 Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

Should Aspirin be replaced with ADP blockers and Anti-GPVI to manage thrombosis?

Hafsa Khan[2], Tahira Ghulam[1], Naseer Ahmed[3], Muhammad Rafai Babar[1], Simon D.J. Calaminus[4] and Muhammad Zuhair Yusuf[1*]

Authors

- **Hafsa Khan**
  International Centre for Chemical and Biological Sciences (ICCBS), Pakistan
- **Tahira Ghulam**
  Aga Khan University Medical College, Pakistan
- **Naseer Ahmed**
  Institute of Basic Medical Sciences, Khyber Medical University, Pakistan
- **Muhammad Rafai Babar**
  Aga Khan University Medical College, Pakistan
- **Simon D.J. Calaminus**
  Hull York Medical School, University of Hull, UK
- **Muhammad Zuhair Yusuf ***
  Aga Khan University Medical College, Pakistan

***Corresponding author**: Muhammad Zuhair Yusuf (zuhair.yusuf@aku.edu)
Department of Biological and Biomedical Sciences
The Aga Khan University Medical College,
Stadium Road, P.O.Box 3500, Karachi 74800, Pakistan

Key words:
Platelets, Endothelial dysfunction, Thrombosis, Aspirin, Clopidogrel, Prasugrel, Ticagrelor, Prostacyclin and Thromboxane

Word count: 4984 (limit is 5000)

Abstract:

Platelets have a pivotal role in maintaining cardiovascular homeostasis. They are kept docile by endothelial derived mediators. Aberration in haemostatic balance predisposes an individual to an elevated risk of a pro-thrombotic environment. Anti-platelet therapy has been a key component to reduce this risk. However, understanding how these medications affect the balance between activation and inhibition of platelets is critical. There is now evidence that a key antiplatelet therapy – aspirin, may not be the most efficacious medicine of choice, as it can compromise both platelet inhibition and activation pathways.

In this review the rationale of aspirin as an anti-thrombotic drug has been critically discussed. This review looks at how recent published trials are asking key questions on the efficacy and safety of aspirin in countering cardiovascular diseases. There is an increasing portfolio of evidence that identifies that although aspirin is a very cheap and accessible drug, it may be used in a manner that is not always beneficial to a patient, and a more nuanced and targeted use of aspirin may increase its clinical benefit and maximize patient response.

The questions around the use of aspirin raises the potential for changes in its clinical use for dual anti-platelet therapy. This highlights the need to ensure that treatment is targeted in the most effective manner, and that other anti-platelet therapies may well be more efficacious and beneficial for CVD patients in their standard and personalized approaches.

Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

Introduction:

Cardiovascular disorders (CVD) have a significant impact on global health that results in CVD being the cause for 32% of worldwide mortality (1). It encompasses conditions that impact the heart and blood vessels of the body; such as coronary heart disease, cerebrovascular disease, peripheral arterial disease, congenital heart disease, arrhythmias, deep venous thrombosis and pulmonary embolism. Amongst them coronary heart disease and cerebrovascular disease are a major cause for CVD mortality (1). A key component in the development of CVD is the platelet. The platelet is a blood cell that has an inherent propensity to respond to any damage to the blood vessel and to generate a thrombus which prevents bleeding. However in patients suffering from CVD these thrombi can become too large, or be caused in a pathological manner, and so block the blood vessel, potentially leading to events such as heart attacks and strokes (1).

Management of CVD disorders relies on the patient following a 'healthy lifestyle'. This involves eating a balanced diet while maintaining an age-appropriate weight, maintaining a physically active life and to avoid smoking or using any other tobacco related product. It also encompasses managing the level of stress in life, maintaining adequate sleep, keeping a check on the levels of high and low density cholesterols and maintaining tightly regulated blood sugar levels (2). It is estimated that maintaining a healthy living helps to prevent 80% of CVD cases (2).

The current pharmacological approach to manage CVD includes a list of anti-thrombotic medications that are either prescribed as a mono and/or combination therapy. The anti-platelets are the mainstay for preventive management of CVD implications via preventing platelets from clumping into a clot while anti-coagulants are effective in slowing down clot formation with appended active monitoring requirement (3).

This review will shed light on the current understanding of thrombus and platelet activity. It will then expand on the current use of a key antiplatelet therapy, aspirin along with the controversy that surrounds it. In addition, it will discuss additional anti-platelet therapies such as the P2Y1 and P2Y12

antagonists that are currently used, and potential new therapies under consideration. Finally, the review will consider the need for a more personalized approach to CVD medications.

Functions of 'Endothelium' and 'Platelet' in the vascular system

Critical to a well-functioning cardiovascular system, are two key players - an intact healthy vascular endothelial layer and healthy platelets. The vascular endothelium is a monolayer of cells that lines the vascular luminal surface, preventing the exposure of the thrombogenic collagen that forms the extracellular matrix. Importantly the endothelial layer produces multiple compounds which help to maintain haemostasis. This is possible, as platelets are found circulating in the blood in close proximity to the endothelial lining and therefore can come into contact with these compounds, such as nitric oxide (NO) and prostacyclin (PGI_2) that help to inhibit their function (4, 5). PGI_2 inhibits platelet function by binding to the IP receptor and activating adenylyl cyclase whilst NO diffuses into the platelet and activates soluble guanylyl cyclase. This causes an increase in either the intracellular cAMP or cGMP levels, respectively. These cyclic nucleotides negatively regulate platelet activation by modulating cytoskeletal reorganisation, inhibiting calcium release, restricting degranulation, and limiting expression of platelet receptors required for further activation and thrombus propagation.

In addition to PGI_2 and NO there are a raft of other compounds which are aimed to maintain haemostasis and prevent unwanted thrombus formation (figure 1). On the surface of the vascular endothelium is a glycocalyx that is designed to keep platelets and other cellular elements at bay to avoid getting unwanted activation by prothrombotic collagen of the sub-endothelial matrix (6, 7). In addition, the endothelial cells contain tissue factor and vWF (von Willebrand Factor) in vesicles beneath the cell membrane which are only expressed upon vascular damage. Finally, vascular endothelial cells produce tissue plasminogen activator (tPA) that triggers fibrinolysis by enabling dissolution of unwanted clots, and also express on their luminal surface the thrombin-thrombomodulin complex (8). This complex activates Protein C in the circulation, which in the presence of Protein S, inactivates the coagulation factors V and VIII, thereby limiting activation of the

Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

coagulation cascade (9). Therefore, the endothelial layer plays a key role in both maintaining inactivated platelets, but also in preventing activation of the coagulation system. Damage to the vascular endothelial layer removes these inhibitory systems, and reveals the prothrombotic extracellular matrix proteins, and so drives thrombus formation.

Thrombus architecture

There are several mechanisms by which platelets are maintained in their quiescent state. Damage to the vascular endothelium or exposure to agonists overcomes the inhibitory signal and platelet activation ensues. Platelets respond by the multitude of receptors and several signalling pathways that enable them to respond to these activatory stimuli as had been illustrated in figure 2.

The platelets tether by binding to endothelial released vWF that is immobilized on collagen, via GPIb-IX-V; that results in platelet rolling and promote Glycoprotein VI (GPVI) interaction with collagen. Increased platelet activity promotes integrin expression on the platelet surface and their engagement with fibrinogen and vWF. To reinforce this interaction the platelet actin cytoskeleton is reorganized, and this simultaneously facilitates release of platelet granule contents [ADP, thromboxane (TxA_2) and fibrinogen]. These released mediators potentiate further platelets activation and hence provide additional stability to the formation of the clot.

During thrombus formation, some platelets generate phosphatidylserine (PS) on their surface and engage the coagulation system for generation of thrombin led fibrin meshwork. Changes in this meshwork is a critical factor to ensure that a clot can effectively form and resist the shear forces of the flowing blood (10).

Research has identified a distinct hierarchical architecture within the thrombus with graded platelet activation, shown in figure 2 (11). This structural organization of the thrombus has a distinct central core. As you move away from the core of the thrombus there is a transition zone and an outer shell (11). Platelets forming the core of the clot are under direct influence of thrombin which is marked by their greater activity and increased packing density, which prevents easy access of compounds into the thrombus core. These platelets fully secrete their granules and so help activate platelets within

the periphery. However, the platelets in the periphery (the shell region) are less affected by thrombin and so rely more on the action of ADP and TxA_2 to mediate their activation. This means they are less activated, and that they achieve reduced packing density, thereby enabling this area of the thrombus to be leakier. This is important as PGI_2 and NO can therefore access the thrombus shell region but cannot effectively access the thrombus core. This makes the shell region more susceptible to the reversal of platelet activation by PGI_2 and NO; to induce thrombus instability, lead to embolization, and prevent excessive thrombus growth (12, 13). Importantly in the presence of a prothrombotic environment, especially one with oxidized LDL (oxLDL), thrombus formation is excessive as the oxLDL helps to prevent the inhibition mediated by PGI_2 and NO and therefore the thrombus can grow more effectively (14, 15). The balance between the activatory and inhibitory signalling is key to the establishment of the core and shell regions of the thrombus and as such ensuring that this balance is maintained effectively is key to attaining a graded thrombus organization (11).

Aspirin – a key anti-platelet therapy

As the platelet plays such a key role in CVD, effective targeting of the platelet can therefore have a profound effect on thrombus formation within CVD patients. Historically, a drug therapy commonly prescribed for preventive and emergency protocols for managing CVDs is aspirin.

Aspirin is classified by World Health Organization (WHO) as “one of the essential drugs, inevitable for any basic health system” (16). Importantly, the WHO categorized low and middle income countries as having greater than three quarters of all global CVD mortalities (1). As such aspirin plays a key role within these low- and middle-income countries to treat CVD, especially as these countries may lack the economic capability to prescribe more expensive therapies for CVD.

Impact of aspirin on platelets and endothelium

Aspirin is an irreversible inhibitor of the cyclooxygenase (COX) enzyme. Physiologically the COX enzyme converts arachidonic acid to produce prostaglandin H_2 (PGH_2). The PGH_2 is then processed in a cell specific manner with platelets converting it to the production of TxA_2, while endothelial cells

Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

produce PGI_2. Interestingly, both prostanoids affect platelets in an opposing manner, TxA_2 activates platelets, whereas PGI_2 inhibits them.

The fact that aspirin causes irreversible inhibition of the COX enzyme is key to its clinical use. Platelets on treatment with aspirin are not able to produce new COX enzyme, as they lack the genetic machinery to do so, and so will no longer be able to produce TxA_2. In contrast although aspirin will also inhibit the COX enzyme in the vascular endothelial cells, these cells have the relevant genetic machinery, to replace the inhibited COX enzyme. Therefore, this helps aspirin's effect to be permanent on the platelet, but transient on the endothelial cell (17). This permanent inhibition of TxA_2, reduces the ability of platelet to activate and form a thrombus.

This reduction in PGI_2 and TxA_2 production is critical to fully understand the effect of aspirin on platelets and thrombus formation. Importantly, it needs to be remembered that the inhibition mediated on platelet by PGI_2 and NO is highly effective as it works in a synergistic manner. Therefore, the action of aspirin in reducing PGI_2 levels could be more profound due to the reduction in the synergistic inhibition of platelet by PGI_2 and NO. Interestingly Taubert *et al* indicated an increase in the circulating levels of NO after aspirin use. This may in part compensate for the reduction in PGI_2 levels and so identify why aspirin is antithrombotic (18).

Aspirin has also been identified to reduce the inflammation associated with thrombus formation. This is attributed to the change in TxA_2 production which in turn reduces the effect of Sphingosine-1 phosphate (S1P) (19). S1P, a pro-inflammatory lipid molecule, is produced and secreted by endothelial cells and platelets upon their activation (20). Research identified that aspirin by reducing TxA_2 synthesis helped to protect the endothelial integrity by inhibiting S1P mediated inflammation (21). In addition, aspirin inhibited COX redirects the arachidonic acid substrate to the lipoxygenase pathway. This relays production of 'aspirin triggered lipoxins' (ATL) and 'resolvins' (ATRv) that helps to counter the inflammatory profile via reducing the production of IL1β, IL6, IL8, IL17, TNF-α and reactive oxygen species (ROS) (22, 23). The reduction in ROS production by aspirin also reduces the NLRP3

inflammasome induced damage to endothelial gap junctions and thereby restores normal endothelial permeability (24).

In a healthy endothelium there is a cytoskeletal lattice present beneath the endothelial cell membrane. This actin cytoskeleton helps to stabilize the vascular lining and is continuously influenced by PGI_2 and NO (25, 26). With an increase in the shear of the flowing blood that can occur within CVD patients, pressure is exerted on the endothelium, thereby activating COX enzymes and eNOS (endothelial nitric oxide synthase) to produce PGI_2 and NO, respectively (27). These mediators synergize to reinforce the actin cortical lattice in endothelial cells to withstand the shearing force of blood flow (figures 1 and 2)(28). Therefore aspirin, by inhibiting PGI_2 production, would also challenge the endothelial protection by limiting the cytoskeletal thickening, thereby rendering the endothelium less able to withstand high shear. With the passage of increased flow of blood, aspirin induced lack of adaptive actin reinforcement would result in damaging the endothelium (29). Apart from possible denudation, the endothelial damage could result in the release of tissue factor that converts prothrombin into thrombin - a strong platelet activator, or expulsion of vWF from the endothelial stores of Weibel Palade bodies that enables circulating platelets to roll and spread on the collagen matrix to initiate clot formation (30, 31). Furthermore, a lack of endothelial integrity decreases the inhibitory influence of the thrombin-thrombomodulin complex and the associated Protein C and Protein S along with reduced production of tPA; thereby predisposing for further platelet activation and thrombus formation.

Therefore, understanding how aspirin affects the production of TxA_2 versus PGI_2 is critical to understanding if it could have an antithrombotic or prothrombotic effect. Depending on the extent to which these mediators traverse the thrombus and the impact they produce on the activity of platelets would then feed-in to control the height, graded structure, and the extent of thrombus (11).

Complexities of using aspirin

A key part of understanding the potential therapeutic benefit of aspirin is the dose at which it is used. It can be used both in a low and high dose form, although preventive cardiology recommends the

Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

usage of 75mg/day of aspirin, which is akin to low dose aspirin therapy (32). High dose aspirin has been used to relieve pain, temperature and swelling along with enhanced inhibition of TxA_2 production (33). However, it also led to a significant reduction in PGI_2 production from the vascular endothelial cells (34). This led the regulatory authorities to shift to using low dose aspirin as it generated a comparable anti-platelet effect to that produced at higher doses of aspirin (32). The low dose aspirin was also believed not to impact the level of the antithrombotic compound-PGI_2 (35). However, later published research showed that even low doses of aspirin also impact both TxA_2 and PGI_2, with a greater inhibiting influence on TxA_2, which then underpins why low dose aspirin is thought to have an anti-platelet effect (36). Importantly, TxA_2 inhibition should at least be 95% for aspirin to be effective as the remaining COX could compensate to produce TxA_2 and reclaim the patient prothrombotic profile. This highlights the need for an individual corrected dosing for patients, while considering the factors of aspirin resistance along with patient compliance.

However, recently the use of aspirin as an antithrombotic has been shown to have mixed effects. The Anti-Thrombotic Trial (ATT) identified that the use of aspirin was not beneficial for all patients, and in fact may induce a prothrombotic phenotype (37). Similarly a meta-analysis by Guirguis-Blake *et al*. stressed a negligible and ineffective response of aspirin use in reducing the risk of cardiovascular mortality (38). These claims were strengthened by major clinical trials – Aspirin to Reduce Risk of Initial Vascular Events (**ARRIVE**), A Study of Cardiovascular Events in Diabetes (**ASCEND**) and Aspirin in Reducing Events in the Elderly (**ASPREE**) that probed the effectiveness of aspirin use in CVD (39-41).

The ARRIVE trial enrolled moderate risk CVD patients and identified aspirin to increase bleeding whilst there was no change in major adverse cardiovascular events (MACE) (40). Similarly, ASPREE, a study conducted on patients aged 70 years old or more, also showed no change in MACE but there was an increase in bleeding tendency and mortality (39). The ASCEND trial was conducted on diabetic patients and showed a reduction in MACE but led to higher bleeding rates (41). Their data identified that prescribing aspirin to different patient groups could induce varying thrombotic responses and challenged its reliance during emergency, primary and secondary prevention for CVD conditions.

Importantly, the age of a patient and the combined risk of developing CVD have critical significance. The current recommendation for primary prevention has a limited age range for aspirin prescription; targeted patients should be aged 40 to 59 years and have a high 10-years risk of CVD development (42). As for secondary prevention and emergency measures of CVD, aspirin still is considered effective, but the increased bleeding risk lingers (43).

All these factors, along with aspirin resistance, highlights the need to develop a nuanced and personalised approach of anti-platelet therapy that should have greater effectiveness and capability of dealing with different patient groups (44). With revised regulations by the United States Preventive Service Task Force (USPSTF) the previously allowed age group of 60 to 69 years have now been excluded for starting aspirin (45). The stricter guidelines necessitate the caution that needs to be advised by weighing the ratio of benefits to harm, in accordance with the dose of aspirin to be used.

Alternative anti-platelet medication avenues

Finding an effective medication to manage thrombotic conditions is the key to lessen the reliance on aspirin for an anti-platelet regimen. Due to use of long-term aspirin monotherapy under question, additional therapies were sought and researched for their potential to work either as monotherapy or as combinations in dual anti-platelet therapy (DAPT).

The 2016 ACC/AHA guidelines for DAPT use comprised of low dose aspirin with $P2Y_{12}$ antagonists. The combination lasted for 12 months and followed indefinitely by aspirin monotherapy (46). Although, DAPT showed better clinical outcomes, but continued use of aspirin even at low doses was questionable due to the bleeding and endothelial cell dysfunction tendency. This potentially inclined the patient to an unknown level of thrombotic risk (47). This prompted ADP blockers and anti-GPVI to be included in the DAPT and reduce the thrombotic risk.

The ADP binds to the receptors; $P2Y_1$ and $P2Y_{12}$, with a significant contribution from $P2Y_{12}$. They potentiate platelet activation via engaging downstream Ca^{2+} mobilization and granule secretion along with inhibiting the PGI_2 - cAMP axis (figure 3) (48). Interestingly recent DAPT guidelines have been proposed where a month of DAPT (aspirin and clopidogrel) is followed by a 12-month clopidogrel

 Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

monotherapy for ACS patients (49). This shift in guidelines highlight the potential need and strengthens the case for finding improved therapies.

The search for improved ADP receptor blockers includes prasugrel that, similar to clopidogrel, require prior activation by liver while ticagrelor have a quicker mode of action, as it does not need activation. The half-life of clopidogrel and prasugrel lasts the lifespan of the platelet (10-14 days), therefore they act as irreversible inhibitors while ticagrelor binds for 3-5 days and is therefore classified as a reversible inhibitor (50). Ticagrelor reaches a maximum plasma concentration in 120 minutes due to its lower bioavailability, while clopidogrel requires 60 minutes and prasugrel 30 minutes only (50). Considering both prior activation and attainment of maximum plasma concentration, ticagrelor and prasugrel are comparable to achieve maximum platelet inhibition at 2 hours and 3 hours, respectively; while clopidogrel necessitate an 8 hours interval to achieve maximal impact on platelets (50).

Johnston SC *et al*, published a clopidogrel and aspirin comparison with 75 mg clopidogrel daily to significantly lower annual rate of vascular death, myocardial infarction, or ischemic stroke (51). The delayed onset of clopidogrel along with the concept of clopidogrel resistance however caused an increase in the risk of post-PCI thrombus development (52). This identified the need to compare ticagrelor, a quicker action ADP receptor blocker, with aspirin on high-risk patients with ACS in the TWILIGHT trial. It identified that ticagrelor alone or in combination with aspirin, reduced bleeding tendency along with no higher risk of myocardial infarction or stroke (53). Contrastingly, the PRINCE trial reported similar rates of overall major bleeding along with significant reduction of cardiovascular and all-cause mortality comparing a combination of aspirin and ticagrelor versus aspirin and clopidogrel; (54). These varied evidence for major bleeds (as classified by TIMI criteria) highlighted a potential variation in response to ticagrelor which was dependent on the patient cohort.

Ticagrelor and prasugrel have shown better outcomes on comparing it with clopidogrel or aspirin. They have comparable efficacies and safety profiles, with prasugrel having a slight increase in bleeding tendency (55). Ticagrelor, on the other hand had mild dyspnoea and ventricular pauses as identified

in the DISPERSE, DISPERSE-2 and the ONSET/OFFSET trials comparing ticagrelor with clopidogrel in patients with coronary artery disease (56).

Supplementary to the recognised effects of ticagrelor to cause direct platelet inhibition; it also resulted in adenosine uptake inhibition by cells that led to an increased plasma adenosine concentration that prompted further inhibition of platelets (57). In addition, ticagrelor caused an increased production of NO and PGI_2, that promotes a healthy endothelium (58). Although with potential clinical variation, the ability of ticagrelor as an anti-platelet does place it a notch above other ADP receptor blockers in the market.

Continuing research on ADP $P2Y_{12}$ receptor blockers have developed their anti-thrombotic impact. Ticagrelor and newer medications in the same drug class have been developed, such as Cangrelor, with improved potency and effectiveness for new patients after PCI (59). Similarly Vicagrel – an analogue of clopidogrel, with far greater efficacy is under clinical development (60). Selatogrel, another $P2Y_{12}$ receptor blocker is under development, with subcutaneous administration and less off-target effects (61). Selatogrel is identified to be effective for managing acute cardiovascular events due to its rapid action along with reduced bleeding risk (61).

In addition to the ADP receptors as potential targets for anti-platelet therapy, the collagen receptor – GPVI is also emerging as a potential anti-platelet target to prevent thrombosis and stroke (figure 3). GPVI has been shown to bind to various extracellular matrix proteins, such as fibrinogen, fibrin, laminin, fibronectin, and collagen (62, 63). GPVI has an important role in the high shear environment of arterial thrombosis, although there is increasing evidence that GPVI may also play a role in venous thrombus formation (64). Furthermore, GPVI has also been reported recently to be overexpressed in stroke patients (65). However, GPVI deficiency, had a minimal impact on bleeding as it did not affect haemostasis (64), potentially as its role can be compensated by vWF or Thrombin. This therefore means that by targeting GPVI you can effectively reduce arterial thrombosis whilst potentially reducing the bleeding diathesis associated with other anti-platelet therapies. However, a combination of GPVI Fc antibodies, which block GPVI signalling, alongside aspirin or $P2Y_{12}$ antagonists could be

 Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

beneficial, as this prevents atherosclerotic plaque mediated thrombus formation without elevating unwanted bleeding (66).

Multiple anti-GPVI approaches have been considered that either inhibit the GPVI receptor or downregulate GPVI surface expression such as that observed in mice with antibodies - JAQ1 and/or activate GPVI cleavage enzymes (67-69). Table 1 expands on the anti- GPVI compounds of which Revacept, Glenzocimab, and DZ-697b have proceeded to clinical trials (Phase I or II).

Glenzocimab (ACT017) is a humanised antibody fragment of the O912 antibody used to target mouse GPVI. It is a selective and reversible inhibitor of the GPVI receptor. ACT017 completed a phase 1 placebo-controlled study in 2019 and showed a favourable safety profile (69). It has since proceeded into Phase II and III trials – ACTIMIS and ACTISAVE, respectively, which are being carried out on patients with acute ischemic stroke (69, 70).

Revacept, is a GPVI-Fc fusion protein that lacks downstream signalling. It competes with GPVI present on platelets, for the exposed collagen binding sites in damaged vascular endothelium. By blocking the ability of the GPVI expressed on the surface of the platelet to bind collagen it can then reduce platelet adhesion and aggregation (71, 72). Unfortunately, addition of revacept did not reduce myocardial injury in low-risk PCI patient already on DAPT in the phase II ISAR_PLASTER trial for improving the anti-thrombotic risk (66, 72-74). However, there is potential that as only low risk patients were used on this trial, revacept may have clinical relevance in higher risk groups. However, it is also being evaluated within patients who have had a transient ischemic attack, or a stroke due to carotid artery stenosis. It will be interesting to see how revacept affects this patient cohort given the lack of effect within the PCI patient group.

A distinct downside with both Revacept and Glenzocimab is that as antibody therapies the drug delivery mechanism requires intravenous injection. This will possibly limit the compliance as many patients take anti-platelet therapy outside a clinical environment. Therefore, the development of a GPVI oral inhibitor would be of great benefit.

DZ-697b is an oral antagonist of GPVI, that inhibits Fc g chain phosphorylation by collagen. Usefully it is not a prodrug and therefore does not require metabolism to its active ingredient. Phase I trials of the compound identified its potential as an anti-platelet therapy as it showed a reduced risk of bleeding in comparison to clopidogrel and aspirin (75, 79). However, the compound has not progressed into phase II trials at present.

Mutalysin II is a snake venom that cleaves both GPVI and the vWF receptor GP1b. As it cleaves GPVI it therefore blocks associated signalling and reduce GPVI mediated platelet aggregation (78). It Is proposed that it has good potential as an anti-platelet but it needs further investigation to see if it can progress into clinical trials.

Although current anti-platelet therapies have shown their ability to manage CVD effectively, an increased bleeding tendency persists as a common side effect. International guidelines have thereby recommended careful evaluation of bleeding risks along with assessing benefit to harm ratio for continuing with the medicament. Moreover, consideration needs to be drawn to other patient factors; such as anaemia, low body weight and chronic kidney disease that imparts an increased risk of major bleeding, as had been appreciated in TICO randomized trial (80).

 Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

Personalised anti-platelet therapy

The idea of personalised anti-platelet therapy is potentially highly useful in CVD patients. It is becoming clearer that there are patients resistant to different anti-platelet therapies, for several different reasons (age, genetic polymorphisms, BMI). Therefore, there is a need to target therapy more effectively to prevent unwanted bleeding, ineffective therapeutic responses, and maximise cost effectiveness of anti-platelet therapy.

There are several different genes (CYP2C8, CYP2C9, CYP2C18, CYP2C19*2, CYP2C19*3, CYP2C19*17, SLCO1B1, UGT2B7, and CYP3A4) that have been associated with ineffective patient responses to aspirin, clopidogrel, prasugrel and ticagrelor (81-83). These therefore have the potential to be used to help screen patients to aide personalised therapy. This notion is under consideration, as the need to assess the extent of anti-platelet impact of these medications is of prime concern. Their sensitivity, specificity is still being screened for worldwide usability, and secondly cost implications too would pose a major hurdle as lower middle-income countries are homing the major brunt of the disease.

Of the identified genes, CYP polymorphisms have been the most investigated. Loss of function CYP450 polymorphisms, such as CYP2C19 are important especially as clopidogrel is a prodrug and therefore requires metabolism in the liver to produce the active metabolite. However, although there is little dispute over the ability of clopidogrel to induce high platelet reactivity in a subset of patients, the reasons for this are still in dispute. The FDA have indicated that patients that metabolise clopidogrel poorly should preferably be treated with other anti-platelet therapies (84). However, trials that have investigated the use of a personalise therapy based on CYP450 polymorphism have had variable outcomes. The most recent trial (TAILOR-PCI) was unclear in how personalised therapy would benefit CVD patients (85). There were indications the study was underpowered, but overall, it indicated that there was little benefit to a personalised therapy approach based upon a pharmacogenetics approach to CYP450 Loss of function mutations. However interestingly further analysis of this trial dataset has indicated that using the ABCD-GENE score could prove to be beneficial in separating different patient groups (86).

The conceptual need of a personalized medication schedule stands to reason. Further research is starting to identify subsets of patients that could carry further genetic polymorphisms or are part of specific patient subsets that would potentially benefit from this personalised approach (87). Supplementary investigations are required to fully understand the benefit of a personalised therapeutic approach to anti-platelet therapy.

Conclusion:

Aspirin is a drug which all can effectively access. However, although aspirin continues to be used in the clinic as a key therapy for managing cardiovascular and thrombotic pathologies, its use is now being challenged. There are questions about aspirin suitability for all age groups? the most beneficial dose to be used? potential issues around how aspirin works within different patient cohorts? and the benefits of longterm aspirin? This review identifies the potential benefits of newer therapies, such as ticagrelor, prasugrel and anti-GPVI drugs. Furthermore, there is a need for a more nuanced, personalised approach for anti-platelet therapy that can effectively balance the bleeding and anti-thrombotic risk of these therapies.

Aspirin along with other antiplatelet medications need careful consideration and individual assessment for identifying the therapeutic advantage in dealing with thrombotic risk. This highlights that it would be beneficial to weigh up the merits of the available medications and to ensure a targeted personalized treatment in the most effective manner, whether to be used as monotherapy or as DAPT or to be replaced by other anti-platelet medication combinations, whilst addressing emergency thrombotic situations or managing preventive CVD outcomes.

Declarations:

- The authors declare they have no financial or any other potential conflict of interest that could be perceived as prejudicing the impartiality of this review.
- This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

 Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

- The contribution of authors includes HK and MZY to conceive the review and to make figures. HK, TG, NA, SC and MZY, have made substantial contribution to draft the write-up. SC, HK and MRB extended the write-up and helped revise it. All authors have read and approved the final manuscript.

Abbreviations:

ACS	Acute coronary syndrome
ACTIMIS	Acute Ischemic Stroke Interventional Study
ADP	Adenosine Di-Phosphate
ARRIVE	Aspirin to Reduce Risk of Initial Vascular Events
ASCEND	A Study of Cardiovascular Events in Diabetes
ASPREE	Aspirin in Reducing Events in the Elderly
ATL	Aspirin triggered lipoxins
ATRv	Aspirin triggered resolvins
ATT	Anti-Thrombotic Trial
CAPRIE	Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events
CLARITY	Clopidogrel use in the setting of ST elevation MI
COX	Cyclooxygenase
CURE	Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial
CVD	Cardiovascular disorders
DAPT	Dual Anti-Platelet Therapy
eNOS	Endothelial nitric oxide synthase
GPVI	Glycoprotein VI
NO	Nitric oxide
PCI	Percutaneous Coronary Intervention
PGI_2	Prostacyclin
PLATO	Study of Platelet Inhibition and Patient Outcomes
PRINCE	Platelet Reactivity in Acute Stroke or Transient Ischaemic Attack
PS	Phosphatidylserine
ROS	Reactive oxygen species
tPA	Tissue plasminogen activator
TxA_2	Thromboxane
vWf	von Willebrand Factor

References:

1. World-Health-Organisation. Cardiovascular diseases (CVDs) France: Studio FFFOG; 2017 [Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
2. School HM. Four keys to prevent cardiovascular disease. Heart Health [Internet]. 2019. Accessed on 03 December 2021. Available from: https://www.health.harvard.edu/heart-health/four-keys-to-prevent-cardiovascular-disease.
3. Health NIo. Blood Thinners2015 Accessed on 03 December 2021. Available from: https://medlineplus.gov/bloodthinners.html.
4. Inagami T, Naruse M, Hoover R. Endothelium - as an Endocrine Organ. Annu Rev Physiol. 1995;57:171-89.
5. Nakayama T. Prostacyclin analogues: prevention of cardiovascular diseases. Cardiovasc Hematol Agents Med Chem. 2006;4(4):351-9.
6. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115(10):1285-95.
7. Pries AR, Secomb TW, Gaehtgens P. The endothelial surface layer. Pflugers Arch. 2000;440(5):653-66.
8. Lin L, Hu K. Tissue Plasminogen Activator: Side Effects and Signaling. J Drug Des Res. 2014;1(1).
9. Fuentes-Prior P, Iwanaga Y, Huber R, Pagila R, Rumennik G, Seto M, et al. Structural basis for the anticoagulant activity of the thrombin-thrombomodulin complex. Nature. 2000;404(6777):518-25.
10. Kattula S, Byrnes JR, Wolberg AS. Fibrinogen and Fibrin in Hemostasis and Thrombosis. Arterioscler Thromb Vasc Biol. 2017;37(3):e13-e21.
11. Stalker TJ, Traxler EA, Wu J, Wannemacher KM, Cermignano SL, Voronov R, et al. Hierarchical organization in the hemostatic response and its relationship to the platelet-signaling network. Blood. 2013;121(10):1875-85.
12. Atkinson L, Yusuf MZ, Aburima A, Ahmed Y, Thomas SG, Naseem KM, et al. Reversal of stress fibre formation by Nitric Oxide mediated RhoA inhibition leads to reduction in the height of preformed thrombi. Sci Rep-Uk. 2018;8(1):3032.
13. Yusuf MZ, Raslan Z, Atkinson L, Aburima A, Thomas SG, Naseem KM, et al. Prostacyclin reverses platelet stress fibre formation causing platelet aggregate instability. Sci Rep-Uk. 2017;7(1):5582.
14. Magwenzi S, Woodward C, Wraith KS, Aburima A, Raslan Z, Jones H, et al. Oxidized LDL activates blood platelets through CD36/NOX2-mediated inhibition of the cGMP/protein kinase G signaling cascade. Blood. 2015;125(17):2693-703.
15. Higgs EA, Higgs GA, Moncada S, Vane JR. Prostacyclin (PGI2) inhibits the formation of platelet thrombi in arterioles and venules of the hamster cheek pouch. Br J Pharmacol. 1978;63(3):535-9.
16. Mikulic M. Bayer - Statistics & Facts. Germany; 2019.
17. Jaffe EA, Weksler BB. Recovery of endothelial cell prostacyclin production after inhibition by low doses of aspirin. J Clin Invest. 1979;63(3):532-5.
18. Taubert D, Berkels R, Grosser N, Schroder H, Grundemann D, Schomig E. Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. Br J Pharmacol. 2004;143(1):159-65.
19. Ulrych T, Bohm A, Polzin A, Daum G, Nusing RM, Geisslinger G, et al. Release of sphingosine-1-phosphate from human platelets is dependent on thromboxane formation. J Thromb Haemost. 2011;9(4):790-8.
20. Yatomi Y, Igarashi Y, Yang L, Hisano N, Qi R, Asazuma N, et al. Sphingosine 1-phosphate, a bioactive sphingolipid abundantly stored in platelets, is a normal constituent of human plasma and serum. J Biochem. 1997;121(5):969-73.
21. Vito CD, Hadi LA, Navone SE, Marfia G, Campanella R, Mancuso ME, et al. Platelet-derived sphingosine-1-phosphate and inflammation: from basic mechanisms to clinical implications. Platelets. 2016;27(5):393-401.

Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

22. Eickmeier O, Seki H, Haworth O, Hilberath JN, Gao F, Uddin M, et al. Aspirin-triggered resolvin D1 reduces mucosal inflammation and promotes resolution in a murine model of acute lung injury. Mucosal Immunol. 2013;6(2):256-66.
23. Pirault J, Back M. Lipoxin and Resolvin Receptors Transducing the Resolution of Inflammation in Cardiovascular Disease. Front Pharmacol. 2018;9:1273.
24. Zhou X, Wu Y, Ye L, Wang Y, Zhang K, Wang L, et al. Aspirin alleviates endothelial gap junction dysfunction through inhibition of NLRP3 inflammasome activation in LPS-induced vascular injury. Acta Pharm Sin B. 2019;9(4):711-23.
25. Baldwin AL, Thurston G, al Naemi H. Inhibition of nitric oxide synthesis increases venular permeability and alters endothelial actin cytoskeleton. Am J Physiol. 1998;274(5):H1776-84.
26. Shen Q, Wu MH, Yuan SY. Endothelial contractile cytoskeleton and microvascular permeability. Cell Health Cytoskelet. 2009;2009(1):43-50.
27. Ando J, Yamamoto K. Flow detection and calcium signalling in vascular endothelial cells. Cardiovasc Res. 2013;99(2):260-8.
28. Hamilos M, Petousis S, Parthenakis F. Interaction between platelets and endothelium: from pathophysiology to new therapeutic options. Cardiovasc Diagn The. 2018;8(5):568-80.
29. Hunt BJ, Jurd KM. Endothelial cell activation. A central pathophysiological process. BMJ. 1998;316(7141):1328-9.
30. Lopez JA, Dong JF. Shear stress and the role of high molecular weight von Willebrand factor multimers in thrombus formation. Blood Coagul Fibrinolysis. 2005;16 Suppl 1:S11-6.
31. Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. Arterioscler Thromb Vasc Biol. 2004;24(6):1015-22.
32. Antithrombotic Trialists C. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ. 2002;324(7329):71-86.
33. Ugurlucan M, Caglar IM, Caglar FN, Ziyade S, Karatepe O, Yildiz Y, et al. Aspirin: from a historical perspective. Recent Pat Cardiovasc Drug Discov. 2012;7(1):71-6.
34. Hall HM, de Lemos JA, Enriquez JR, McGuire DK, Peng SA, Alexander KP, et al. Contemporary patterns of discharge aspirin dosing after acute myocardial infarction in the United States: results from the National Cardiovascular Data Registry (NCDR). Circ Cardiovasc Qual Outcomes. 2014;7(5):701-7.
35. Hanley SP, Bevan J, Cockbill SR, Heptinstall S. Differential inhibition by low-dose aspirin of human venous prostacyclin synthesis and platelet thromboxane synthesis. Lancet. 1981;1(8227):969-71.
36. Davi G, Custro N, Novo S, Mattina A, Strano A. The effect of two low doses of aspirin on whole blood thromboxane and prostacyclin generation in healthy subjects. Thromb Haemost. 1983;50(3):669-70.
37. Antithrombotic Trialists C, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373(9678):1849-60.
38. Guirguis-Blake JM, Evans CV, Senger CA, O'Connor EA, Whitlock EP. Aspirin for the Primary Prevention of Cardiovascular Events: A Systematic Evidence Review for the U.S. Preventive Services Task Force. Ann Intern Med. 2016;164(12):804-13.
39. Group AI. Study design of ASPirin in Reducing Events in the Elderly (ASPREE): a randomized, controlled trial. Contemp Clin Trials. 2013;36(2):555-64.
40. Gaziano JM, Brotons C, Coppolecchia R, Cricelli C, Darius H, Gorelick PB, et al. Use of aspirin to reduce risk of initial vascular events in patients at moderate risk of cardiovascular disease (ARRIVE): a randomised, double-blind, placebo-controlled trial. Lancet. 2018;392(10152):1036-46.
41. Group ASC, Bowman L, Mafham M, Wallendszus K, Stevens W, Buck G, et al. Effects of Aspirin for Primary Prevention in Persons with Diabetes Mellitus. N Engl J Med. 2018;379(16):1529-39.
42. Spencer FA, Guyatt G. Who should take aspirin for Primary Prevention? 2021. In: Patient education: Aspirin in the primary prevention of cardiovascular disease and cancer (Beyond the Basics)

[Internet]. Wolters Kluwer. Available from: https://www.uptodate.com/contents/aspirin-in-the-primary-prevention-of-cardiovascular-disease-and-cancer-beyond-the-basics#H371637445.
43. Jacobsen AP, Raber I, McCarthy CP, Blumenthal RS, Bhatt DL, Cusack RW, et al. Lifelong Aspirin for All in the Secondary Prevention of Chronic Coronary Syndrome: Still Sacrosanct or Is Reappraisal Warranted? Circulation. 2020;142(16):1579-90.
44. Godoy LC, Farkouh ME. Personalised Approaches to Improving the Effect of Anti-platelet Agents: Where Do We Stand? Eur Cardiol. 2019;14(3):179-80.
45. Bibbins-Domingo K, Force USPST. Aspirin Use for the Primary Prevention of Cardiovascular Disease and Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med. 2016;164(12):836-45.
46. Levine GN, Bates ER, Bittl JA, Brindis RG, Fihn SD, Fleisher LA, et al. 2016 ACC/AHA Guideline Focused Update on Duration of Dual Antiplatelet Therapy in Patients With Coronary Artery Disease. Journal of the American College of Cardiology. 2016;68(10):1082-115.
47. Yeh RW. Thirty months of DAPT linked to better outcomes in stable, unstable patients2015. Available from: https://www.healio.com/news/cardiology/20150316/dapt-analysis-30-months-of-dapt-linked-to-better-outcomes-in-stable-unstable-patients
48. Leon C, Ravanat C, Freund M, Cazenave JP, Gachet C. Differential involvement of the P2Y1 and P2Y12 receptors in platelet procoagulant activity. Arterioscler Thromb Vasc Biol. 2003;23(10):1941-7.
49. Park DY, An S, Kumar A, Malhotra S, Jolly N, Kaur A, et al. Abbreviated versus Standard Duration of DAPT after PCI: A Systematic Review and Network Meta-analysis. American Journal of Cardiovascular Drugs. 2022.
50. Teng R. Ticagrelor: Pharmacokinetic, Pharmacodynamic and Pharmacogenetic Profile: An Update. Clin Pharmacokinet. 2015;54(11):1125-38.
51. Johnston SC, Easton JD, Farrant M, Barsan W, Conwit RA, Elm JJ, et al. Clopidogrel and Aspirin in Acute Ischemic Stroke and High-Risk TIA. N Engl J Med. 2018;379(3):215-25.
52. Gurbel PA, Bliden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol. 2005;46(10):1820-6.
53. Mehran R, Baber U, Sharma SK, Cohen DJ, Angiolillo DJ, Briguori C, et al. Ticagrelor with or without Aspirin in High-Risk Patients after PCI. N Engl J Med. 2019;381(21):2032-42.
54. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2009;361(11):1045-57.
55. Schupke S, Neumann FJ, Menichelli M, Mayer K, Bernlochner I, Wohrle J, et al. Ticagrelor or Prasugrel in Patients with Acute Coronary Syndromes. N Engl J Med. 2019;381(16):1524-34.
56. Rosa GM, Bianco D, Valbusa A, Massobrio L, Chiarella F, Brunelli C. Pharmacokinetics and pharmacodynamics of ticagrelor in the treatment of cardiac ischemia. Expert Opin Drug Metab Toxicol. 2016;12(12):1491-502.
57. Cattaneo M, Schulz R, Nylander S. Adenosine-mediated effects of ticagrelor: evidence and potential clinical relevance. J Am Coll Cardiol. 2014;63(23):2503-9.
58. Reiner M.F., Stivala S., Akhmedov A, Spescha R.D., Savaerese G., Luescher T.F., et al. Cell-specific off-target effects of ticagrelor but not clopidogrel-active metabolite in endothelial dysfunction. European heart journal. 2014;35.
59. Fugate SE, Cudd LA. Cangrelor for treatment of coronary thrombosis. Ann Pharmacother. 2006;40(5):925-30.
60. Qiu Z, Li N, Wang X, Tian F, Liu Q, Song L, et al. Pharmacokinetics of vicagrel, a promising analog of clopidogrel, in rats and beagle dogs. J Pharm Sci. 2013;102(2):741-9.
61. Crescence L, Darbousset R, Caroff E, Hubler F, Riederer MA, Panicot-Dubois L, et al. Selatogrel, a reversible P2Y12 receptor antagonist, has reduced off-target interference with haemostatic factors in a mouse thrombosis model. Thromb Res. 2021;200:133-40.
62. Bergmeier W, Hynes RO. Extracellular matrix proteins in hemostasis and thrombosis. Cold Spring Harb Perspect Biol. 2012;4(2).

Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

63. Xu RG, Gauer JS, Baker SR, Slater A, Martin EM, McPherson HR, et al. GPVI (Glycoprotein VI) Interaction With Fibrinogen Is Mediated by Avidity and the Fibrinogen αC-Region. Arterioscler Thromb Vasc Biol. 2021;41(3):1092-104.
64. Nieswandt B, Brakebusch C, Bergmeier W, Schulte V, Bouvard D, Mokhtari-Nejad R, et al. Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. EMBO J. 2001;20(9):2120-30.
65. Induruwa I, McKinney H, Kempster C, Thomas P, Batista J, Malcor JD, et al. Platelet surface receptor glycoprotein VI-dimer is overexpressed in stroke: The Glycoprotein VI in Stroke (GYPSIE) study results. PLoS One. 2022;17(1):e0262695.
66. Mojica Munoz AK, Jamasbi J, Uhland K, Degen H, Munch G, Ungerer M, et al. Recombinant GPVI-Fc added to single or dual antiplatelet therapy in vitro prevents plaque-induced platelet thrombus formation. Thromb Haemost. 2017;117(8):1651-9.
67. Nieswandt B, Schulte V, Bergmeier W, Mokhtari-Nejad R, Rackebrandt K, Cazenave JP, et al. Long-term antithrombotic protection by in vivo depletion of platelet glycoprotein VI in mice. J Exp Med. 2001;193(4):459-69.
68. Bender M, Hagedorn I, Nieswandt B. Genetic and antibody-induced glycoprotein VI deficiency equally protects mice from mechanically and FeCl(3) -induced thrombosis. J Thromb Haemost. 2011;9(7):1423-6.
69. Voors-Pette C, Lebozec K, Dogterom P, Jullien L, Billiald P, Ferlan P, et al. Safety and Tolerability, Pharmacokinetics, and Pharmacodynamics of ACT017, an Antiplatelet GPVI (Glycoprotein VI) Fab. Arterioscler Thromb Vasc Biol. 2019;39(5):956-64.
70. Renaud L, Lebozec K, Voors-Pette C, Dogterom P, Billiald P, Jandrot Perrus M, et al. Population Pharmacokinetic/Pharmacodynamic Modeling of Glenzocimab (ACT017) a Glycoprotein VI Inhibitor of Collagen-Induced Platelet Aggregation. J Clin Pharmacol. 2020;60(9):1198-208.
71. Massberg S, Konrad I, Bultmann A, Schulz C, Munch G, Peluso M, et al. Soluble glycoprotein VI dimer inhibits platelet adhesion and aggregation to the injured vessel wall in vivo. FASEB J. 2004;18(2):397-9.
72. Ungerer M, Rosport K, Bultmann A, Piechatzek R, Uhland K, Schlieper P, et al. Novel antiplatelet drug revacept (Dimeric Glycoprotein VI-Fc) specifically and efficiently inhibited collagen-induced platelet aggregation without affecting general hemostasis in humans. Circulation. 2011;123(17):1891-9.
73. Mayer K, Hein-Rothweiler R, Schupke S, Janisch M, Bernlochner I, Ndrepepa G, et al. Efficacy and Safety of Revacept, a Novel Lesion-Directed Competitive Antagonist to Platelet Glycoprotein VI, in Patients Undergoing Elective Percutaneous Coronary Intervention for Stable Ischemic Heart Disease: The Randomized, Double-blind, Placebo-Controlled ISAR-PLASTER Phase 2 Trial. JAMA Cardiol. 2021;6(7):753-61.
74. Schupke S, Hein-Rothweiler R, Mayer K, Janisch M, Sibbing D, Ndrepepa G, et al. Revacept, a Novel Inhibitor of Platelet Adhesion, in Patients Undergoing Elective PCI-Design and Rationale of the Randomized ISAR-PLASTER Trial. Thromb Haemost. 2019;119(9):1539-45.
75. Shibutani T, Iijima T, Kaneda Y, Muramatsu S, Ogihara Y, Shibano T. Anti-Thrombotic Action of DZ-697b, a Novel Anti-Platelet Agent, on Photochemically Induced Thrombosis with Lower Bleeding Risk in Guinea Pigs. Blood. 2005;106(11):1870-.
76. Onselaer m-b, Nagy M, Pallini C, Pike J, Perrella G, Quintanilla L, et al. Comparison of the GPVI inhibitors losartan and honokiol. Platelets. 2019;31:1-11.
77. Lu W-J, Tsai C-H, Chen R-J, Huang L-T, Chen T-Y, Chen L-C, et al. Artesunate as a glycoprotein VI antagonist for preventing platelet activation and thrombus formation. Biomedicine & Pharmacotherapy. 2022;153:113531.
78. Sanchez EF, Alvarenga VG, Oliveira LS, Oliveira DL, Estevao-Costa MI, Flores-Ortiz R, et al. A fibrinolytic snake venom metalloproteinase, mutalysin-II, with antiplatelet activity and targeting capability toward glycoprotein GPIbalpha and glycoprotein GPVI. Biochimie. 2021;184:1-7.

584 79. Zafar MU, Ibanez B, Choi BG, Vorchheimer DA, Pinero A, Jin X, et al. A new oral antiplatelet
585 agent with potent antithrombotic properties: comparison of DZ-697b with clopidogrel a randomised
586 phase I study. Thromb Haemost. 2010;103(1):205-12.
587 80. Cho JY, Lee SY, Yun KH, Kim BK, Hong SJ, Ko JS, et al. Factors Related to Major Bleeding After
588 Ticagrelor Therapy: Results from the TICO Trial. J Am Heart Assoc. 2021;10(7):e019630.
589 81. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, et al. Association of
590 Cytochrome P450 2C19 Genotype With the Antiplatelet Effect and Clinical Efficacy of Clopidogrel
591 Therapy. JAMA. 2009;302(8):849-57.
592 82. Cuisset T, Loosveld M, Morange PE, Quilici J, Moro PJ, Saut N, et al. CYP2C19*2 and *17 Alleles
593 Have a Significant Impact on Platelet Response and Bleeding Risk in Patients Treated With Prasugrel
594 After Acute Coronary Syndrome. JACC: Cardiovascular Interventions. 2012;5(12):1280-7.
595 83. Varenhorst C, Eriksson N, Johansson Å, Barratt BJ, Hagström E, Åkerblom A, et al. Effect of
596 genetic variations on ticagrelor plasma levels and clinical outcomes. European Heart Journal.
597 2015;36(29):1901-12.
598 84. Pena A, Collet JP, Hulot JS, Silvain J, Barthélémy O, Beygui F, et al. Can we override clopidogrel
599 resistance? Circulation. 2009;119(21):2854-7.
600 85. Pereira NL, Farkouh ME, So D, Lennon R, Geller N, Mathew V, et al. Effect of Genotype-Guided
601 Oral P2Y12 Inhibitor Selection vs Conventional Clopidogrel Therapy on Ischemic Outcomes After
602 Percutaneous Coronary Intervention: The TAILOR-PCI Randomized Clinical Trial. JAMA.
603 2020;324(8):761-71.
604 86. Capodanno D, Angiolillo DJ, Lennon RJ, Goodman SG, Kim SW, O'Cochlain F, et al. ABCD-GENE
605 Score and Clinical Outcomes Following Percutaneous Coronary Intervention: Insights from the TAILOR-
606 PCI Trial. J Am Heart Assoc. 2022;11(4):e024156.
607 87. Angulo-Aguado M, Panche K, Tamayo-Agudelo CA, Ruiz-Torres D-A, Sambracos-Parrado S,
608 Niño-Orrego MJ, et al. A Pharmacogenetic Study of CYP2C19 in Acute Coronary Syndrome Patients of
609 Colombian Origin Reveals New Polymorphisms Potentially Related to Clopidogrel Therapy. Journal of
610 Personalized Medicine. 2021;11(5):400.

611

612

 Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022

FIGURE LEGENDS

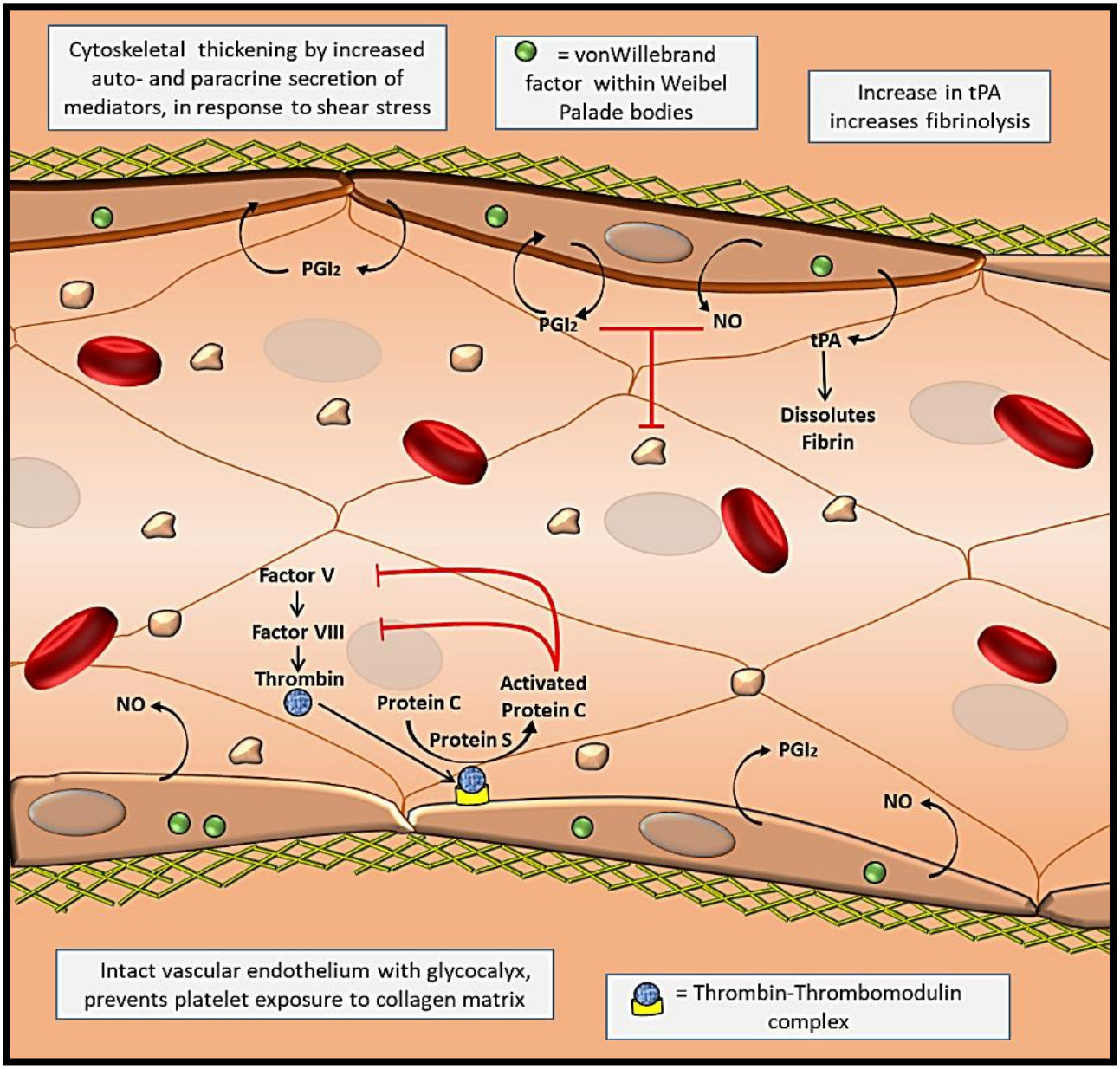
Figure 1: Endothelial and platelet perspective to haemostasis. The diagram shows the endothelial and platelet factors engaged in maintaining haemostasis. The intact endothelial lining with an overlying glycocalyx (not shown) deters direct interaction of platelets to pro-coagulant sub-endothelial matrix. The endothelium stores the tissue factor and von Willebrand factor; which on release promotes formation of a clot. The intact endothelium activates thrombin and adhering circulating platelets to exposed matrix, respectively. Endothelial mediators, such as NO and PGI_2, act as vessel vasodilators, platelet inhibitors and thickens the sub-endothelial cytoskeleton.

Figure 2: Schematic of thrombus architecture and blood flow dynamics: Blood flows in a (1) laminar approach during normal circulation. (2) Endothelial damage or accumulation of prothrombotic factors predisposes an individual to atheroma or thrombus formation via platelet activation. This damage exposes the underlying matrix along with the release of vWF from the endothelium to bind platelets. Activation of further platelets results in the formation of (3) clot with a graded architecture having a defined core (blue) and a shell (mauve). Increase in the size of the clot, shifts the blood flow to (4) non-linear dynamics and increases shear on the endothelial lining. The endothelium responds by generating greater amounts of mediators such as PGI_2. The produced (5) PGI_2 responds by inhibiting platelet activation and to also thicken the endothelial cytoskeletal framework; providing a reduction in the size of the thrombus and endothelial capacity to withstand shear force of the flowing blood.

Figure 3: **Platelet therapeutic modulations**: The figure illustrates the targets of Aspirin, ADP receptor blockers and GPVI receptor blockers along with the downstream impact on platelet activation. In the platelet, aspirin inhibits Cox enzyme to prevent the production of TxA_2, a platelet activator. Simultaneously aspirin also hinders endothelial PGI_2 production that relieves the inhibitory impact on platelets and prevents the endothelial cytoskeletal thickening thereby exposing the endothelium to high pressure of flowing blood that leads to denudation and dysfunction. The ADP receptor blockers (e.g. Clopidogrel, Prasugrel and Ticagrelor) and GPVI receptor blocker (e.g. Revacept) help modulating the platelet activity while sparing the endothelium.

640

Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022



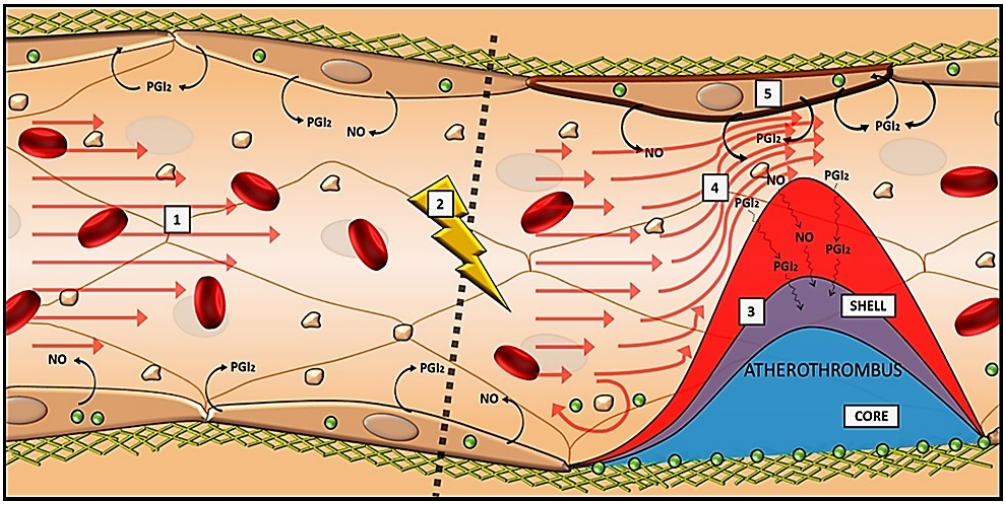
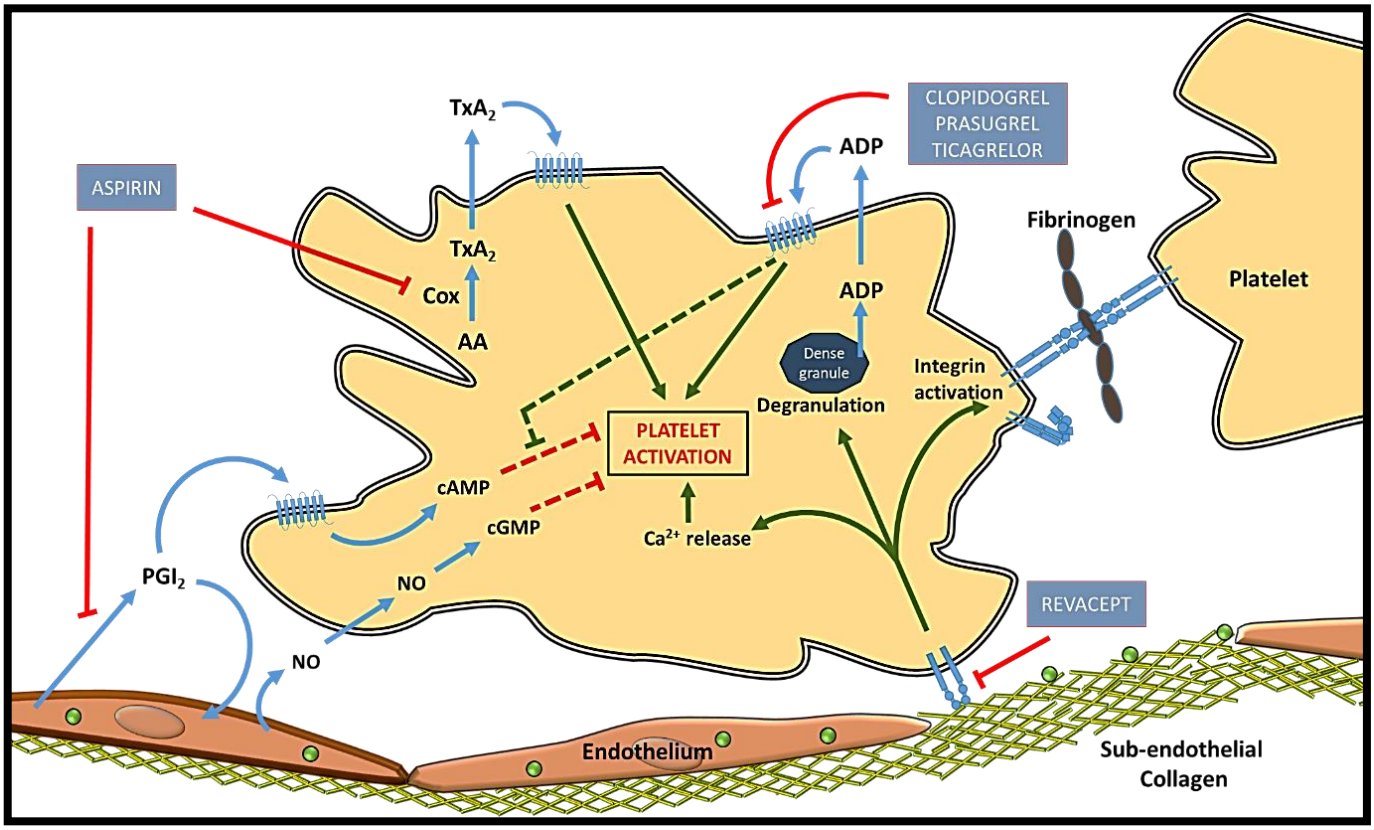


Figure 2: Schematic of thrombus architecture and blood flow dynamics: Blood flows in a (1) laminar approach during normal circulation. (2) Endothelial damage or accumulation of prothrombotic factors predisposes an individual to atheroma or thrombus formation via platelet activation. This damage exposes the underlying matrix along with the release of vWF from the endothelium to bind platelets. Activation of further platelets results in the formation of (3) clot with a graded architecture having a defined core (blue) and a shell (mauve). Increase in the size of the clot, shifts the blood flow to (4) non-linear dynamics and increases shear on the endothelial lining. The endothelium responds by generating greater amounts of mediators such as PGI2. The produced (5) PGI2 responds by inhibiting platelet activation and to also thicken the endothelial cytoskeletal framework; providing a reduction in the size of the thrombus and endothelial capacity to withstand shear force of the flowing blood.

166x82mm (150 x 150 DPI)

Accepted Manuscript published as VB-22-0010.R1. Accepted for publication: 31-Oct-2022



ANTI-GPVI APPROACHES			
COMPOUND		STRATEGY	REFERENCES
Antibodies	Glenzocimab (ACT-017)	Binds to GPVI active site on platelets and reversibly compete with collagen interaction.	(69, 70)
	Revacept	Inhibits GPVI receptor downstream signalling	(66, 71-74)
	JAQ1	Downregulates GPVI expression in mice	(67)
Small molecule inhibitors	DZ-697b	A novel compound that inhibits interactions amongst GPVI + collagen and GPIbα + fibrinogen	(75)
	Losartan	Selectively inhibit collagen-mediated platelet activation by binding to GPVI and blocking downstream signalling	(76)
	Artesunate		(77)
	Mutalysin-II	An enzyme that cleaves GPVI and GPIbα.	(78)

Table 1 ***List of Anti-GPVI approaches:*** The table enlists currently researched antibodies and small molecule inhibitors that target the interaction of GPVI and collagen that climaxes in platelet activation.