A STUDY ON CYP2C19 GENETIC VARIATIONS OF CLOPIDOGREL AND TICAGRELOR TREATMENT AMONG CORONARY ARTERY DISEASE (CAD) PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION (PCI)

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UNIVERSITI SAINS MALAYSIA

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by

MOHAMMED AHMED IMRAN AK-KAIF

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AA	Arachidonic acid
ACEI	Angiotensin converting enzyme inhibitor
ACS	Acute coronary syndrome
ADP	Adenosine diphosphate
APPT	Activated partial thromboplastin time
ARB	Angiotensin II reuptake blocker
BMI	Body mass index
BMS	Bare-metal stents
C	Clopidogrel
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
cAMP	Cyclic adenosine monophosphate
CCBs	Calcium channel blocker
CCS	chronic coronary syndrome
CES1	Carboxylesterase 1
CFR	Coronary flow reserve
CHD	Coronary heart disease
CI	Confidence intervals
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
COX-1	Cyclooxygenase
CPG	Clinical practice guidelines
CR	Clopidogrel resistance
CVD	Cardiovascular disease
CVDs	Cardiovascular diseases
DAPT	Dual antiplatelet therapy
DD	Droplet digital
DES	Drug-eluting stents
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor

ESC	European Society of Cardiology
FFR	Flow fraction reserve
FMD	Flow-mediated dilation
GOF	Gain of function
Hb	Haemoglobin
HCT	Haematocrit
HES1	Hairy and enhancer of split-1
HPP	Hospital Pulau Pinang
HTPR	High treatment platelet reactivity
HUVECs	Human umbilical vein endothelial cells
IAP	Interventional angiographic procedure
ID	Identification code
IHD	Ischaemic heart disease
IMR	Index of microvascular resistance
INR	International normalized ratio
KDQOI	Kidney Foundation, Kidney disease quality outcome initiative
LD	Loading dose
LDL	Low-density lipoprotein
LOF	Loss of function
MACE	Major adverse cardiac events
MD	Maintenance dose
MI	Myocardial infraction
MREC	Medical Research and Ethics Committee
n	Number
NASM-PCR	Nested allele-specific multiplex PCR
ND	No data
NSCD	Non-significant coronary disease
NSTEMI	Non-ST elevation myocardial infarction
OAD	Oral antidiabetic drugs
P-gp	P-glycoprotein
PAF	Platelet-activating factor
PAR	Protease-activated receptor
PAT	Peripheral arterial tonometry
PCI	Percutaneous coronary intervention

PCR	Polymerase chain reaction
PD	Pharmacodynamic
PFTs	Platelet function tests
PLATO	Platelet inhibition, and Patient Outcomes
PLT	Platelet
PPIs	Proton pump inhibitors
PRI	Platelet reactivity index
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
PRU	P2y12 reaction unit
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothrombin time
RBC	Red blood cell
RCTs	Randomized controlled trials
RHI	Reactive hyperemia index
rpm	Revolutions per minute
RR	Risk ratio
RT	Room temperature
SCAD	Stable coronary artery disease
SD	Standard deviation
SIRT1	Sirtuin 1
SNPs	Single nucleotide polymorphisms
ST	Stent thrombosis
STEMI	ST-elevation myocardial infarction
T	Ticagrelor
TIMI	Thrombolysis in Myocardial Infarction
TP	TXA2 receptor
TXA2	Thromboxane A2
UA	Unstable angina
UI	Unique identifier
UV	Ultraviolet device
V	Visit
VASP	Vasodilator-associated stimulated phosphoprotein
WBC	White blood cell
WHO	World health organization

LIST OF APPENDICES

KAJIAN MENGENAI VARIASI GENETIK CYP2C19 BAGI RAWATAN CLOPIDOGREL DAN TICAGRELOR DALAM KALANGAN PESAKIT PENYAKIT ARTERI KORONARI (CAD) YANG MENJALANI INTERVENSI KORONARI PERKUTANEUS (PCI)

ABSTRAK

Perencatan reseptor P2Y12, terutamanya clopidogrel, adalah ubat antiplatelet yang biasa digunakan untuk mencegah penyakit kardiovaskular berulang dalam kalangan pesakit dengan penyakit arteri koronari (CAD) yang menjalani intervensi koronari perkutaneus (PCI). Terdapat beberapa faktor genetik yang boleh menjejaskan keberkesanan terapi antiplatelet, termasuk variasi genetic dalam enzim CYP450. Pembawa bagi kehilangan fungsi (LOF) gen *CYP2C19* adalah dikaitkan dengan pengurangan metabolisme clopidogrel kepada metabolit aktif melalui enzim CYP2C19, yang membawa kepada penurunan perencatan platelet. Ini menyebabkan peningkatan risiko penyakit kardiovaskular berulang. Pihak berkuasa pengawalan dadah mencadangkan penggunaan perencat P2Y12 alternatif, iaitu ticagrelor. Oleh itu, kajian ini bertujuan untuk menyiasat kesan buruk, dan variasi genetik CYP2C19 bagi clopidogrel dan rawatan ticagrelor di kalangan pesakit CAD yang menjalani PCI. Kajian reka bentuk prospektif, rawak, selari telah dijalankan dalam kalangan pesakit CAD yang stabil dan menjalani PCI. Kaedah pengenotipan untuk polimorfisme *CYP2C19*2* dan **3* telah dibangunkan menggunakan teknik NASM-PCR. Pesakit yang memenuhi kriteria kemasukan telah digenotipkan untuk polimorfisme *CYP2C19*. Pesakit dirawat secara rawak dengan sama ada ticagrelor atau clopidogrel selepas PCI. Selepas 4 jam dos pemuatan, pesakit diuji untuk PRI, dan kemudian susulan dijalankan selepas 30 hari untuk menilai hasil klinikal. Dalam kalangan 94 pesakit yang diambil,

41 (43.62%) adalah pembawa bagi varian kedua-dua alel (*l/*2, *2/*2, *l/*3). Daripada 84 pesakit yang dinilai PRI, (44 mengambil clopidogrel dan 40 mengambil ticagrelor), 23 (52.3%) pesakit mempunyai PRI ≥ 50 dan hanya 1 (2.5%) pesakit yang mengambil ticagrelor mempunyai PRI ≥ 50. Pesakit yang mengambil ticagrelor menunjukkan tindak balas antiplatelet yang lebih baik berbanding dengan clopidogrel, untuk kedua-dua kumpulan dengan alel jenis liar dan alel varian (P=0.005 dan <0.001, masing-masing). Kesimpulannya kajian ini menunjukkan potensi penggunaan ujian genetik CYP2C19 dalam pesakit CAD untuk memandu pemilihan terapi antiplatelet selepas prosedur PCI.

A STUDY ON CYP2C19 GENETIC VARIATIONS OF CLOPIDOGREL AND TICAGRELOR TREATMENT AMONG CORONARY ARTERY DISEASE (CAD) PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION (PCI)

ABSTRACT

The P2Y12 receptor inhibitors, especially clopidogrel, are common antiplatelet drugs used for the prevention of recurrent adverse cardiovascular events among patients with coronary artery disease (CAD) undergoing percutaneous coronary intervention (PCI). Several factors may affect the effectiveness of antiplatelet therapy, including genetic variations in CYP450 enzymes. Loss of function (LOF) carriers of the *CYP2C19* gene are associated with the decreased metabolism of clopidogrel into active metabolites via CYP2C19 enzyme, leading to decreased platelet inhibition. This causes an increased risk of recurrent cardiovascular events. Drug regulatory authorities suggested using alternative P2Y12 inhibitor, which is ticagrelor. Therefore, this study aims to investigate the adverse effects, and *CYP2C19* genetic variations of clopidogrel and ticagrelor treatment among CAD patients undergoing PCI. A prospective, randomized, parallel design study was conducted among patients with stable CAD and undergoing PCI. The genotyping for *CYP2C19*2* and **3* polymorphisms was optimized using the NASM-PCR technique. Patients who met the inclusion criteria were genotyped for *CYP2C19* polymorphisms. Patients were randomly treated with either ticagrelor or clopidogrel after PCI. After 4 hours of the loading dose, patients were tested for the PRI, and then were followed-up after 30 days to assess the clinical outcomes. Among 94 recruited patients, 41 (43.62%) were carriers of the variant type of both alleles (*l/*2, *2/*2, *l/*3). Out of the 84 PRI-assessed patients, (44 taken

clopidogrel and 40 taken ticagrelor), 23 (52.3%) patients had PRI ≥ 50 and only 1 (2.5%) patient taken ticagrelor had PRI ≥ 50. Patients taken ticagrelor were shown to have a better antiplatelet response as compared to clopidogrel, for both groups with wild type alleles and variant alleles (P= 0.005 and <0.001, respectively). Other non-genetic factors showed no significant association with the outcomes. As a conclusion, this study demonstrates the potential of adopting *CYP2C19* genetic testing among CAD patients to guide the selection of antiplatelet therapy after PCI procedure.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Cardiovascular diseases (CVDs) are labelled as the leading cause of death in Asian countries including Malaysia (Chan et al., 2022; Joseph et al., 2022). The World Health Organization (WHO) predicts that coronary artery disease (CAD) will be the leading cause of mortality in many nations around the world, of which confirmed by the European Society of Cardiology (ESC) (Timmis et al., 2022; WHO, 2021). With CAD accounting for the most deaths globally, finding proper treatments for CAD has emerged as a major challenge in cardiology practice.

CVDs are associated with 17.9 million deaths annually around the world due to CAD and stroke, which representing 32% of all global deaths (WHO, 2021). In Malaysia, CAD has been denoted as the leading cause of mortality and it accounts to approximately 17% of the 109,155 medically certified deaths in 2020 (Ministry of Health Malaysia, 2021b). CAD is often caused by atherosclerosis, which restricts blood flow to the heart and results to a heart attack (Chan & Ramji, 2022; Jebari-Benslaiman et al., 2022). A person will suffer from CAD when there is an inadequate supply of oxygen-rich blood and nutrients to the cardiac muscles due to the narrowing or even blockage of the coronary arteries caused by atherosclerosis (Chan & Ramji, 2022). The pathogenesis of atherosclerosis also involves the processes of vascular injury, inflammation, degeneration, and thrombosis (Chan & Ramji, 2022).

Percutaneous coronary intervention (PCI) is a recognized and popular treatment for CAD worldwide, including Malaysia (Ahmad & Bang, 2016).It is a non-surgical procedure that uses stent implantation to widen the narrowed or blocked

coronary arteries without open-heart surgery (Khan & Ludman, 2022). PCI is often followed by bare metal stents (BMS) and drug-eluting stents (DES), which are commonly preferred among patients. Currently, DES is a more popular option to significantly reduce the risk of restenosis and improve clinical outcomes (Weiss et al., 2022). PCI is a mainstay in the management of high-risk coronary artery disease and requires antiplatelet therapy, depending on stent type and admission diagnosis (Khan & Ludman, 2022).

Platelet aggregation induces thrombotic events in patients undergoing PCI. Therefore, inhibition of the platelet aggregation pathway by dual antiplatelet therapy (DAPT) is crucial for preventing platelet activation, followed by reducing the risk of further major adverse cardiovascular events (MACE) and stent-related complications (Berg et al., 2021; Lee et al., 2018). Using DAPT after placement of DES is a well-established practice for CAD patients undergoing PCI since both ischaemic and bleeding risks, as measured by MACE, are associated with worse outcomes after DES. The long-term efficacy of DAPT after PCI, regardless of clinical symptoms, has been demonstrated in general guidelines (Members et al., 2022). For instance, 12-months use of aspirin and a P2Y12 receptor inhibitor has become the standard of care for alleviating thrombotic events in patients with acute coronary syndrome or after PCI (Members et al., 2022; Valgimigli et al., 2018).

Although the efficacy of DAPT has been confirmed, some patients may still develop subsequent thrombotic events. This may be associated with broad interindividual variability among different ethnicities in the clinical response of antiplatelet drugs (Akkaif et al., 2021; Amin, Chin, Noor, et al., 2017; Tamargo et al., 2022). The optimal duration of DAPT after PCI is also controversial because it is

influenced by the patient's risk factors and the type of stent used. Canadian, Australian, US, and UK guidelines have shown that DAPT should be given up to 12 months after placement of bare-metal stents (BMS) or first-generation DES based on CREDO study results (Alsadat et al., 2019; Ibanez et al., 2018; Mehta et al., 2018; Members et al., 2022; Natsuaki et al., 2018; Natsuaki et al., 2022). In Malaysia, clopidogrel and ticagrelor are used although ticlopidine and prasugrel are permitted according to the book of Clinical Practice Guidelines (CPG) for management of Non-ST Elevation Myocardial Infarction (NSTE-ACS) in Malaysia 2021 (Ministry of Health Malaysia, 2021a).

DAPT has always been considered the cornerstone of treatment for patients with CAD, which consisted of aspirin being coupled with an adenosine diphosphate (ADP) receptor antagonist. clopidogrel is the first therapeutic option, while ticagrelor is a relatively recent alternative (Jourdi et al., 2022; Wallentin et al., 2009). The PLATO study discovered that ticagrelor was more effective than clopidogrel, whereby it is more well-tolerated by acute coronary syndrome (ACS) patients and it is not associated with an increased risk of major bleeding in relative to clopidogrel (Dhillon, 2015; Wallentin et al., 2009). This was confirmed by several recent studies that ticagrelor should be used as an alternative to clopidogrel, as clopidogrel is more likely to be subjected to resistance among patients in comparison to ticagrelor (Akkaif et al., 2021; Biswas et al., 2021; Yoon et al., 2020).

Aside from that, the pharmacodynamic (PD) effects of clopidogrel are considered erratic, albeit it is classified as one of the most common P2Y12 inhibitor. Some patients who receive the clopidogrel treatment tend to have high treatment platelet reactivity (HTPR) and are at increased risk of thrombosis in comparison to

other antiplatelet agents (Aradi et al., 2019; Li et al., 2021). This discrepancy in clopidogrel PD has been attributed to genetic polymorphisms of the cytochrome CYP2C19 enzyme, which is the major enzyme involved in clopidogrel metabolism (Akkaif et al., 2021; Duarte & Cavallari, 2021; Lee et al., 2022).

Several single nucleotide polymorphisms (SNPs) have been reported for the gene encoding for CYP2C19 enzyme, including *CYP2C19 *2*, **3* alleles. The *CYP2C19**1 allele refers to a normal allele or a wild-type allele and is associated with normal drug metabolism mediated by *CYP2C19* (Li-Wan-Po et al., 2010). Both *CYP2C19 *2* and **3* alleles are the loss-of-function (LOF) variant alleles associated with decreased functional metabolic activity. The genetic defect of *CYP2C19*2* is caused by a 681 G>A substitution in exon 5, resulting in a defective splice site; whereas *CYP2C19*3* contains a 636 G>A point mutation in exon 4, which leads to an early stop codon (Ieiri et al., 1996; Yin et al., 2004). The loss of function (LOF) carriers of the *CYP2C19* gene are namely labelled as the poor metaboliser phenotype, which denoted significant decreased generation of the active metabolite clopidogrel, decreased platelet inhibition, and increased rates of thrombotic events (Beitelshees et al., 2022; Yamani et al., 2022; Yu et al., 2021; Zhang et al., 2022).

Thus, drug regulatory authorities cautioned about the reduced efficacy of clopidogrel among individuals with *CYP2C19* LOF carriers and suggested using alternative P2Y12-inhibiting therapies (e.g., ticagrelor) for these individuals (Food & Drug Administration, 2010; Use, 2020). However, the implementation of the genotype-guided selection strategy for the oral P2Y12 inhibitor is still limited in clinical practice (Empey et al., 2018; Moon et al., 2018; N. L. Pereira et al., 2019).

The interaction of cardiovascular risk factors between the molecular and biochemical complexities that lead to poor response to treatment with platelet inhibitors has hampered clinicians' ability to prescribe more effective and personalized antiplatelet therapy. So great strides have been made in many individual areas of study, including genetics, pharmacology, and haematology. It has therefore become apparent that research efforts need to move away from traditional approaches and use interdisciplinary and integrative systems biology study designs to bridge the gap between genotype, phenotype, disease manifestations, and/or recurrence.

1.2 Research problem statements

Atherosclerosis is a complex process (Basiak et al., 2022). Thus, despite the prolonged use of DAPT, the risk of recurrent thrombotic and arterial ischaemic disease remains. This may be related to the wide interindividual and interethnic variation in the clinical response to the antiplatelet effect of clopidogrel (Akkaif et al., 2021; Gulizia et al., 2018). East Asian populations have a lower response to clopidogrel (40.1-63.5%). Therefore, rates of ischaemic events after PCI are increasing if compare it with the clopidogrel response in the western populations (20–35%) (Amin, Chin, Noor, et al., 2017; Aradi et al., 2014; Kim et al., 2013).

The antiplatelet drugs used in Malaysia to treat CAD patients undergoing PCI are clopidogrel and ticagrelor with aspirin. They are used as the first-line medications based on some factors that physicians may consider when deciding between clopidogrel and ticagrelor, including the patient's clinical characteristics and the available evidence. The choice depends primarily on personal experience. Due to the advantages of ticagrelor in the treatment of CAD patients, its efficacy being higher than that of clopidogrel, and its insensitivity to the *CYP2C19* gene variation, it may be

suggested that clopidogrel should be replaced by ticagrelor as a suitable option in Malaysian hospitals. However, this suggestion could be controversial.

The most recent study by Amine et al. 2017 demonstrates that clopidogrel resistance is widespread among CAD patients because the prevalence of *CYP2C19*2* and **3* variant alleles in Malaysia. The authors indicates the need to develop new tools to personalize appropriate antiplatelet therapy in this population. Therefore, it must be investigated whether deciding to switch the patient to a potent antiplatelet therapy (e.g., ticagrelor) is the best option (Amin, Chin, Noor, et al., 2017).

Studies reporting the prevalence of *CYP2C19*2* and **3* variant alleles in Malaysian multi-ethnic groups remain insufficient (Amin, Chin, Noor, et al., 2017; Ang et al., 2016; Bakar, 2021; Goh et al., 2017). Besides, the use of pharmacogenetics testing and platelet function testing (PFT) to predict platelet aggregation levels among patients undergoing PCI remains debatable. Further investigations and confirmations are therefore required to provide further insight into the prevalence of these variant alleles and effective antiplatelet therapy (Moon et al., 2018).

Therefore, this study uses PRI as a comprehensive method for predicting response to antiplatelet therapy (clopidogrel and ticagrelor) by combining it with the *CYP2C19* genotyping information proposed. For example, personalizing therapy based on genetic testing for *CYP2C19*2* and **3* genetic variants would offer an ideal guide for decision-makers in practices, where *CYP2C19*2* or **3* mutation carriers would receive ticagrelor and *CYP2C19*2* or **3* non-carriers of the mutation, they will receive clopidogrel. This approach will provide the clinicians with comprehensive information about antiplatelet response and enable them to provide individualized treatment.

1.3 Research hypothesis

This study hypothesised that the CYP2C19 genotype-guided antiplatelet therapy approach could be utilized to personalize which appropriate antiplatelet therapy (clopidogrel and ticagrelor) among CAD patients undergoing PCI.

1.4 Research objectives

1.4.1 General objective

to investigate the CYP2C19 genetic variations of clopidogrel and ticagrelor treatment among CAD patients undergoing PCI.

1.4.2 Specific objective

1. To optimize a nested allele-specific multiplex PCR (NASM-PCR) genotyping method for *CYP2C19* SNPs detection.

2. To identify the frequency of *CYP2C19 *2* and *3* alleles among recruited patients.

3. To investigate the effects of CYPC19 genotypes on PFT among patients receiving clopidogrel and ticagrelor therapy.

4. To evaluate non-genetic factors affecting the PFT among patients receiving clopidogrel and ticagrelor therapy.

5. To evaluate the association between CYP2C19 polymorphism and clinical outcomes

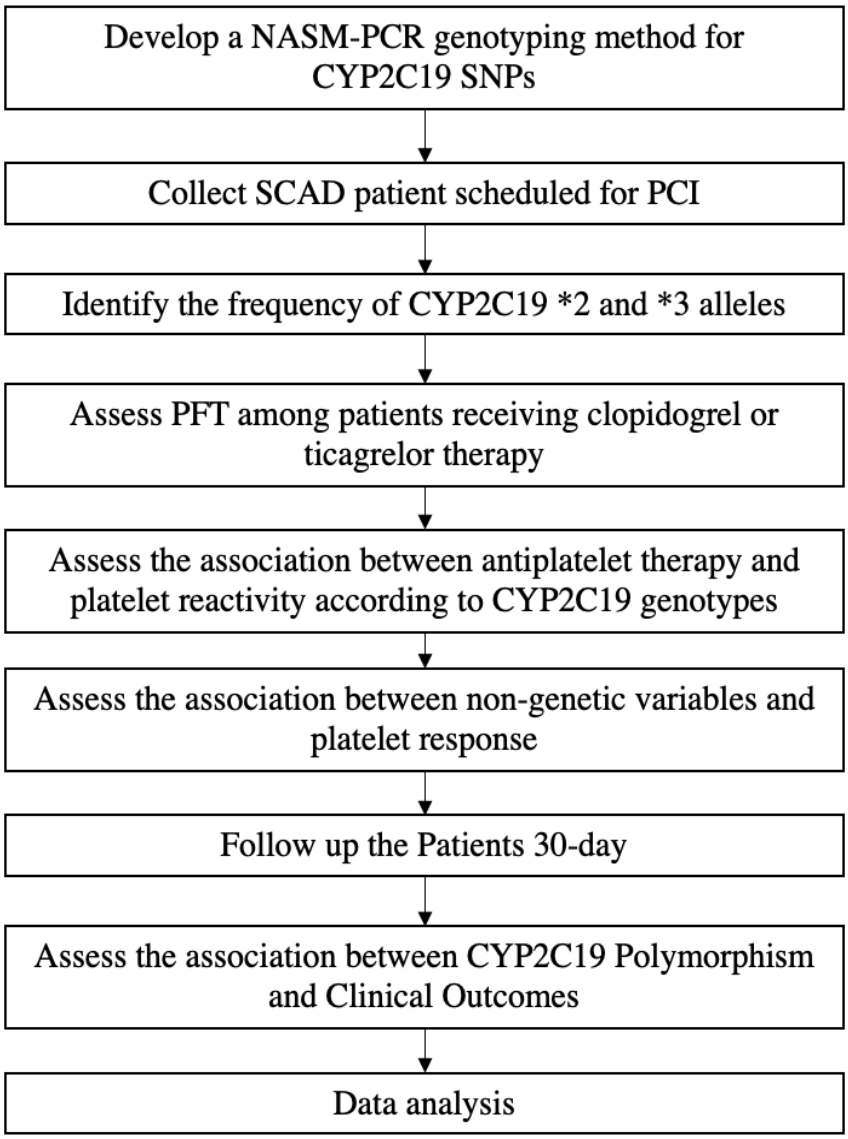

Figure 1.1 A flowchart summarizing the research activities. NASM-PCR: nested allele-specific multiplex PCR PCI: percutaneous coronary intervention ; PCR: Polymerase chain reaction; SCAD= Stable coronary artery disease, PFT: Platelet function test

CHAPTER 2

LITERATURE REVIEW

2.1 Coronary artery disease (CAD)

Coronary artery disease, also known as coronary heart disease (CHD), coronary atherosclerosis, and ischaemic heart disease (IHD), is a branch of cardiovascular disease and is a common form of heart disease (Shahjehan & Bhutta, 2021). CAD is considered a serious disease and a major source of death in countries worldwide, including Malaysia (Figure 2.1) (Ministry of Health Malaysia, 2021b; Wang et al., 2022; WHO, 2021).

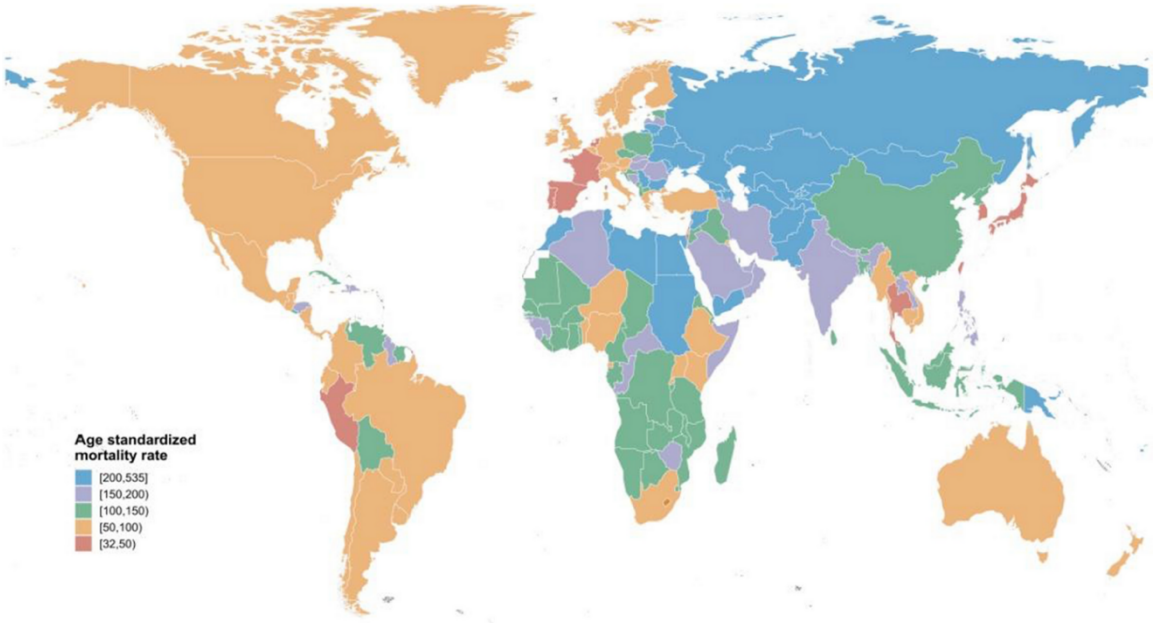

Figure 2.1 Global age- standardised mortality rate per 100,000 people of ischaemic heart disease for both sexes combined in 195 countries and territories in 2017 (Wang et al., 2022)

Coronary artery disease causes more than 7 million deaths yearly and remains the leading worldwide cause of death (WHO, 2021). CAD affects mostly men, and its incidence increases with age (Bots et al., 2017). CAD usually results from a focal thickening of the innermost layer of the coronary arteries, a process known as

atherosclerosis. Then, stenosis occurs in the affected artery causing myocardial ischaemia (Chan & Ramji, 2022; Jebari-Benslaiman et al., 2022). Classification of coronary artery disease is typically done as under stable/ chronic coronary artery disease (SCAD) and acute coronary syndrome (ACS) (Shahjehan & Bhutta, 2021).

2.1.1 Chronic coronary artery disease

The chronic coronary syndrome (CCS) is characterised by recurrent episodes of chest pain and shortness of breath and is defined as a state of coronary artery ischaemia or hypoxia (Katz & Gavin, 2020; Montalescot et al., 2013). Exercise, emotional stress, or other stresses may lead to this condition, which often arises from an imbalance between the heart muscle's metabolic supply and demand (Montalescot et al., 2013). The significant stenosis (narrowing) of the epicardial coronary artery (>50% - 70%) increases coronary blood flow while increasing myocardial oxygen demand. Therefore, coronary artery occlusion caused by coronary atherosclerosis and vasospasm is the main cause of angina pectoris (Montalescot et al., 2013).

The diagnosis and clinical evaluation of coronary artery obstruction require specific diagnostic tests, including anatomical and functional tests (Knuuti et al., 2020). Also, the common risk factors such as diabetes mellitus, hyperlipidemia, and a family history of CAD must be evaluated (Montalescot et al., 2013). In addition, resting ECG must be performed for every patient suspected of having CCS although normal findings cannot exclude the presence of coronary artery stenosis. Dynamic and reversible changes in the ST segment during symptoms can help diagnose vasospasm (Montalescot et al., 2013).

An echocardiography should be performed on all patients who acquire new symptoms. Regional wall movement anomalies raise the possibility of coronary artery

stenosis and may suggest an afflicted vessel. Furthermore, in patients with CCS, left ventricular function is an essential predictive indicator (Montalescot et al., 2013). The gold standard for diagnosing CAD is invasive coronary angiography. Flow fraction reserve (FFR) measures can be used to identify important prognostic parameters such as the severity and location of coronary artery stenosis. Previous research has found that only one-third of patients who undergo invasive coronary angiography for chest discomfort have obstructive CAD (Douglas et al., 2015; Greenwood et al., 2016). As a result, an invasive diagnostic strategy should be considered depending on the severity of symptoms and the risk of a cardiovascular event (Knuuti et al., 2020). Therefore, to improve the diagnostic effectiveness of invasive coronary angiography, rigorous clinical evaluation is required.

2.1.2 Acute coronary syndrome

There are three distinct clinical entities grouped under the term acute coronary syndrome (ACS): unstable angina (UA), ST-elevation myocardial infarction (STEMI) and non-ST elevation myocardial infarction (NSTEMI) (Bergmark et al., 2022). The results of the electrocardiogram (ECG) may provide an indication for treatment differentiation, as shown in Figure 2.2. Since acute coronary occlusion, typically represented by ST elevations in patients with acute symptoms, is associated with high mortality, this disease must be treated immediately (Ibanez et al., 2018).

Patients with NSTEMI—those who do not have ST elevations on their ECG but have elevated troponin levels represent a wide clinical spectrum, ranging from asymptomatic people to those who have suffered cardiac arrest due to ischaemia. Depending on risk factors and clinical presentation, treatment and the urgency of the treatment may differ (Basit et al., 2022; Bergmark et al., 2022).

Unstable angina (UA) is a subtype of ACS that does not exhibit ST elevation, or a biomarker rise (Figure 2.4). However, myocardial ischaemia is not characterised pathophysiologically by cardiac cell death (Bergmark et al., 2022).

When there is acute myocardial ischaemia, cardiomyocytes become necrotic, which is what myocardial infarction (MI) is known for (Thygesen et al., 2012). As seen in the below figure, the identification of MI depends on ischaemic symptoms, alterations in the ECG, and an elevated cardiac biomarker (typically cardiac troponin). MI can be divided into two categories from a pathophysiological standpoint; the first category is characterised by reduced myocardial blood flow, typically brought on by the rupture of an atherosclerotic plaque and the subsequent formation of an intraluminal thrombus, which leads to myocardial necrosis. The second category is caused by myocardial oxygen demand, and supply is out of balance, unrelated to unstable coronary plaque. In addition, anemia, heart arrhythmias, hypotension, and other conditions can contribute to this (Vergallo & Crea, 2020).

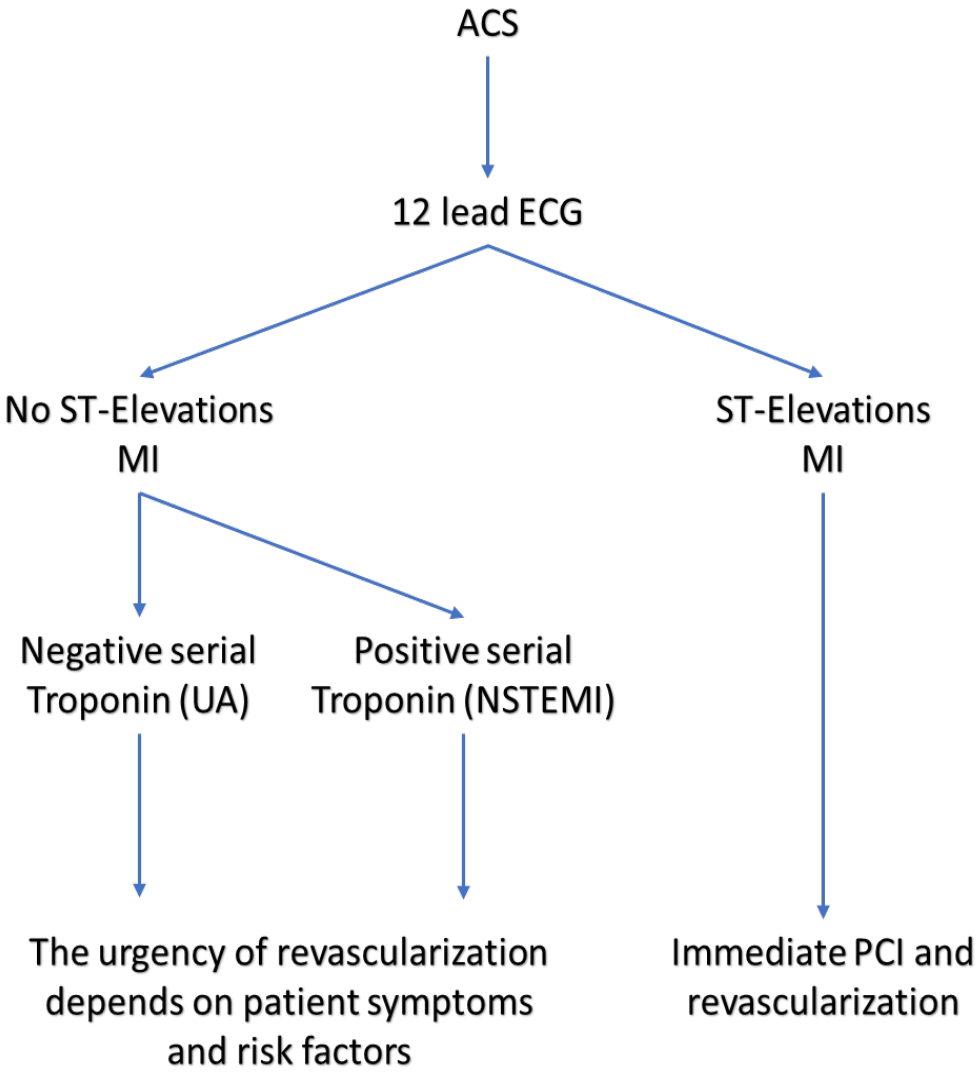

Figure 2.2 Diagnostic flowchart in ACS

2.1.3 Pathophysiology of coronary artery disease

The formation of atherosclerotic plaque is a defining feature of the pathophysiology of CAD. A build-up of fatty substances called plaque causes the artery lumen to become congested and obstructs blood flow. The development of a "fatty streak" is the initial stage of the procedure. Subendothelial deposition of lipid-rich macrophages, which are often called foam cells, causes fatty streaks to form. A vascular injury causes the intima layer to

tear. Monocytes then move into the subendothelial area, where they change into macrophages. Foam cells are created when these macrophages take up oxidized low-density lipoprotein (LDL) particles. T cells get activated, which release cytokines only to aid in the pathologic process. When growth factors are released, smooth muscles become active. These muscles also pick up oxidized LDL particles and collagen, deposit them with activated macrophages, and make more foam cells. This process leads to the formation of subendothelial plaque (Shahjehan & Bhutta, 2021) (Figure 2.3).

This plaque may increase in size over time or become stable. A fibrous cap will form and calcify if it becomes stable. The plaque can become hemodynamically significant enough that insufficient blood reaches the myocardial tissue at increased demand, and symptoms of angina pectoris may occur. However, symptoms will go away at rest as the need for oxygen decreases. For a lesion to cause angina at rest, it must be at least 90% circumscribed. Some plaques can break, which lets tissue factors get into the blood and cause a blood clot. This thrombosis can cause complete or partial obstruction of the lumen. Depending on the level of injury, it can lead to acute coronary syndrome (ACS) in the form of unstable angina, NSTEMI, or STEMI (Nakahara et al., 2017).

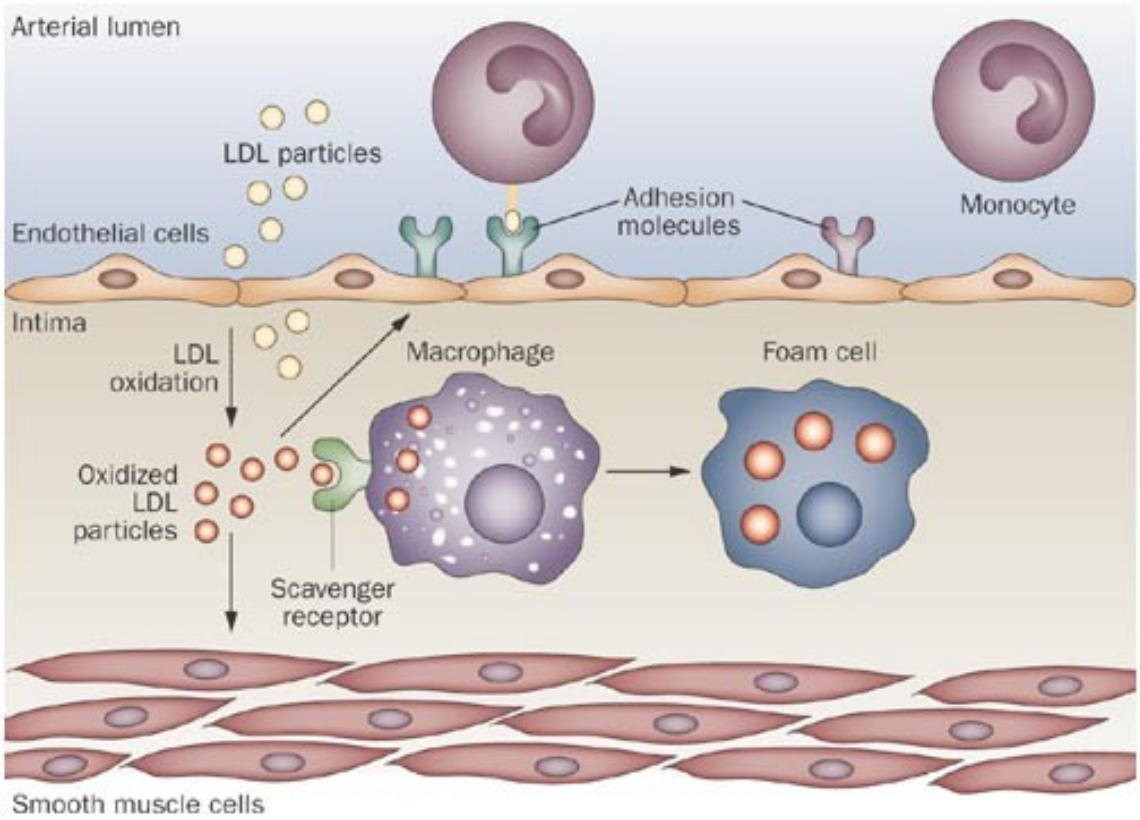

Figure 2.3 Pathophysiology of coronary artery disease (Rocha & Libby, 2009)

2.1.4 Risk factor of coronary artery disease

The common risk factors for CAD include age, gender, and race accounting for 63% to 80% of the prognostic performance (Pencina et al., 2019). The prevalence of CAD increases after 35 years in men and women. The lifetime risk of CAD in men and women after 40 years of age is 49% and 32%, respectively (Sanchis-Gomar et al., 2016). Men are more at risk compared to women, and Blacks, Latinos, Hispanics, and Southeast Asians, are ethnic groups with an increased risk of CAD and mortality (Carnethon et al., 2017; Rodriguez et al., 2014; Volgman et al., 2018). Family history is also a significant risk factor. Patients with early heart disease who are less than 50 years old have an increased risk of death from CAD (Hajar, 2017). Siblings of patients with CVD have a 40% increased

risk, while premature offspring of parents with cardiovascular disease (CVD) have an increased risk of 60% to 75% (Kolber & Scrimshaw, 2014). In addition, hypertension, hyperlipidemia, diabetes mellitus, etc., are common risk factors (Benjamin et al., 2019; Cavero-Redondo et al., 2017; Malakar et al., 2019). Obesity is an independent risk factor for CAD and increases the risk of other coronary heart disease risk factors, including hypertension, hyperlipidemia, and diabetes mellitus (Ades & Savage, 2017) (Figure 2.4)

One recent study found that obese patients were twice as likely to get coronary heart disease (hazard ratio 2.0, 95% confidence interval 1.67–2.40), even after considering demographics, smoking, physical activity, and alcohol consumption, which is also a major risk factor (Ndumele et al., 2016).

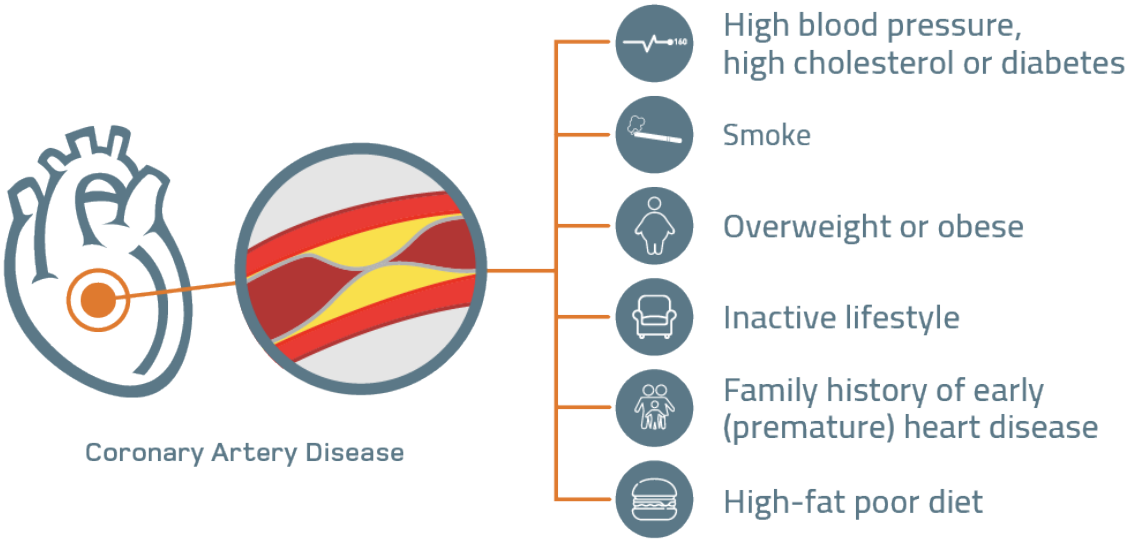

Figure 2.4 Risk factor of coronary artery disease (OpSens Medical, 2022)

2.2 Percutaneous coronary intervention (PCI)

In Malaysia, PCI has become a recognized and popular treatment for patients with atherosclerotic CAD (Lee et al., 2017; Ministry of Health Malaysia, 2009). PCI, which is commonly known as angioplasty with stent, is a non-surgical procedure that uses a catheter, balloon and stent to help to open and widen narrowed or blocked coronary arteries without open-heart surgery (Davis, 2015).

During the process of PCI, a thin flexible hollow tube called the catheter is inserted into the blood vessels, either through the femoral artery or hand radial artery. The catheter is threaded through the blood vessels and is moved up into the affected coronary artery using the help of an X-ray called fluoroscopy. The tip of the catheter contains a tiny balloon covered with a small expandable metallic tube called stent. When the catheter reaches the site of the narrowed or blocked coronary artery, the balloon is inflated to compress the plaques in the coronary artery and hence widen the coronary artery. The stent is expanded

and placed permanently in the coronary artery after the initial balloon dilation. Then, the balloon will be deflated and withdrawn from the coronary artery. This stent can help to reduce the risk of recurrent narrowing or blockage of the coronary artery. Thus, PCI will help to restore and improve the blood flow from the coronary artery to the heart muscles, hence reducing angina and preventing heart attacks (Figure 2.5) (Davis, 2015).

Stents are now commonly used in PCI procedures followed by balloon angioplasty. There are five types of stents available in Malaysia which are bare metal stents (BMS), drug eluting stents (DES), endothelial progenitor cell capture stents, covered stents and biodegradable (bioabsorbable) stents (Ministry of Health Malaysia et al. 2009). BMS and DES were commonly preferred compared to the other stents (Wu et al., 2022). Many studies reported that endothelial progenitor cell capture stents and covered stents are associated with higher rates of MACE and stent-related complications which require further investigations and evaluations (Krackhardt et al., 2018; Lee et al., 2017; Ministry of Health Malaysia, 2009; Wu et al., 2022). However, DES is a more popular option to significantly reduce the risk of restenosis and improve clinical outcomes when compared to BMS (Weiss et al., 2022).

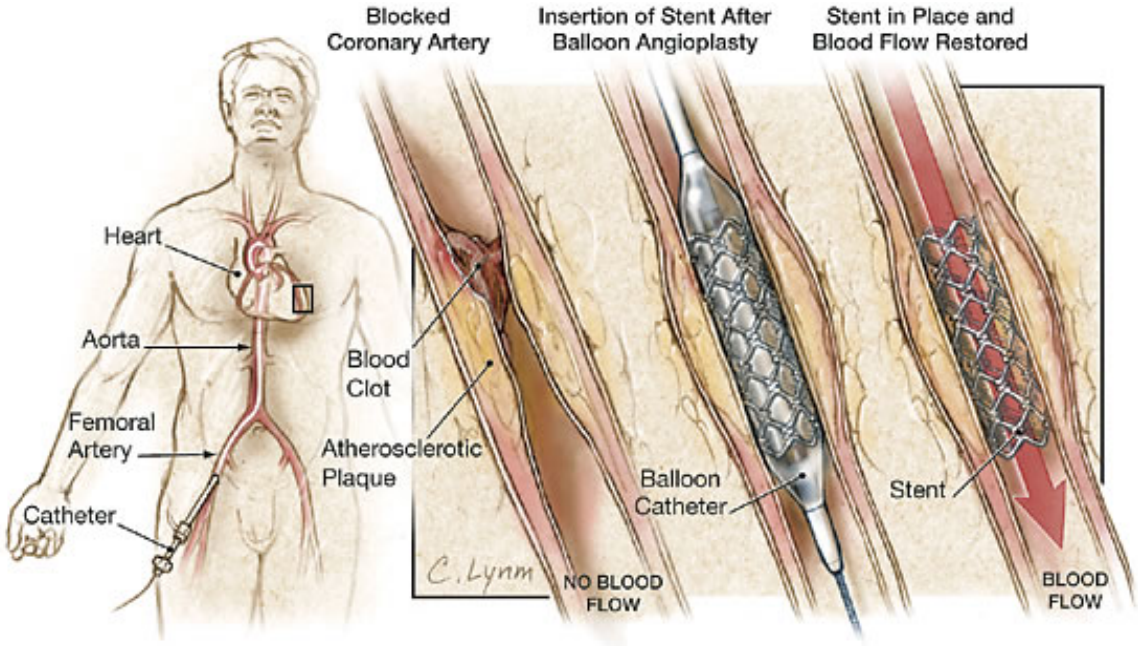

Figure 2.5 Percutaneous Coronary Intervention (PCI) (Medgaget, 2018)

2.3 Platelet activation and thrombosis

Platelets stay in a steady state and are inactive when there is an intact endothelium near the surface. This is because endothelial cells have properties that prevent them from sticking together (Ghoshal & Bhattacharyya, 2014). Damage to the blood vessels turns on these platelets, which stick to the damaged endothelium and interact with leukocytes and the endothelium. These interactions usually act to reduce inflammatory and thrombotic events by secreting cytokines. Leukocytes are attracted to endothelium-associated platelets in a multistep process that involves tethering, rolling, and stable adhesion through integrins. The initial interactions between platelets and leukocytes are mainly attributed to P-selectin. This adhesive molecule is stored in α-granules and, in response to activation signals, translocated to platelet membranes. The main receptor for P-selectin, called P-selectin glycoprotein ligand-1 (PSGL-1), is found on the surface of most leukocytes (Layne et al., 2018). Pools of P-selectin, PSGL-1 and platelet leukocytes modulate atherosclerosis and

may be important biomarkers in identifying patients at increased risk of stroke (Franks et al., 2010). Therefore, the interaction of platelets with leukocytes, vessel walls, and the subendothelial matrix is mediated by integrins, selectins, several platelet receptors, and their respective ligands, some of which are identified in Table 2.1 (Ghoshal & Bhattacharyya, 2014).

Table 2.1 Platelet receptors and their agonists

Platelet receptors	**Platelet agonists**
$P2X_1$, $P2Y_1$, $P2Y_{12}$	ADP
PAR-1, PAR-4, GPIbα	Thrombin
α2A	Epinephrine
TP	Thromboxane A_2
PAF-R	PAF and PAFLL
Subendothelial matrix constituents	
$\alpha_2\beta_1$ (GPIa-IIa)	Collagen
$\alpha_5\beta_1$ (Fibronectin Receptor)	Fibronectin
GPVI	Laminin, Collagen
GPIb-V-IX (GP Ib-IX-V Complex)	VWf
$\alpha_{IIb}\beta_3$ (GP IIb/IIIa)	VWf, fibrinogen, fibronectin

*Original source: (Ghoshal & Bhattacharyya, 2014)

Platelets are activated by thrombin, adenosine diphosphate (ADP), and thromboxane A2 (TXA2). These are all well-known activators of platelets. They attach to the corresponding receptors on the surface of the platelets and start signal transduction, which makes the platelets work (Mederle et al., 2015; Schrottmaier et al., 2015) (Figure 2.6). Of these, thrombin and TXA2 are strong platelet activators that work by binding to the protease-activated receptor (PAR) and the TXA2 receptor (TP), respectively, on the platelet membrane to cause platelets to stick together without granule secretion (de

Stoppelaar et al., 2014). Thrombin turns on PARs by cutting off the extracellular domain of the receptor. This shows a locked, encrypted ligand inside the molecule that binds to the receptor (Vu et al., 1991). ADP primarily binds to the G protein-coupled receptor P2Y12 on the platelet membrane. A high dose of ADP can cause the platelets to release their ADP and granules, causing them to stick together in a way that can't be undone (Thomas & Storey, 2015). The P2Y12 receptor binds to the Gi protein and stops adenylyl cyclase from working. This lowers cyclic adenosine monophosphate (cAMP) and phospho-vasodilator-triggered phosphoprotein levels inside the cell. This, in turn, helps the glycoproteins GPIIb and GPIIIa stick together on the surface of platelets to form the GPIIb/IIIa complex (Rywaniak et al., 2015). The GPIIb/IIIa complex then changes its shape and structure, making a place where fibrinogen can bind. When platelets bind to fibrinogen in the blood, they form a platelet-fibrinogen complex. This causes platelets to stick together, making a stable platelet thrombus (Keane et al., 2010).

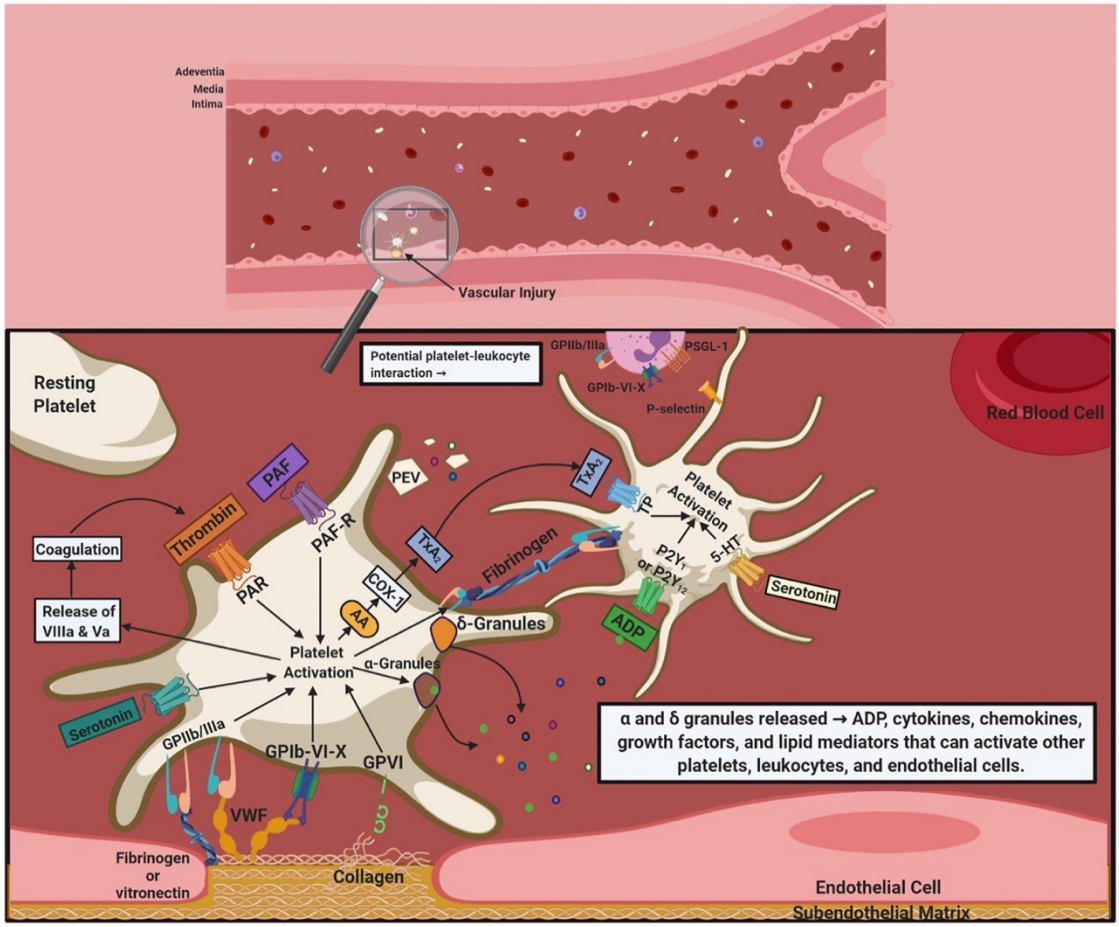

Figure 2.6 Dynamic Reactions in Platelet Activation and Inflammatory Responses to Endothelial Vascular Injury. (Lordan et al., 2021).

2.4 Antiplatelet medications

Antiplatelet medications were produced to change how platelets operate. These medications aim for the inhibition of platelets in atherosclerotic diseases. They constitute an integrated therapeutic approach in the management of cardiovascular diseases.

These currently prescribed medications can be categorised into three groups based on their mechanism of action.

A. Cyclooxygenase inhibitors: Aspirin

B. GPIIb/IIIa Inhibitors: Abciximab, tirofiban, and eptifibatide

C. P2Y12 receptor:

- thienopyridine (clopidogrel, ticlopidine and prasugrel)

Non-thienopyridine (ticagrelor and cangrelor).

2.4.1 Cyclooxygenase inhibitors (Aspirin)

Aspirin is classified as a non-steroidal anti-inflammatory drug, and acetylsalicylic acid is its principal component (Patrono, 1994). It is widely used as an effective antiplatelet agent as it binds to cytochrome c oxidase I (COX1) that is responsible for the production of prostanoids with physiological, protective functions and inhibits thromboxane A2-mediated activation of platelets. Deficient TxA2 synthesis impairs platelet stimulation by other agonists such as ADP and thrombin. Aspirin inhibits platelet aggregation more than adhesion.

Several randomized trials and meta-analyses have confirmed the statistically significant and clinically significant benefits of aspirin use in CAD (Calderone et al., 2022; Gaziano et al., 2018; McNeil et al., 2018). Aspirin is an essential component of antiplatelet therapy in patients undergoing PCI (Chiarito et al., 2020).

The pharmacokinetic and pharmacodynamic effects of aspirin vary in duration. When taken orally, aspirin is absorbed in the stomach and upper intestine, with plasma levels peaking in 30 to 40 minutes (3 to 4 hours if enteric coated). The plasma half-life (t1/2) in the blood is only about 15 to 20 minutes long, which is enough to exert its effect (Patrono et al., 2004). On the other hand, its pharmacodynamic effect lasts much longer because it Inhibits platelet aggregation based on irreversible cyclooxygenase (COX-1) inhibition by acetylating serine, which stops nucleated platelets from working for the rest of their lives (Vane & Botting, 2003). In contrast, nucleated cells such as megakaryocytes and endothelium can inhibit COX-1 resynthesis, and thus inhibiting aspirin in eukaryotic

cells is temporary (Félétou et al., 2011). In addition, since aspirin inhibits TXA2 synthesis but not its actions via the thromboxane receptor, the inhibitory platelet can still be activated by TXA2 released by non-inhibited platelets.

2.4.2 GPIIB/IIIA receptor blockers (abciximab, tirofiban, and eptifibatide)

GPIIB/IIIA drugs such as abciximab, tirofiban, and eptifibatide inhibit the last common pathway of platelet aggregation, preventing the cross-binding of platelets to fibrinogen. These drugs cause potent and effective platelet inhibition with an increased risk of bleeding. Current medications are available in an intravenous form and are used in patients with ACS undergoing PCI (Investigators, 1994). These drugs are used as additional antiplatelet agents along with aspirin and P2Y12 receptor blockers (Peng & Li, 2022; Revilla-Marti et al., 2022).

2.4.3 P2Y12 receptor Blockers

The P2Y12 receptor is a G-protein-bound receptor activated by ADP. ADP binds to the P2Y12 receptor, which leads to the inhibition of adenyl cyclase and decreases intracellular levels of cAMP (cyclic adenosine monophosphate). The cAMP reduction reduces the phosphorylation of vasodilator-stimulated phosphoprotein (VASP) that leads to the activation of the glycoprotein IIb/IIIa receptors (Foye, 2008). Activation of the glycoprotein IIb/IIIa receptors increases thromboxane production and, therefore, platelet aggregation (Damman et al., 2012). These drugs antagonize the P2Y12 platelet receptors, decreasing platelet aggregation and inhibiting thrombus formation.

The group of thienopyridines includes ticlopidine, clopidogrel and prasugrel; these are prodrugs and need to be converted to an active metabolite which causes irreversible inhibition of the P2Y12 receptor. Thienopyridines are metabolised in the liver and the