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Emerging Novel Diagnostic Measure: Prothrombin Fragment 1+2

Niharika Bharti PhD[1], Ashish Gupta PhD[2*], Sudeep Kumar MD[3]

[1,2]Centre of Biomedical Research, SGPGIMS Campus, Raebareli Road, Lucknow, India
[3]Department of Cardiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India
*Corresponding author: ashishg24@yahoo.co.in

Abstract:
One potential useful tool for diagnosing thrombosis is the prothrombin fragment 1+2 (PF1+2) that is generated when factor Xa cleaves prothrombin. The realisation that thrombosis plays a critical role in the genesis of vascular diseases has generated growing interest in the relationship between PF1+2 and clinical characteristics. The goal of the current review is to stimulate more translational and clinical research on this topic by offering an up-to-date update on the studies investigating whether PF1+2 measurements may be utilised as a diagnostic technique for vascular disorders. Hence; we performed a comprehensive review to describe the reports of elevated PF1+2 levels in venous thromboembolism, inflammation, cancer, sepsis, acute coronary syndromes, stroke, atrial fibrillation, rheumatoid arthritis, liver and kidney disorders, and in the post-operative period. Systematic searches in the English language were conducted in the Pubmed database. It may also be useful in assessing the efficacy of different treatments, in addition to its potential prognostic and diagnostic value. Although, elevations occur in the presence of overt thrombosis as well as the hypercoagulable state. However, to date, little is known about the diagnostic accuracy of the cut-off level to be employed for the definition of elevations. Therefore, additional research is necessary to develop a non-invasive technique for the evaluation of PF1+2 levels in the laboratory in order to predict prognosis in a variety of vascular disorders. This review comprises the clinical application of PF1+2 in hemorrhagic and cardiovascular diseases.

Keywords: Prothrombin Fragment 1+2, hemostasis, thrombosis, hypercoagulable state, blood coagulation, fibrinogen, prothrombin time

Running head: Prothrombin Fragment 1+2 as a diagnostic measure

1. Introduction:

Increasing evidence indicates that the hemostatic system plays an important role in the pathogenesis of various vascular diseases. The coagulation pathway involves a sequence of proteolytic events involving enzymes similar to trypsin, which aid in the production of thrombin to form a clot. This pathway can be reflected in routine clinical laboratory tests such as the TF-factor VII pathway by Prothrombin Time and the Factor XI activation by the Activated Partial Thromboplastin Time (APTT) test. The conversion of prothrombin to thrombin is the central event in the coagulation cascade, and it is mediated by the action of factors Xa and Va in the complex formed on the membrane surfaces (Fig. 1).

This complex, which acts on prothrombin and is known as the "prothrombinase" complex, is present on the membrane together with Ca^{2+}.

Prothrombin is a 72kDa plasma protein found in human blood at a concentration of 0.1mg/ml[1]. It was first described in 1959 by Loeliger as a cofactor for a circulating anticoagulant in patients with hypoprothrombinemia[2]. It is synthesized in hepatocytes, neurons, and astrocytes[3]. Prothrombin molecules are composed of three domains: prothrombin fragment 1, prothrombin fragment 2, and prethrombin. Cleavage of Arg 271 or Arg 286 in the presence of plasma proteins yields prothrombin fragments 1+2 (PF1+2) derived from the NH_2 terminus of human prothrombin[4] (Fig. 1). Prothrombin fragments 1 and 2 have an almost similar homology. Prothrombin fragment 1 is a vitamin-K-dependent protein that contains ten GLAs (-carboxyglutamic acids) and aids in the binding of prothrombin molecules to negatively charged phospholipids and Ca^{2+} ions during activation via factor X. However, the prothrombin fragment 2 domain has a weak calcium-binding ability and shows interaction with factor V during activation.

In the presence of Ca^{2+}, activated factor X cleaves prothrombin to produce thrombin (factor IIa), an active serine protease[5]. The resulting fibrin monomers then polymerize to form an insoluble extracellular matrix, which also promotes local inflammation. PF1+2 is a polypeptide with a plasma half-life of 90 minutes that is released from prothrombin during its activation to thrombin by the prothrombinase complex[5,6]. Therefore, measurement of circulating levels of PF1+2 has been considered a precise indicator of in vivo thrombin production in various diseases (Table 1).

The aim of the present review is to provide a current update on the studies looking into whether PF1+2 measurements could be used as a clinical index for vascular illnesses in order to encourage more translational and clinical studies on this topic.

2. Methods

The present review adheres to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines and is presented in accordance with the PRISMA statement (Fig. 2)[7].

2.1 Search strategy and selection criteria

The following phrases combined to search PubMed for prospective studies and systemic reviews published in English between January 1990 and February 2023: prothrombin, prothrombin fragment, intervention with (prothrombin fragment 1+2 OR hypercoagulable state), intervention with (Cardiovascular disease OR Cancer OR Pregnancy OR Transplantation OR Type 2 Diabetes) intervention with (Plasma OR Urine). First, prothrombin fragment was used to screen the titles and abstracts of all identified studies. In a later, more thorough examination into prothrombin fragment, selected article titles and complete texts were meticulously evaluated for PF1+2 using blood and urine, even including experimental models. The following factors were used to mine the studies for PF1+2: kind of sample, sample size, type of disease research findings, validation, levels of PF1+2, and outcome.

3. Emergence of PF1+2 in different diseases

3.1 Cardiovascular Disease

All normal individuals have a measurable amount of activation markers in their circulation, confirming that low-grade coagulation is a continuous process[8-9]. Increased amounts of activation products are produced during systemic or even local coagulation activation. Therefore, the assessment of activation markers has been proposed to determine the hypercoagulable status.

The PF1+2 levels examined in antihypertensive drug-treated individuals were higher than in the untreated hypertension patients[10]. Koczko et al. also discovered an augmented level of PF1+2 and thrombin-antithrombin-III complexes (TAT) in the plasma of newly diagnosed, essential hypertension patients[11], suggesting an intravascular thrombin generation. However, PF1+2 levels were comparable in patients with idiopathic pulmonary arterial hypertension (IPAH) and in the controls[12]. Agorasti et al. conducted a study comprising treated hypertension having nocturnal dips in blood pressure (dippers) and those not having dips in blood pressure (non-dippers) patients. Plasma levels of factors VIII and IX, fibrinogen, PF1+2, and TAT were significantly higher in non-dippers than in dippers[13]. This suggests that hypertensive patients are at increased risk for cardiovascular disease, and that a prothrombotic state in these patients is associated with organ damage. In individuals with essential hypertension, PF1+2 levels were linked with the presence and severity of target-organ damage (TOD), which may be a factor in the development of atherosclerotic disease in these patients[14]. In hypertensive individuals with modestly reduced creatinine clearance, the presence of high plasma PF1+2 which may indicate that the coagulation system is active in uremic patients, raising their cardiovascular risk[15].

Elevated PF1+2 levels have been found to be correlated to the presence of CVD risk factors such as age, smoking, and dyslipidemia[16]. In a study of 2,964 men who were chronic smokers, those who were still smoking had the highest levels of PF1+2, followed by those who had quit; those who hadn't smoked had the lowest amounts[17]. The intima-media thickness (IMT) of the carotid artery can predict the incidence of CVD[18]. In a population of 181 adults without clinically overt atherosclerotic disease, the plasma levels of F1+2 were significantly associated with carotid IMT, suggesting a relationship between thrombin generation and the development of atherosclerosis[19].

It is evident that inflammation plays a vital role in coronary heart disease (CHD). The protein C (PC) anticoagulant pathway is an important mechanism for limiting the coagulation response to injury or inflammation by down-regulating the thrombin feedback loop[20]. This pathway includes circulating PC and protein S and the integral endothelial cell membrane proteins, thrombomodulin (Tm), and endothelial PC receptors (EPCR)[21]. Dysfunctional variants of EPCR such as Ser219Gly may reduce the antithrombotic/anti-inflammatory effects of the pathway and may lead to the development of atherosclerosis and thrombosis in CHD[22]. The investigation conducted by Ireland et al. revealed a substantial rise in plasma PF1+2 levels for the 219Gly variant[22]. This implies that increased shedding of the Gly allele from the endothelial cell surface links increased the CHD risk and thrombin generation.

Coronary artery disease (CAD) is characterized by LDL accumulation and plaque formation due to activation of intimal inflammation and by an immune response triggered by endothelial injury and dysfunction, as well as activation of the haemostatic system. When compared to patients with angina pectoris and angiographically normal coronaries, the patients with coronary atherosclerosis had higher PF1+2 levels; however, no correlation was identified with the severity of atherosclerosis[23]. However, the investigation by Giannitsis et al. revealed that patients with CAD had significantly higher levels of PF1+2 than healthy controls (HC). Within the CAD group, those with severe coronary atherosclerosis (> or = 2 vessel disease) also had significantly higher values for PF1+2[24].

A myocardial infarction (MI) typically results from the formation of a coronary thrombus; hence, coagulation-related variables may play a pivotal role in this pathophysiological process. Acute MI and unstable angina both cause activation of the blood coagulation system. Patients with MI and unstable angina have elevated plasma PF1+2 levels[25]. In patients with MI, carotid artery IMT predicted the

incidence of cardiovascular disease. The IMT was significantly and positively associated with prothrombin[26].

In another study of patients with MI, late diastolic filling time and mitral E/A (mitral early-to-late flow velocity ratio) were linked to cIMa (calculated intima-media area) of common carotid, brachial arteries, and PF1+2, pointing to a connection between atherosclerosis, thrombin generation, and diastolic dysfunction[27].

Halvorsen et al. conducted a NORwegian study on District treatment of ST-elevation MI (NORDISTEMI) to examine the effects of early angioplasty versus standard care on prothrombotic markers in STEMI (acute ST-elevation myocardial infarction) patients treated with thrombolysis and found that the PF1+2 levels were elevated[28]. Moreover, in STEMI patients, the levels of PF1+2 were substantially linked to myocardial necrosis, as determined by peak Troponin T (TnT)[29]. High levels of these coagulation markers as PF1+2 in patients with low left ventricular ejection fraction (LVEF) and high N-terminal pro b-type natriuretic peptide (NTproBNP) were used to assess LV impairment, which may indicate a hypercoagulable state in patients with impaired myocardial function[29]. In the Martnez-Sales Vet et al. study, which examined the thrombotic activity in MI patients using 200 mg of aspirin daily for two years after their MI, persistent formation of PF1+2 was discovered[30].

A significant amount of thrombin is generated during coronary artery bypass grafting surgery (CABG) under cardiopulmonary bypass. Even after accounting for marker clearance, hemodilution, blood loss, and transfusion, the reperfusion following CABG led to a spike in the rate of thrombin production[31]. The activation of coagulation in patients undergoing cardiopulmonary bypass (CPB) surgery was examined, using the activation marker PF1+2, which is a gauge of overall thrombin generation. Moreover, graft thrombotic occlusion is a frequent complication in patients following aorto-coronary bypass grafting with noticeable coagulation activation, as shown by higher PF1+2 concentrations[32]. However, in minimally invasive valve surgery (MIVS), PF1+2 is reduced during and after the operation[33].

The most typical heart arrhythmia that might manifest as thrombosis is atrial fibrillation (AF)[34]. Nowadays, factor Xa inhibitors are recommended for the treatment of nonvalvular AF[35]. AF is associated with elevated levels of $PF1+2$[36-37]. PF1+2 exhibits a significant and adverse connection with plasma Factor Xa inhibitor concentrations in patients with AF who are taking Factor Xa inhibitors such as *rivaroxaban* and *apixaban*[38]. *Rivaroxaban* is an oral anticoagulant that prevents thromboembolic complications with fixed doses that do not require laboratory monitoring, whereas Warfarin administration with an adjusted dose does necessitate routine monitoring[39].

In a comparative study of patients with non-valvular AF treated with warfarin and rivaroxaban, the observation revealed that the prothrombin time (PT) values did not significantly differ between the two groups. However, the PF1+2 level, a marker of thrombin generation, was significantly higher in the rivaroxaban group than in the warfarin group[39]. Thus, this study suggests that warfarin treatment may inhibit thrombin generation more aggressively than rivaroxaban. Moreover, this study raises the possibility that rivaroxaban may not be as effective at inhibiting thrombin production as warfarin. Compared with AF patients who were treated with aspirin alone, AF patients treated with an adjusted dose of warfarin or in combination with aspirin had a significantly higher prothrombin time, as measured by the INR (international normalized ratio), which was associated with decreased thrombin generation, as measured by the PF1+2 level, than AF patients alone treated with aspirin[40].

AF can activate the expression of atrial endocardial endothelia and platelet (PLT) inflammatory mediators such as adhesion molecules like P-selectin, as thrombogenesis found to be associated with inflammation[41-

[42]. A study by Jing et al. on a rat model of AF also showed elevated levels of plasma PF1+2, which strongly correlated with the inflammatory mediator P-selectin ($r = 0.916$, $p < 0.05$)[43]. In AF, sustained sinus rhythm for 6 months had no impact on PF1 + 2 but discontinuation of warfarin was associated with significantly higher levels of PF1 + 2 compared with the reference group[44]. PF1+2 was more abundant in NVAF stroke patients than in sinus rhythm stroke patients[45]. Patients with chronic non-rheumatic AF who were not on anticoagulation medications showed higher PF1+2 compared with control subjects[46-47]. The presence of matrix degradation in AF and its association with PF1+2 was demonstrated in a study by Marnet et al.[46]. According to the Liles et al. study, despite the use of traditional (Warfarin) or newer anticoagulants (*apixaban* and *rivaroxaban*), prothrombotic biomarkers such as PF1+2 were still produced at elevated levels in patients with AF[48]. AF patients with diabetes who needed insulin had considerably higher levels of PF1+2 than those without diabetes and higher levels than those with diabetes who were taking oral anti-diabetic medications. Thus, in AF patients receiving oral anticoagulation medications, those with diabetes regardless of the type (with or without insulin therapy), and those without diabetes had a comparably high thrombotic generation[49].

3.2 Venous thromboembolism:

Venous thromboembolism (VTE) is a common disease with an estimated incidence of 1:1000 individuals per year in Western countries[50]. Deep vein thrombosis (DVT) and pulmonary embolism (PE) are two manifestations of hypercoagulability. Studies by Wexels et al. regarding patients with imaging-confirmed VTE found significantly higher levels of PF1+2 in plasma and urine compared to patients without VTE. They also suggested that PF1+2 ex vivo generates thrombin in plasma and urine in the same way it does in vivo[6,51].

Furthermore, statistically significant higher levels of PF1+2 were found in patients with DVT compared with those with PE, but there were no differences in uPF1+2 concentrations between the two groups[52-53]. Higher urine PF1+2 levels were found in DVT patients ($p<0.001$) and DVT-positive patients with ongoing malignancy, but not in DVT-positive patients with infection or trauma[52]. van Es et al. also found high levels of PF1+2 in both plasma and urine of VTE patients; however, there was no significant difference in urine concentration compared to HC[54] (Table 1).

PF1+2 is a more suitable serum marker involved in coagulation for risk identification in cancer patients with venous thromboembolism[55]. In the CATS (Vienna Cancer and Thrombosis Study), PF1+2 levels were significantly higher in patients with VTE than in patients without VTE[56-57], predicting a twofold increased risk of VTE[56]. The highest hazard ratio (HR) for VTE was found in patients who had both elevated PF1+2 (HR, 3.6) and an increased incidence of VTE at 6 months in patients with various malignancies. Therefore, elevated PF1+2 levels were strongly associated with VTE risk; however, they had no significant influence on overall survival. PF1+2 levels may therefore be helpful for the early diagnosis of VTE in cancer patients.

Unfractionated heparin (UFH) was once the go-to treatment for deep vein thrombosis (DVT), but more recently, low-molecular-weight heparins (LMWH), such as enoxaparin, have been accessible to the modification of conventional heparin by enzymatic or chemical hydrolysis. Compared with the UFH group, plasma PF1+2 concentrations in the enoxaparin group were steadily reduced over time. This finding suggests that LMWH is more effective in suppressing ongoing thrombosis in vivo than UFH in patients with venous thrombosis[58]. With an incidence rate ranging from 17 to 53%, depending on the technique of prophylaxis, patients having total knee arthroplasty (TKA) were at high risk for venous thromboembolism

(VTE)[59]. A study by Yang et al. exhibited that patients with VTE had significantly higher levels of plasma PF1+2 than patients without VTE on the first and third day after surgery[60]. This study also revealed that PF1+2 may be utilized to predict VTE following TKA because it has higher diagnostic accuracies than other biomarkers including PAF-1, TAT, and D-dimer. In a similar vein, research by Borris LC demonstrated an elevated urine PF1+2 level that predicted postoperative VTE following total hip replacement and TKA[61-62]. To identify individuals who are at risk of VTE after surgery, the measurement of urine PF1+2 may offer a quick, non-invasive clinical diagnostic method.

3.3 Stroke

Stroke occurs in 9–16% of AF patients, and AF patients have a five times higher chance of having a stroke than the general population[63]. Stroke incidence in individuals with AF has been demonstrated to drop by more than 50% when receiving oral anticoagulation medication, which includes factor Xa inhibitors such as Apixaban, Rivaroxaban, etc.[64]. Elevated PF1+2 levels have been described in stroke patients with AF and, as PF1+2 levels reflect thrombin generation, they have been suggested as a better marker for stroke prediction[65]. Apixaban treatment has been linked with a lesser reduction in thrombin production but PF1+2 reduces more significantly in stroke patients treated with warfarin[66]. According to this observation, almost 85% of strokes are ischemic or are brought on by a blood clot that causes a thrombus or embolism, which results in an abrupt loss of blood flow in a major cerebral artery.

In the Acute Embolic Stroke Trial, including different types of strokes, stroke severity (OR, 1.09) and PF1+2 level (OR, 1.77) were independently associated with a poor outcome at 3 months[67]. The presence of large atherosclerotic plaques in the proximal segment of the aorta have been shown to be associated with an increased risk of ischemic stroke. In stroke patients, an increase of PF1+2 was observed with increased plaque thickness[68]. Principally, when compared to patients without plaque, patients with big plaques showed a significant increase in PF1+2 levels (Table 1).

3.5 Cancer

Cancer induces a high risk of VTE, with an incidence rate of 8 per 1,000 people per year in cancer patients[50]. Cancer patients with VTE have a higher mortality rate than cancer patients without VTE[57, 69]. It could be due to the release of tissue factor from tumor cells, which causes thrombin generation and hypercoagulability. Consequently, measuring the concentration of prothrombin fragments may also help to recognize coagulation abnormalities in cancer patients.

According to a recent study, individuals with localized head and neck cancer and low-stage primary lung cancer have higher PF1+2 concentrations than HC[70]. In patients with NSCLC (non-small cell lung cancer), PF1+2 was a more sensitive hypercoagulability marker than TAT (thrombin anti-thrombin III complexes), which indicates the risk of thromboembolic disorders after tumor resection[71]. According to research by Iversen et al., people with localized colorectal disease have higher F1+2 levels than those with benign colorectal disease[72]. High expression of prothrombin fragment with VEGF in human colon cancer demonstrates a functional interrelationship between thrombin generation and angiogenesis[73]. In pre-treated gynaecological cancer patients, a higher level of plasma PF1+2 indicates that hemostasis activation has occurred. Ovarian cancer shows a higher level of PF1+2 than cervical and endometrial cancer when compared to HC[74].

In a study on gastric cancer, it was discovered that plasma PF1+2 and PT-INR were significant predictors of lymph node metastasis[75]. So, it is indicated that early detection and treatment of DIC, as well

as the measurement of plasma PF1+2, are essential in order to follow the activation of the coagulation system in patients with malignancies. The risk of having thromboembolic complications is much higher in a sizeable fraction of patients (20-30%) with brain tumors, especially gliomas. In both lower- and higher-grade gliomas, PF1+2 were found, indicating local activation of blood coagulation (Table 1)[76].

3.6 Pregnancy

Pregnancy causes the maternal coagulation mechanism to be upregulated in women, which results in an overall increase in thrombin generation and may be the cause of an increased risk of venous thrombosis[77]. Dargaud et al. also found elevated levels of PF1+2 in the first, second, and third trimesters of pregnancy[78]. In APAS (antiphospholipid antibody syndrome) pregnancies, plasma concentrations of PF1+2 were found to be higher than in healthy pregnancies. Pregnant women with a history of recurrent abortions and APA have significantly higher prothrombin activation than healthy pregnant females[79]. PF1+2 may be used to modify low-molecular-weight heparin (LWMH) prophylaxis in high-risk pregnant women with thrombophilia[80]. In a study conducted by Simeone et al. pregnant women with thrombophilia treated with LWMH and healthy pregnant women served as the control group, the exhibited increased levels of PF1+2 in pregnant women exposed to heparin prophylaxis were significantly lower than those in normal pregnant women during gestation[80]. PF1+2 levels were also higher in pre-eclamptic women than in normal pregnancies[81-82]. Furthermore, the fact that PF1+2 was markedly elevated in women who had experienced one or more miscarriages suggests that termination of a pregnancy may be associated with an excessively hypercoagulable state, which could result in a moderate risk of thrombosis throughout the various trimesters of pregnancy (Table 1)[83].

3.7 Kidney disease

Patients with end-stage renal disease are at risk for hemorrhagic complications as well as for a variety of thrombotic complications. Molino et al. conducted a study comprising hemodialysis patients with no thrombotic complications (NTC) and hemodialysis patients with thrombotic complications (TC), and HC blood donors. Compared to controls, the plasma level of PF1+2 in NTC and TC was higher. Moreover, the level of PF1+2 was augmented in NTC compared to TC[84]. Compared to controls, patients with chronic renal disease have shown characteristics of a hypercoagulable condition and have higher levels of PF1+2[85]. Renal transplant recipients (RTR) were also shown to have elevated levels of PF1+2, D-Dimer, and fibrinogen. This suggests a persistent prothrombotic state that may be a factor in the RTR population's higher risk of CVD[86].

The best long-term treatment for chronic renal insufficiency is renal transplantation (RT); however, the risk of VTE is particularly high for RT patients. Upon discontinuation of oral anticoagulant medications, RTR with VTE were shown to have higher levels of PF1+2 than RTR without VTE and VTE recipients without impairments in their renal function[87]. With noticeably increased median plasma concentrations of PF1+2, hemolytic-uremic syndrome is a thrombotic consequence of *Escherichia coli* O157:H7 infection that worsens renal damage[88].

Acute kidney injury (AKI) is a common complication following cardiac surgery. The levels of PF1+2 were significantly higher in the AKI group, and they were independently associated with an estimated glomerular filtration rate reduction[89]. This suggests that thrombin generation is increased in patients with deteriorating renal function and is an independent risk factor for AKI. The PF1+2 levels were evaluated in individuals with hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura

(TTP). It was found to be increased in all patients compared to controls and also remained higher than normal after treatment with plasma exchange (Table 1)[90].

3.8 Liver disease

Patients with chronic liver disease have considerably greater plasma levels of PF1+2 compared to controls, which suggests intravascular coagulation[91] (Table 1). Hepatic endothelial cells were harmed by high-dose chemotherapy or radiation, resulting in microthrombosis in hepatic venules, which leads to veno-occlusive disease (VOD) of the liver. Patients with VOD have higher levels of PF1+2 after stem cell transplantation (SCT)[92]. In a study conducted by Fota-Markowska et al., it was demonstrated that neither patients with stable liver cirrhosis nor those with chronic hepatitis C (CHC) had significantly different serum concentrations of PF1+2 compared to controls. However, elevated serum levels of PF1+2 were found in 16.7% of patients with cirrhosis and in 35.3% of patients with CHC[93].

Patients with liver disease often suffer from bleeding complications; to overcome this, a fresh frozen plasma transfusion was performed. Patients with liver disease who get prophylactic fresh frozen plasma transfusions experience a prothrombotic impact and experience a 38% rise in PF1+2 levels[94]. In addition, PF1+2 was discovered to be considerably higher in individuals with cirrhosis with or without portal vein thrombosis (PVT), hepatocellular carcinoma (HCC), cholangiocarcinoma, or metastatic liver cancers compared to HC[95].

3.9 Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a hypercoagulable state. T2DM-related problems with coagulation and fibrinolysis increase the risk of macrovascular consequences such as MI and ischemic stroke[96]. In T2DM patients, micro-albumin-urea (MAU) is also associated with an increased risk of cardiovascular disease. Plasma PF1+2 levels were considerably higher in individuals with MAU than in those with normo-albuminuria (NAU), indicating that MAU is linked to a prethrombotic condition that may increase the risk of cardiovascular disease[97]. T2DM is a major, interrelated public health problem that is frequently treated with statins and angiotensin-converting enzyme (ACE) inhibitors[98], and it was discovered that simvastatin and ramipril worked better together than either medicine alone to lower PF1+2 levels[98-99]. In poorly controlled T2DM patients, hyperglycemia and hyperinsuleminia frequently coexist. This combination promotes a procoagulant condition that raises plasma PF1+2 and may put these patients at risk for sudden cardiovascular events[100].

Recent findings in AF patients receiving oral anticoagulation showed that diabetic patients on insulin had a higher thromboembolic risk than those without insulin therapy. Patients with diabetes requiring insulin had significantly higher levels of PF1+2 than those without diabetes and diabetic patients on oral anti-diabetic drugs (Table 1)[49].

3.10 Rheumatoid Arthritis

Levels of PF1+2 have been reported to be raised in patients with rheumatoid arthritis (RA) and parallels the clinical disease activity and levels of acute phase proteins. Reduction in levels of PF1+2 parallel reduced disease activity following treatment with Tocilizumab[101]. Similarly, systemic lupus erythematosus (SLE) disease activity is associated with raised markers of thrombin anti-thrombin complexes, PF1+2, and soluble thrombomodulin. It suggests that the inflammation induced hypercoagulability[102]. A monoclonal antibody against PF1 has been reported to behave like lupus

anticoagulant[103]. In systemic sclerosis (SSc) patients with peripheral ischemia, infusion of Iloprost has been reported to significantly lower the levels of PF1+2[104].

4. Discussion

A number of epidemiological and clinical research have been conducted to examine the potential of hemostatic factors and new indicators of hemostasis activation to enhance the risk prediction of thrombotic events, as may be inferred from the aforementioned literature. The two fundamental metrics employed in the analysis of the hypercoagulable state in diverse illnesses are the levels of hemostatic factors and the levels of activation products. The two fundamental metrics employed in the analysis of the hypercoagulable state in various diseases are the levels of hemostatic factors and the levels of activation products.

Many substances can be detected during the activation of hemostasis, such as peptides generated during the activation of coagulation factors (such as PF1+2), complexes of activated hemostatic factors with their inhibitors (such as TAT), and the breakdown products of fibrin (e.g., D-dimer)[105]. PF1+2 is a polypeptide released from prothrombin during its activation to thrombin by the prothrombinase complex and is regarded as the most accurate indicator of in vivo thrombin generation. PF1+2 and Thrombin-antithrombin Complex (TAT) were measured to assess thrombogenesis. Tissue-type plasminogen activator (tPA) antigen and the plasmin-anti-plasmin complex (PAP) were measured to characterize the activation of fibrinolysis while D-dimer reflects both processes. A study by Wexels et al showed that plasma D-dimer had the 93% sensitivity and 94% NPV compared to plasma PF1+2, whereas urine PF1+2 had the 74% sensitivity and 85% NPV[6].

Elevated levels of PF1+2 have been investigated as a risk factor for first and recurrent thrombotic events. Plasma PF1+2 was connected to elements that raised the risk of CVD[106-107]. High PF1+2 has been linked to coronary atherosclerosis, peripheral arterial disease, and the presence of traditional CAD risk factors such age, smoking, and dyslipidemia[16]. PF1+2 is significantly higher in smokers than in nonsmokers, and higher in subjects with a family history of ischaemic heart disease than in those without. In patients with ST-elevation MI, there was a strong inter-correlation between D-dimer and PF1+2 ($r = 0.504$, $p<0.001$).

There is evidence from a number of studies that IMT is associated with higher PF1+2, which may make it easier to find asymptomatic individuals who would benefit from antithrombotic treatment for coronary atherosclerosis[19]. Hence, PF1+2 was assessed to aid in oral anticoagulant strategies and to minimize thrombotic events following surgery. Postoperative thrombosis could occur if the thrombin production during cardiac surgery is not suppressed[108]. In non-valvular AF patients treated with warfarin and rivaroxaban, PT values did not significantly change while PF1+2 levels were significantly higher in the rivaroxaban group than in the warfarin-treated group[39]. This study also raises a novel thought that rivaroxaban may not be as effective at inhibiting thrombin production as warfarin. Warfarin inhibits thrombin generation more aggressively than rivaroxaban.

Similarly, PF1+2 levels were discovered to be significantly higher than HC in diseases such as liver cirrhosis, renal disease, T2DM, transplantation, malignancies, etc. Their levels are linked to the prognosis of the disease or risk factors[109]. High D-dimer and PF1+2 levels independently predict occurrence of VTE in patients with cancer. It has been demonstrated that several conventional anticoagulants, including warfarin, unfractionated heparin, and low-molecular-weight heparin, lower the levels of circulating PF1+2[110]. Such lower levels may be because increased PF1+2 most likely indicates a

hypercoagulable state that can be affected by anticoagulation. Therefore, in those who are most at risk for thrombotic events, laboratory evaluation of PF1+2 may assist in directing further preventive or therapeutic treatments.

5. Summary

Laboratory tests that measure hemostatic activity, such as PF1+2, can serve to distinguish patients at high risk from those at lower risk for thrombosis, which may facilitate the administration of thromboprophylaxis. Increased PF1+2 levels have been linked to risk factors in diseases with hypercoagulable states and independently predict the occurrence of thrombotic events. Therefore, establishing a cost-effective and non-invasive assessment of PF1+2 in a laboratory to evaluate disease prognosis is critically required.

6. Present and future perspective opinion

The augmented proof specifies that the hemostatic system plays an imperative role in the pathophysiology of various vascular ailments. The foregoing discussions of the production of PF1+2 in different vascular illnesses and their risk of thrombosis events pertain to these conditions. Although various international scientific groups have tried to measure the PF1+2 fragment in different diseases, the outcomes are very encouraging. Few scientific groups have demonstrated that PF1+2 can be used for diagnosis, prognosis, and monitoring of post-operative measures in the underlying diseases. In the inference, we propose that PF1+2 measurements can be utilized as a novel clinical index for a variety of vascular illnesses, but to date, including it as a routine clinical measure has been totally disregarded. In light of all the above-mentioned investigations, it appears that PF1+2 will eventually be used as a clinical indicator for a variety of disorders. There should be brainstorming sessions to decide how PF1+2 will be incorporated into standard clinical measures.

Highlights

1. This review highlights the role of prothrombin fragments 1+2 in various underlying diseases.
2. Clinical applications of prothrombin fragment 1+2 in various diseases have exhibited the possibility that it may be a novel diagnostic measure.
3. The biggest challenge is, "Can prothrombin fragment 1+2 be a novel diagnostic measure?"
4. Prothrombin fragment 1+2 has been neglected, but it has potential and is a significant emerging novel diagnostic measure.
5. Prothrombin fragment 1+2 can be considered an emerging novel diagnostic measure to detect various diseases at an early stage.

Author contributions

N. B., A.G. and S.K. searched data for this review article. N.B. and A.G. wrote the manuscript, made figures, and tables. S.K. V.A., and A.G. edited the manuscript. S.K. and V.A. suggested and wrote clinical part of the manuscript. All authors made substantial contributions to discussions of content and reviewed and edited the manuscript before submission.

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Abbreviation:
PF1+2: Prothrombin fragment 1+2; APTT: Activated Partial Thromboplastin Time; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; TAT: Thrombin-antithrombin-III; IPAH: Idiopathic pulmonary arterial hypertension; TOD: Target-organ damage; CVD: Cardiovascular Disease; IMT: Intima-media thickness; CHD: Coronary heart disease; Tm: Thrombomodulin; EPCR: Endothelial PC receptors; CAD: Coronary artery disease; MI: Myocardial Infarction; cIMa: calculated intima-media area; NORDISTEMI: NORwegian study on District treatment of ST-elevation MI; STEMI: acute ST-elevation myocardial infarction; TnT: Troponin T; LVEF: Left ventricular ejection fraction; NTproBNP: N-terminal pro b-type natriuretic peptide; CABG: coronary artery bypass grafting surgery; CPB: cardiopulmonary bypass; MIVS: minimally invasive valve surgery; AF: atrial fibrillation; PT: prothrombin time; INR: international normalized ratio; NVAF: non-valvular AF; VTE: Venous thromboembolism; DVT: Deep vein thrombosis; PE: pulmonary embolism; CATS: Vienna Cancer and Thrombosis Study; HR: hazard ratio; UFH: Unfractionated heparin; LMWH: low-molecular-weight heparins;
TKA: total knee arthroplasty; OR: Odd ratio; NSCLC: non-small cell lung cancer; VEGF: Vascular endothelial growth factor; APAS: antiphospholipid antibody syndrome; TC: thrombotic complications; RTR: Renal transplant recipients; AKI: Acute kidney injury; HUS: hemolytic uremic syndrome; TTP:thrombocytopenic purpura; VOD: veno-occlusive disease; SCT: stem cell transplantation; CHC: chronic hepatitis C; PVT: portal vein thrombosis; HCC: hepatocellular carcinoma; T2DM: Type 2 diabetes mellitus; MAU: micro-albumin-urea; ACE: angiotensin-converting enzyme; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; tPA: Tissue-type plasminogen activator; PAP: plasmin- anti-plasmin complex.

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Table 1: Emergence of Prothrombin fragment 1+2 measures in different diseases

Clinical Condition	Sample	Study Population	Result	References
Hypertension	Plasma	43 treated and 11 untreated patients	PF1+2 more in untreated hypertensive patients.	10
	Plasma	27 consecutive patients with idiopathic pulmonary arterial hypertension (IPAH) and 16 controls without	PF1+2 shows no significant change in both group (p=0.92).	12

		pulmonary hypertension		
Cardiovascular Disease (CVD)	Plasma	study cohort of 5201 patients with CVD and 399 persons free of CVD	High level of PF1+2 was correlated with age, smoking, triglyceride, creatinine, C-reactive protein, low levels of glucose. This suggests PF1+2 levels were associated with cardiac risk factors and progression of atherosclerosis	16
	Plasma	181 asymptomatic men free of overt clinical atherosclerotic disease	PF1+2 upper range (>0.55nmol/L) showed significantly higher IMT, suggests development of atherosclerosis	19
Coronary Heart Disease (CHD)	Plasma	3052 men were recruited and PF1+2 done in 2442 individuals with Northwick Park Heart Study	PF1+2 were higher for those men present with glycine allele	22
Coronary Atherosclerosis	Plasma	225 patients with angina pectoris	PF1+2 levels were increase in patients with angiographically verified coronary atherosclerosis compared to patients with angina pectoris only and normal coronaries.	23
	Plasma	57 patients with verified and graded Coronary artery Disease (CAD) and 21 HC	PF1+2 higher in patients with CAD compared to controls. Also in CAD patients with coronary atherosclerosis (>or=2vessel disease) had significantly higher values for PF1+2 (1.89 vs 1.57nmol/L; p=0.04).	24
Myocardial Infarction (MI)	Plasma	Consecutive patients with unstable angina (n=81) or acute MI (n=32)	PF1+2 significantly higher in patients with unstable angina and acute MI compared with stable angina and healthy controls	25
	Plasma	123 patients with a history of MI	PF1+2 levels were associated with late diastolic filing time and mitral E/A and calculated intima media area	26

	Plasma	246 patients with MI	PF1+2 levels were found to be elevated.	28
Cardiac Surgery	Plasma	100 patients undergoing revascularization of whom 81 underwent shunt angiography	A significant increase was observed immediately after surgery.	32
	Plasma	79 patients undergoing mitral and aortic valve procedures	PF1+2 levels reduced during and after the operation, suggest reduced coagulopathy in minimally invasive valve surgery (MIVS) patients.	33
Atrial Fibrillation (AF)	Plasma	591 patients with non-valvular atrial fibrillation (NVAF) and 129 control subjects	PF1+2 levels increased along with the increase in the risk ($p<0.001$) and were significantly suppressed by warfarin	36
	Plasma	75 patients who underwent radiofrequency catheter ablation and 80 patients in an outpatient clinic	PF1+2 showed modest and inverse association with plasma concentration of rivaroxaban and apixaban in patients with AF.	38
Venous thromboembolism (VTE)	Plasma and Urine	Out of 720 patients, 150 patients present with VTE	PF1+2 elevated in urine and plasma both but present with higher diagnostic accuracy in plasma	6
	Plasma and Urine	VTE was diagnosed in 117 of 591 patients	PF1+2 in plasma and urine reflect thrombin generation exvivo in the same manner. This indicates that urine may be an alternative substitute to quantify a procoagulate state.	51
Stroke	Plasma	55 patients with AF, 20 patients were induced into AF, 20 patients with atrial and 15 were controls	PF1+2 levels increase in stroke patients with AF.	65
	Plasma	4850 patients randomized to treatment with	PF1+2 levels were decreased by 25% with apixaban and by 59% with warfarin	66

		apixaban or warfarin		
	Plasma	431 patients with acute embolic stroke	PF1+2 levels were independently associated with a poor outcome of disease.	67
	Plasma	255 patients with first acute ischemic stroke and 209 controls	Coexistence of large aortic plaques and blood hypercoagulability measured by PF1+2, also associated with an increased risk of recurrent stroke and death	68
Cancer	Plasma Plasma	124 healthy individuals, 86 with low stage primary lung cancer 57 with localized head and neck cancer, 24 non-small cell lung cancer (NSCLC) patients and 24 healthy controls	PF1+2 may be used for identifying hyper-coagulation in cancer patients as it found elevated in cancer patients than healthy controls. PF1+2elevated in cancer patients than healthy controls.	70 71
	IHC in tissue	59 colon cancer patients	PF1+2 were more sensitive hypercoagulability marker than TAT.	73
	Plasma	110 patients with adenocarcinoma of stomach	High PF1+2 shows presence of lymph node metastasis.	75
Pregnancy	Plasma	28 women at first trimester, 33 at 2nd and 32 at 3rd trimester of pregnancy	PF1+2 levels were elevated in all group, therefore, pregnancy can induce thrombosis.	78
	Plasma	21 pregnant women affected by thrombophilia treated with LWMH and 20 untreated normal pregnant women as controls	PF1+2 levels were low in treated pregnant women.	80
	Plasma	14 normal and 29 pregnant women	PF1+2 higher in pre-eclampsia condition	81

		with pre-eclampsia		
Kidney Disease	Plasma	20 Hemodialysis patients with no thrombotic complication and 20 with thrombotic complication	Showed high PF1+2 levels in thrombotic group than without thrombotic complications and controls.	84
	Plasma	66 CKD patients and 36 healthy controls	PF1+2 elevated in CKD patients.	85
	Plasma	484 renal transplant patients, 34 develop VTE and 84 patients without renal history	PF1+2 levels were high in patients with VTE undergoing renal transplant than without renal history.	87
Liver Disease	Plasma	44 patients with cirrhosis and 30 healthy controls	PF1+2 found to be elevated in liver cirrhosis patients than controls.	91
Type 2 diabetes	Plasma	17 microalbuminuric patients and 17 normalbuminuric	PF1+2 were high in microalbuminuric patients and associated with cardiovascular risks.	97
	Plasma	29 with type 2 diabetes on oral drugs and 31 dependent on insulin and 30 without diabetes	PF1+2 were higher in diabetic patients dependent on insulin than with oral drugs and non-diabetic patients.	49
Rheumatoid Arthritis (RA)	Plasma	15 RA patients and 15 RA patients with tocilizumab treatment	PF1+2 were higher in RA patients and PF1+2 level were reduced after tocilizumab treated patients	101

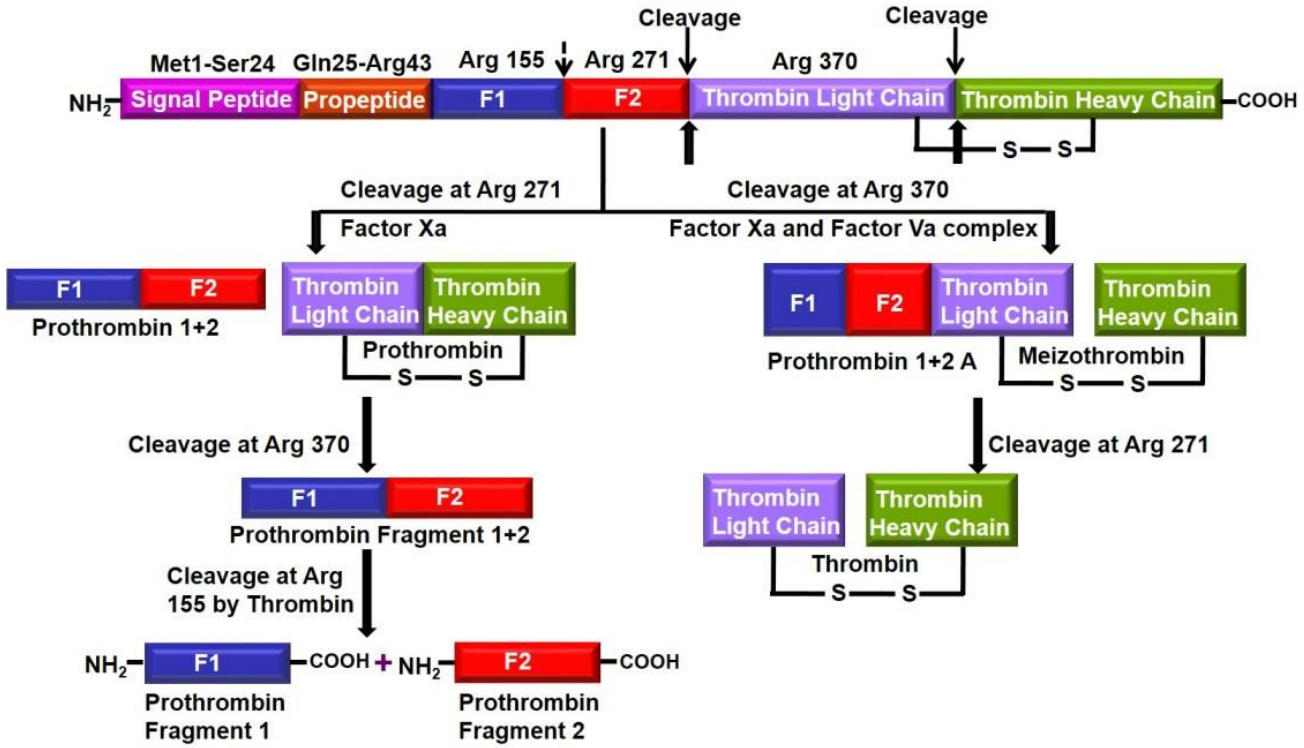

Figure 1: Prothrombin Fragment 1+2 and thrombin generation during prothrombin activation by Factor Xa and cofactors in blood coagulation cascade and prothrombin fragments degradation by thrombin into the prothrombin fragment 1 and 2.

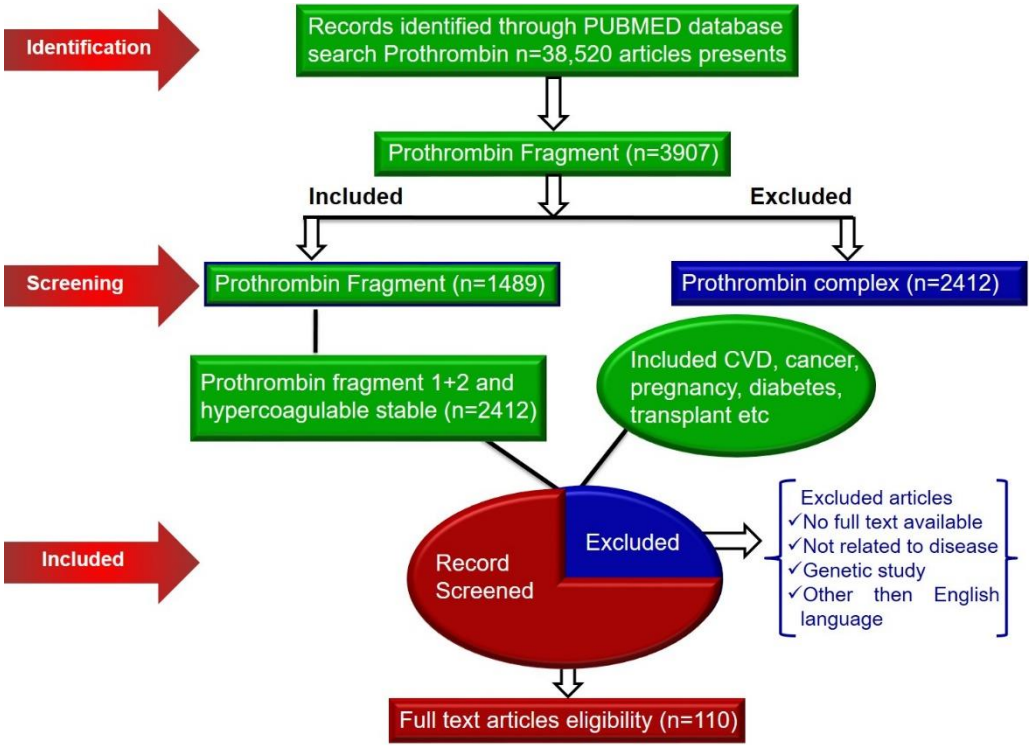

Figure 2: PRISMA-derived flowchart of the literature search