	29	Patients	Males	55	0	0	1997	MCA-PAC	130	82	Yes	Yes	Yes	No	No	11.2	4.62	15.6	95.7	301	2		53.9	13.5	28.6	1.8	22.5	5.6
P030	30	Patients	Males	58	0	0	2002	LMCA	120	76		2	Yes	Yes	No	7.6	5.8	14.4	74.4	260	3.4		51.6	8.4	37.7	2.7	14	
P031	31	Patients	Males	65	0	0	2004	TIA	126	72	No	No	No	No	No	6.4	4.99	16.2	94.9	173	4.1		59.2	9.7	30.2	3.8	21.9	21.3
P032	32	Patients	Females	71	0	0	2002	TIA	130	84	No	Yes	Yes	No	No	8.1	5.01	15.1	90.2	319	1.7		57.5	4.1	2.8		3	
P033	33	Patients	Females	59	0	0	2002	LMCA	120	80	No	No	No	No	No	6.3	4.28	12.6	85.5	326	0.8		86.2	15.2	39.3	3.9	28.6	5.1
P034	34	Patients	Females	64	0	0	2001	LMCA	130	90	Yes	Yes	Yes	Yes	No	12.3	4.71	14.2	89.8	507	1.2		59.6	5.4	38.4	2.9	0	0
P035	35	Patients	Males	76	0	0	2002	RMCA	130	72	Yes	Yes	No	Yes	No	8.9	5.07	15.5	86.7	148	2.9		64.2	22.8	44.6	0	22.5	14.1
P036	36	Patients	Females	74	0	0	2001	LMCA	132	86	No	Yes	No	No	No	9.1	4.87	13.7	83.5	628	4.1		70.4	10.9	11.6		7.7	
P037	37	Patients	Females	80	0	0	2001	RMCA	136	90	No	Yes	No	No	No	7.5	3.9	12.7	95.1	329	3.6		65.4	1.8	22	2.6	3.4	
P038	38	Patients	Males		0	0	2002	TIA	128	82	No	No	Yes	Yes	No	6.6	4.88	14.3	85.5	179	5.5		60.9	27.6	39.6	4.8	26.8	9.1
P039	39	Patients	Females	55	0	0	1998	RMCA	130	84		2	Yes	No	No	6.1	5.02	16.9	97.8	214	3.2		45.3	2.8	27.6	0.7	2.9	
P040	40	Patients	Females	49	2	1	1997	RMCA	118	82	No	No	Yes	No	No	7.8	4.56	15.6	98	201	7.6		67.3	46.5	14.6	8.9	46.6	24.3
P041	41	Patients	Females	59	0	0	2001	PACI	138	90	No	Yes	No	No	No	6.9	5.21	15.5	84.9	339	0	0.9	61.1	12.2	15.8	2.1	19.3	8.8
P042	42	Patients	Males	56	0	0	1987	RMCA	140	80		2	No	Yes	No	7.1	4.22	15.1	105.8	238	6.6		60.8	3.5	14.1	0.5	0	0
P043	43	Patients	Females	26	0	0	2004	TIA	120	78	Yes	Yes	Yes	No	No	12.5	5.49	17.2	89.2	425	2.2		74.6	4.3	16.1		2.4	
P044	44	Patients	Males	73	0	0	2004	TIA	120	82	No	Yes	Yes	No	No													
P045	45	Patients	Males	72	0	0	2000	TIA	130	72	No	Yes	Yes	No	No	6.4	4.14	14.3	99.4	267	0.5		53.1	10.8	19.4	2.2	15.2	

																	FBC					aa		adp		adr		coll	
No.	id	group	gender	age	resist	resist01	strdate	strtype	sbp	dbp	smoke	highbp	highchol	dm	af	wbc	rcc	hb	mcv	plt	hi	low	hi	low	hi	low	hi	low
P046	46	Patients	Males	38	0	0	2000	TIA	126	82	No	No	No	No	No	8.1	4.88	15.3	88.8	413	0.5		64.8	8.2	38.8	4.5	20.3	4.2
P047	47	Patients	Males	69	0	0	1995	TIA	130	86	2	Yes	Yes	No	No	6.9	5.2	15.1	87.7	208	3.3		64.9	6.3	34.1	0.6	23.9	12.7
P048	48	Patients	Females	49	0	0	2004	RMCA	120	80	Yes	No	No	No	No	11.1	4.92	16	96.9	288	-2.1		61	3.9	50.9	4.9	25	7.2
P049	49	Patients	Females	76	0	0	2001	TIA	126	84	2	Yes	No	No	No	6.1	3.7	12.3	98.2	373	-3		73.7	14	42.1	6.2	27.2	16.4
P050	50	Patients	Males	78	2	1	2000	MCA-PAC	122	78	No	No	No	No	No	7.2	5.12	15.5	87.4	244	23.2	0	77	41.4	78.1	20.4	39.9	10.8
P051	51	Patients	Females	54	0	0	2004	TIA	130	90	Yes	Yes	Yes	No	No	9	4.5	12.5	85.7	404	1.8		49.1	5.1	14.8		7.2	
P052	52	Patients	Males	79	0	0	1985	LMCA	128	82	2	Yes	Yes	No	No	10.9	5.05	16.2	98.3	324	-0.7		29.3	5.6	48.7	4.1	13.9	
P053	53	Patients	Males	57	0	0	2003	RMCA	126	84	Yes	Yes	No	Yes	No	8.3	4.84	17	101.3	266	0		70.6	8.2	20.9	-0.3	13.4	
P055	55	Patients	Males	62	0	0	2004	RMCA	130	72	No	Yes	Yes	No	No	5.6	5.32	16.2	90.2	251	2.9		80.4	25.2			57.8	7.8
P056	56	Patients	Females	59	0	0	2004	TIA	132	84	Yes	Yes	Yes	No	No	8.9	4.8	14.7	89.2	408	2.1		78.8	14.8	36.3	19.5	57.9	5.6
P057	57	Patients	Females	92	2	1	2002	RMCA	126	86	No	Yes	No	No	No	6.4	4.75	14.5	89.6	260	2.7		95.2	40.1	41.1	26	22.8	3.1
P058	58	Patients	Females	85	0	0	1999	RMCA	130	82	No	Yes	No	No	No	5.6	4.97	15.2	91.5	263	0		67.9	15.8	23.2	14.4	12.7	
P059	59	Patients	Females	82	0	0	2002	LMCA	132	88	No	Yes	No	No	No						3.3		64.3	16.7	27.5	17.9	18.8	0
P060	60	Patients	Females	81	0	0	1994	LMCA	126	80	No	No	No	No	No	10.1	4.57	14.7	95.3	333	2.7		66	15	20.8	12	0	
P061	61	Patients	Males	91	0	0	2003	RMCA	120	80	No	No	Yes	Yes	No	7.6	3.32	10.8	96.2	345	2.5		77.9	19.1	50.8	29	13.5	
C001	1	Controls	Males	40	0	0			130	90	Yes	No	No	No	No	8.3	5.88	16.2	81.2	297	0.5		72.5	8.7	50.7	38.3	23.2	7.6
C002	2	Controls	Females	30	0	0			130	86	Yes	No	No	No	No	8.3	4.4	13.3	88.1	474	1.8		70.6	0	26.1	0	55	0
C003	3	Controls	Females	32	0	0			120	80	No	No	No	No	No	9.2	4.5	15.9	101.3	175	1.6		69.9	0	13.6		31.8	2.9
C004	4	Controls	Males	61	0	0			130	88	No	Yes	No	No	No	7.2	5.03	15.2	87.7	220	1.6		58.1	11.9	21.7	7.1	35.5	0.2
C005	5	Controls	Females	32	0	0			110	80	No	No	No	No	No	5.8	4.82	14.8	88.4	260	2		72.4	10.1	22.2	3.6	19.4	
C006	6	Controls	Females	42	0	0			120	80	No	No	No	No	No	7	4.73	15.2	93.9	273	3.9		58.9	0	26.9	4.2	19.3	
C007	7	Controls	Males	68	0	0			126	82	No	Yes	Yes	No	No	6.4	5.32	17	93	255	2.3		58.3	9.6	12.3	2.7	30.2	0.4
C008	8	Controls	Males	37	0	0			120	78	No	No	No	No	No	4.5	4.79	13.9	80.8	207	2.8		66	19.8	26.2	3.9	53.9	16.3
C009	9	Controls	Males	91	0	0			110	70	No	No	No	No	No	5.7	4.42	14.4	91.4	179	3.6		59.7	10.9	33.6	2	47.2	6.3
C010	10	Controls	Females	76	0	0			124	84	No	Yes	Yes	Yes	No	8.1	4.67	15.4	94	234	1.9		69.9	17.1	34.7	4.8	59	13
C011	11	Controls	Females	53	0	0			128	82	No	Yes	Yes	Yes	No	8.4	4.53	14	91	338	0.6		67.7	5.1	20.2	1.5	22.4	3.8
C012	12	Controls	Females	29	0	0			120	80	No	No	No	No	No	7.4	4.66	13.9	83.6	314	3.3		81.9	7.7	21	2.7	27.3	0
C013	13	Controls	Females	65	0	0			122	84	No	No	No	No	No	6.9	4.77	14.8	89.4	203	6		51.4	20	46.6	10.4	53.2	13.4
C014	14	Controls	Females	25	0	0			120	80	No	No	No	No	No	6.1	4.46	14.4	90.5	329	0.5		75.1	14.5	28.3	3	41.2	0
C015	15	Controls	Females	39	0	0			122	82	No	No	No	No	No	5.1	4.25	13.3	88.5	315	1.6		83.7	14	16.5	6.3	29	1
C016	16	Controls	Males	58	0	0			126	82	No	No	No	No	No	5.4	4.94	16.1	94.1	220	2.8		62.7	5.9	33	2.6	40	3.5
C017	17	Controls	Females	51	0	0			130	90	No	Yes	No	No	No	6.7	5.02	14.9	85.1	399	0.3		68.6	-2.6	13.2		3.1	
C018	18	Controls	Females	65	0	0			130	86	No	No	No	No	No	7.4	4.5	14.8	95.3	255	4		73.4	18.6	40.7	6	28.8	2.2
C019	19	Controls	Females	68	0	0			126	88	No	No	No	No	No	5.7	4.65	14.4	90	261	1.3		53.8	7.9	30.2	1.8	4	
C020	20	Controls	Males	62	0	0			120	80	No	No	No	No	No	6	5.19	16.9	92.8	209	1.4		54	11	31.1	5.2	13.7	
C021	21	Controls	Females	28	0	0			130	90	No	No	No	No	No	8.1	5.01	15.1	87.8	266	1.3		74.8	11.4	33.2	2.3	18.5	0.4
C022	22	Controls	Females	58	0	0			128	88	No	No	No	No	No	7.1	4.19	12.9	90.2	298	2.5		60.4	19.3	25.9	1.8	30	0.2
C023	23	Controls	Males	61	0	0			120	80	No	No	No	No	No	7	4.95	14.8	86.7	247	2.9		62.4	17.8	42.3	5.3	22.2	5.1
C024	24	Controls	Females	33	0	0			132	88	No	No	No	No	No	7.1	4.48	14.3	90.5	300	1.6		61.9	5.8	23.7	1.9	30.4	2.2
C025	25	Controls	Females	60	0	0			132	88	No	No	No	No	No	9.2	5.27	16.2	87.2	219	3.4		70	21.5	33.3	3.1	31	14.7
C026	26	Controls	Females	41	0	0			130	80	No	No	No	No	No	9.9	4.74	13.6	84.6	325	1		52.6	2.4	19.8	0.3	3.3	
C027	27	Controls	Females		0	0					No	No	No	No	No	4.6	4.67	13.1	82.3	286	0.3		81.6	17.7	30.9	4.1	34.9	1.3
C028	28	Controls	Females	40	0	0			120	80	No	No	No	No	No														
C029	29	Controls	Females	60	0	0			128	86	No	No	No	No	No	9.6	4.38	14.4	93.4	191	0.7		75.3	7	20.8	2.6	30.9	12.9
C030	30	Controls	Females	34	0	0			120	80	No	No	No	No	No	6.3	4.8	15.7	95.6	271	-1		67	11.3	26.7	3	7.8	

																FBC					aa		adp		adr		coll	
No.	id	group	gender	age	resist	resist01	strdate	strtype	sbp	dbp	smoke	highbp	highchol	dm	af	wbc	rcc	hb	mcv	plt	hi	low	hi	low	hi	low	hi	low
C031	31	Controls	Males	32	0	0			124	82	No	No	No	No	No	5.7	5.97	16.9	82.4	305	4.3		64.1	2.1	47.1	22.4	11.1	
C032	32	Controls	Males	44	0	0			122	86	No	No	No	No	No	6.7	5.78	16.8	85.8	189	2.8		65.6	9.4	40.3	21.8	33.1	9.1
C033	33	Controls	Males	30	0	0			120	82	No	No	No	No	No	4.7	5.49	17.4	89	175	2.8		62.1	6.2	53.7	6.9	59.4	9
C034	34	Controls	Males	34	0	0			120	86	No	No	No	No	No	3.5	5.41	17	89.1	250	2.5		61	0.8	21.3	4.6	25.2	1.6
C035	35	Controls	Females	50	0	0			130	84	No	No	No	No	No	6.6	4.52	13.3	86.9	260	1.5		48.2	4.5	22.1	-2.2	26.4	0
C036	36	Controls	Males	55	0	0			132	88	No	No	No	No	No	5.6	5.6	16.7	86.7	276	2.4		66.7	13.1	29.5	4.4	43.4	6.5
C037	37	Controls	Females	56	0	0			130	82	No	Yes	No	No	No	5.8	4.55	14.4	90.5	193	1.4		75.9	11.8	73.7	3.9	69.2	24.7
C038	38	Controls	Males	65	0	0			126	82	Yes	No	No	No	No	6	4.72	15.1	90.5	232	1.2		71.9	12.4	42.5	10.1	47.6	13.2
C039	39	Controls	Females	55	0	0			130	88	No	Yes	Yes	No	No	7.6	4.57	14.3	89.7	301	2.1		51.7	7.7	12.2		18.5	0
C040	40	Controls	Females	72	0	0			130	86	No	No	No	No	No	6.8	4.65	11.6	78.5	276	0.3		58.7	7.7	32.3	0.8	12.1	
C041	41	Controls	Females	56	0	0			134	84	No	No	No	No	No	8.6	4.59	14.5	90.4	422	5.1		57.3	3.1	14.1	3	1.3	
C042	42	Controls	Females	56	0	0			130	82	No	Yes	No	No	No	6.1	5.02	14.9	85.4	230	2.5		59.7	5.1	23.3	1.7	1.6	
C043	43	Controls	Females	54	0	0			132	86	No	Yes	No	No	No	6.9	5.14	15.3	88.9	321	5.1		75.6	4.6	14.7	1.6	0.2	
C044	44	Controls	Females	40	0	0			130	82	No	Yes	No	No	No	4.5	4.33	12.7	87	296	0		73.5	7.3	24.8	5.3	4	
C045	45	Controls	Females	54	0	0			128	82	No	No	No	No	No	7.4	5.68	13.2	72.7	464	5.5		53.7	3.6	22.7	0	7	
C046	46	Controls	Males	64	0	0			128	86	No	No	Yes	No	No	9.3	5.57	17.3	88.9	242	0.9		70	7.8	26	4.3	0	
C047	47	Controls	Females	57	0	0			122	82	No	No	No	No	No	5.4	4.63	14.6	90.8	360	2.4		75.1	2.3	13.5	0	1.6	
C048	48	Controls	Males	40	0	0			136	84	No	No	No	No	No	5.7	5.06	16.9	97.3	270	5.4		80.3	12.9	44.5	3.8	29.1	1.5
C049	49	Controls	Females	46	1	1			132	86	No	No	No	No	No	4.9	4.44	11.6	80.1	352	0		70.1	12.7	35.3	3.7	14.2	
C050	50	Controls	Females	50	0	0			126	82	No	No	No	Yes	No	9.2	5.73	10.3	58.8	418	33.3	16.2	47	37.4	48.8	7.3	11.1	
C051	51	Controls	Males	42	0	0			130	86	No	No	No	No	No	4.8	4.67	15.1	93.3	280	0		63.6	5.9	34.4	2.5	26.1	0.8
C052	52	Controls	Males	48	0	0			126	82	No	No	No	No	No	7	5.36	17.1	92.7	250	1.4		64.7	28.7	42.6	5.3	43.8	6.2
C053	53	Controls	Females	53	0	0			128	86	No	No	No	No	No	7.8	4.87	15.5	94.8	264	6		90.5	20.3	63.1	4.4	61.8	7
C055	55	Controls	Females	42	0	0			130	88	No	No	No	No	No	7.6	4.63	15	91.4	321	0.5		76.2	4.6	38.9	6.5	17.9	
C056	56	Controls	Females	50	0	0			126	84	No	No	No	No	No	5.7	4.4	14	91.6	332	0		65.5	0	27		0	
C057	57	Controls	Females	42	0	0			132	80	No	No	No	No	No	4.8	4.7	14.9	92.7	264	0		73.7	6.4	47.6	4.4	5.8	
C058	58	Controls	Females	48	0	0			132	88	No	No	No	No	No	8.3	4.65	14.6	89.1	312	0		84.3	0	14.1	0.7	8.6	
C059	59	Controls	Females	68	0	0			128	82	No	No	No	No	No	8.2	4.31	14	92.5	263	2.3		96	9.7	26.2	5.3	17.6	
CO60	60	Controls	Females	48	0	0			130	86	No	No	No	No	No	7.3	4.82	15.1	90.6	382	1.5		75.9	0	18.3	0.5	0.5	
CO61	61	Controls	Females	49	0	0			128	84	No	No	No	No	No	6.7	4.66	13.8	87.3	248	2.4		75.1	2.3	13.5	0	1.6	

8. REFERENCES

(1) Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *The Lancet* 2006; 367: 1747-57.

(2) Norman R, Bradshaw D, Schneider M, Pieterse D, Groenewald P. Revised burden of disease estimates for the comparative risk factor assessment, South Africa, 2000. *Cape Town: Medical Research Council* 2006.

(3) Bhatt DL, Topol EJ. Scientific and therapeutic advances in antiplatelet therapy. *Nature Reviews* 2003; 2(1): 15-17.

(4) Hoffbrand AV, Pettit JE, Moss PAH. Platelets, blood coagulation and haemostatis. In: Hoffbrand AV, Pettit JE, Moss PAH, editors. *Essential Haematology*. Blackwell Science Ltd, 2001: 239-41, 245-47.

(5) Fogarty PF, Rick ME. Disorders of Haemostasis II. In: Rodgers GP, Young NS, editors. *Bethesda Handbook of Clinical Haematology*, Philadelphia: Lippincott Williams & Wilkins, 2005: 86-87, 290.

(6) Donnan GA, Fisher M, Macleod M., Davis SM. Stroke. *The Lancet* 2008; 371: 1612-23.

(7) Bradshaw D, Schneider M, Dorrington R, Bourne D and Laubscher R. South African cause-of-death profile in transition – 1996 and future trends. *S Afr Med J 2002;* 92(8): 618-23.

(8) Pestana J, Steyn K, Leiman A. and Hartzenberg G. The direct and indirect costs of cardiovascular disease in South Africa in 1991. *S Afr Med J 1996;* 86(6): 679-84.

(9) Connor M. Stroke in patients with Human Immunodeficiency Virus infection. *J Neurol Neurosurg Psych* 2007; 78: 1291.

(10) Corral, I, *et al*. Cerebrovascular ischaemic events in HIV-1-infected patients receiving highly active antiretroviral therapy (HAART): Incidence and risk factors. *Cerebrovasc Dis* 2009; 27: 559-63.

(11) Shuaib A, Hachinski VC. Mechanisms and amangement of stroke in the elderly. *CMAJ* 1991; 145: 433-43.

(12) Stam J. Thrombosis of the cerebral veins and sinuses. *N Eng J Med* 2005; 352(17): 1791-98.

(13) Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *The Lancet* 1991; 337(8756): 1521-26.

(14) Bamford JM. The role of the clinical examination in the subclassification of stroke. *Cerebrovasc Dis* 2000; 10 (Suppl 4): 2-4.

(15) Kidwell C, Chalela J, Saver J. et al. Comparison of MRI and CT for detection of acute intracerebral haemorrhage. *JAMA* 2004; 292(15): 1823-30.

(16) Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction and stroke in high risk patients. *BMJ* 2002; 324: 71-86.

(17) Cattaneo M. Resistance to antiplatelet drugs: molecular mechanisms and laboratory detection. *J Thromb Haemost* 2007; 5 (Suppl 1): 230-37.

(18) Cattaneo M. Aspirin and clopidogrel. Efficacy, safety and the issue of drug resistance. *Arterioscler Thromb Vasc Biol* 2004; 24: 1980-87.

(19) Mega JL. Cytochrome P-450 Polymorphisms and Response to Clopidogrel. *N Engl J Med* 2009; 360: 354-62.

(20) Collet J. Cytochrome P-450 2C19 polymorphism in young patients treated with clopidrogrel after myocardial infarction: a cohort study. *Lancet* 2009; 373: 309.

(21) Diener HC, Bogousslavsky J, Brass LM *et al*. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-controlled trial. *Lancet* 2004; 364 (9431): 331-37.

(22) Tcheng JE, Kandzari DE, Grines CL, *et al*. CADILLAC Investigators. Benefits and risks of abciximab use in primary angioplasty for acute myocardial infarction: the Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications (CADILLAC) trial. *Circulation* 2003; 108(11): 1316-23.

(23) Hartzman GD, *et al*. Non-Peptide Fibrinogen Receptor Antagonists. Discovery and Design of Exosite Inhibitors. *J Med Chem* 1992; 35: 4640-42.

(24) Halkes PH, *et al*. Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): randomised controlled trial. *Lancet* 2006; 367(9523): 1665-73.

(25) Lewis HD, *et al*. Protective effects of aspirin against acute myocardial infarction and death in men with unstable angina. Results of a Veterans Administration Cooperative Study. *New Engl J Med* 1983; 309: 396-403.

(26) Belch J, MacCuish A, Campbell I, *et al*. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. *Br Med J* 2008; 337: 1840.

(27) Vane JR, Flower RJ, Botting RM. History of aspirin and its mechanism of action. *Stroke* 1990; 21: IV12-23.

(28) Somasundaram S, *et al*. Uncoupling of intestinal mitochondrial oxidative phosphorylation and inhibition of cyclooxygenase are required for the development of NSAID-enteropathy in the rat. *Aliment Pharmacol Ther* 2000; 14: 649-50.

(29) McCarty MF, Block KI. Preadministration of high-dose salicylates, suppressors of NF-κB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. *Integr Cancer Ther* 2006; 5(3): 252-68.

(30) Hartwig OH. Pharmacokinetic considerations of common analgesics and antipyretics. *Am J Med* 1983; 75: 30-37.

(31) Raithel M, Baenkler HW, Naegel A, *et al*. Significance of salicylate intolerance in diseases of the lower gastrointestinal tract. *J Physiol Pharmacol* 2005; 56(5): 89-102.

(32) Chan, *et al*. Long-term Use of Aspirin and NSAIDs and Risk of Colorectal Cancer. *JAMA* 2005; 294(8): 914-23.

(33) Moysich KB, Menezes RJ, Ronsani, A, *et al*. Regular aspirin use and lung cancer risk. *BMC Cancer* 2002; 2: 31.

(34) Wolff, *et al*. Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res* 1998; 58(22): 4997-5001.

(35) Patrono C. Aspirin resistance: definition, mechanisms and clinical read-outs. *J Thromb Haemost* 2003; 1: 1710-13.

(36) Hankey GJ, Eikelboom JE. Aspirin resistance. *The Lancet* 2006; 367: 606-17.

(37) Campbell CL, Steinhubl SR. Variability in response to aspirin: do we understand the clinical relevance? *J Thromb Haemost* 2005; 3: 665-69.

(38) Gum, Kottke-Marchant K, Welsh P, White J. and Topol E. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol* 2003;41(6):961-65.

(39) Helgason, C, Bolin, K, Hoff J, Winkler S, Mangat A, Tortorice K and Brace L. Development of aspirin resistance in persons with previous ischaemic stroke. *Stroke* 1994;25(12):2331-36.

(40) Grotemeyer KH., Scharafinski HW., Husstedt IW. Two-year follow-up of aspirin responder and aspirin non-responder. A pilot-study including 180 post-stroke patients. *Throm Res* 1993;71:397-403.

(41) Crawford, KM and Talbert RL. Antiplatelet therapy in secondary stroke prevention. *Expert Opin Pharmacother2001;* 2(10):1609-13.

(42) Weber A, Przytulski, B, Schanz A, Hohlfeld T and Schror K. Towards a definition of aspirin resistance: a typological approach. *Platelets 2002;*13:37-40.

(43) Christie DJ, Avari T, Carrington LR, Cohen E, DeBiase BA, Harrison P, et al. Platelet function testing by aggregometry; Approved guideline. *Clin and Lab Stds Inst Doc H58-A* 2008; 28(31): 1.

(44) Marcus AJ. Platelet Aggregation. In: Coleman RW, Hirsh J, Marder VJ, Salzamn EW, editors. *Haemostasis and Thrombosis: Basic Principles and Clinical Practice*. J B Lippincott Co, Philadelphia, 1992: 380-89.

(45) Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 1962; 194: 927.

(46) Bio/Data Corporation. Collagen (package insert). Technical Bulletin No. 101563.

(47) Laffan M, Manning R. Investigation of haemostasis. In: Lewis SM, Bain BJ, Bates I, editors. *Dacie and Lewis Practical Haematology*. Churchill Livingstone, Philadelphia, 2006: 427-33.

(48) APACT 2 – Operator Manual – Version 1.0.

(49) Haematology Working Group. Standard Operating Procedure (SOP) for Platelet aggregation and sensitivities on the APACT 2 ON Helena Aggregometer™. *NHLS Dept Haem* 2005; 1-4.

(50) Ivandic BT, *et al*. Determination of clopidogrel resistance by whole blood platelet aggregometry and inhibitors of the P2Y12 receptor. *Clin Chem* 2006; 52: 383-88.

(51) Heptinstall S, *et al*. Clopidogrel resistance. *Catheter Cardiovasc Interv* 2004; 63: 397-98.

(52) Hayward CP, *et al*. The Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and plate function. *J Thromb Haemost* 2006; 4: 312-19.

(53) Fontana P, Nolli S, Reber G, de Moerloose P. Biological effects of aspirin and clopidogrel in a randomized cross-over study in 96 healthy volunteers. *J Thromb Haemost* 2006; 4: 813-19.

(54) Anderson K, Hurlen M, Arnessen H, Seljeflot I. Aspirin non-responsiveness as measured by PFA-100 in patients with coronary artery disease. *Thromb Res* 2003; 108: 37-42.

(55) Harrison P, *et al*. Screening for aspirin responsiveness after transient ischaemic attack and stroke: comparison of 2 point-of-care platelet function tests with optical aggregometry. *Stroke* 2005; 36: 1001-05.

(56) Frelinger AL III, *et al*. Residual arachidonic acid-induced platelet activation via an adenosine diphosphate-dependent but cyclooxygenase-1- and cycloxoygenase-2-independent pathway: a 700-patient study of aspirin resistance. *Circulation* 2006; 113: 2888-96.

(57) Agarwal S, Coakley M, Reddy K, Riddell A, Mallet S. Quantifying the effect of antiplatelet therapy: a comparison of the platelet function analyzer (PFA-100) and modified thromboelastography (mTEG) with light transmission platelet aggregometry. *Anesthesiology* 2006; 105: 676-83.

(58) Patrono C, *et al.* Estimated rate of thromboxane secretion into the circulation of normal humans. *J Clin Invest* 1986; 77: 590-94.

(59) Eikelboom JW, *et al*. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation* 2002; 105: 1650-55.

(60) Ohmori T, *et al*. Aspirin resistance detected with aggregometry cannot be explain by cyclooxygenase activity: involvement of other signalling pathway(s) in cardiovascular events of aspirin-treated patients. *J Thromb Haemost* 2006; 4:
1271-78.

(61) Faraday N, *et al*. Relation between atherosclerosis risk factors and aspirin resistance in a primary prevention population. *Am J Cardiol* 2006; 98: 774-79.

(62) Campbell CL, Steinhubl SR. Variability in response to aspirin: do we understand the clinical relevance? *J Thromb Haemost* 2005; 3: 665-69.

(63) Macchi L, *et al*. Resistance *in vitro* to low-dose aspirin is associated with platelet PLA1 (GP IIIa) polymorphism but not with C807T (GP Ia/IIa) and C-5T kozak (GP 1bα) polymorphisms. *J Am Coll Cardiol* 2003; 42: 1115-19.

(64) Undas A, *et al*. PLA2 polymorphism of β3 integrins is associated with enhanced thrombin generation and impaired antithrombotic action of aspirin at the site of microvascular injury. *Circulation* 2001; 104: 2666-72.

(65) Toppol EJ and Quinn MJ. Common variations in platelet glycoproteins: pharmacogenomic implications. *Pharmacogenomics* 2001; 2: 341-52.

(66) Maree AO, *et al*. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. *J Thromb Haemost* 2005; 3: 2340-45.

(67) Hankey GJ and Eikelboom JW. Aspirin resistance editorial. *BMJ* 2004; 328: 477-79.

(68) Cox D, *et al.* Effects of enteric coating on antiplatelet activity of low-dose aspirin in healthy volunteers. *Stroke* 2006; 37: 2153-58.

(69) Catella-Lawson F, *et al*. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med* 2001; 345: 1809-17.