Review

# Novel Diagnostic and Monitoring Tools in Stroke: an Individualized Patient-Centered Precision Medicine Approach

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**Central to the pathogenesis of ischaemic stroke are the normally protective processes of platelet adhesion and activation. Experimental evidence has shown that the ligand-receptor interactions in ischaemic stroke represent a thrombo-inflammatory cascade, which presents research opportunities into new treatment. However, as anti-platelet drugs have the potential to cause severe side effects in ischaemic stroke patients (as well as other vascular disease patients), it is important to carefully monitor the risk of bleeding and risk of thrombus in patients receiving treatment. Because thrombo-embolic ischaemic stroke is a major health issue, we suggest that the answer to adequate treatment is based on an individualized patient-centered approach, inline with the latest NIH precision medicine approach. A combination of viscoelastic methodologies may be used in a personalized patient-centered regime, including thromboelastography (TEG®) and the lesser used scanning electron microscopy approach (SEM). Thromboelastography provides a dynamic measure of clot formation, strength, and lysis, whereas SEM is a visual structural tool to study patient fibrin structure in great detail. Therefore, we consider the evidence for TEG® and SEM as unique means to confirm stroke diagnosis, screen at-risk patients, and monitor treatment efficacy. Here we argue that the current approach to stroke treatment needs to be restructured and new innovative thought patterns need to be applied, as even approved therapies require close patient monitoring to determine efficacy, match treatment regimens to each patient's individual needs, and assess the risk of dangerous adverse effects. TEG® and SEM have the potential to be a useful tool and could potentially alter the clinical approach to managing ischaemic stroke. As envisaged in the NIH precision medicine approach, this will involve a number of role players and innovative new research ideas, with benefits that will ultimately only be realized in a few years. Therefore, with this ultimate goal in mind, we suggest that an individualized patient-orientated approach is now available and therefore already within our ability to use.**

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**Key words:** Ischaemic stroke, Coagulation, Scanning electron microscopy, Thromboelastography, Treatment monitoring

## Introduction

Stroke is considered the second leading cause of dementia and primary cause of chronic disability in the United States[1]. It is a highly prevalent condition with approximately 17 million people worldwide suffering a stroke every year[2], and it is currently the second leading cause of death globally[3]. The rise in population age and the stroke risk factors related to unhealthy lifestyle that are not properly managed have been noted to progressively augment the already heavy burden of this disease[4]. Stroke is classified as either ischaemic, resulting from a thrombotic or embolic occlusion of a blood vessel, or haemorrhagic, which is caused by a rupture or leakage of a blood vessel. Ischaemic stroke is the most common, accounting for approximately 87% of all strokes[5]. Because thrombo-embolic ischaemic stroke is a major health issue, we suggest that the answer for adequate treatment is based on an individualized patient-centered precision

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medicine approach[6]. Because of the vast extent of the various risk factors for stroke and the variability of these factors, an innovative approach is needed. A precision medicine approach may be particularly successful and is already suggested, where researchers aim to use individualized approaches to treat cerebrovascular disorders[7]. In stroke management, it is suggested that big data may be leveraged to identify and manage stroke risk with specific treatments utilizing an improved neuroimaging infrastructure, data collection, and analysis[7]. Risk factors have always played a major role in stroke, but many of the risk factors relate to the general risk factors of cardiovascular disease and metabolic syndrome; thus, making stroke one of the most complex conditions.

In this study, we focus on thrombo-embolic ischaemic stroke and in particular how it is measured via viscoelastic methods. In thrombo-embolic ischaemic stroke, thrombi as well as a hyperactivated coagulation system are involved. We suggest that a combination of viscoelastic methodologies should be used as a personalized patient-centered regime that may eventually be added to the envisaged precision medicine approach. As mentioned in the new approach to precision medicine, this will involve a number of role players with benefits that will ultimately only be realized in a few years. However, with this ultimate goal in mind, we suggest that an individualized patient-orientated approach is now already within our ability.

## Pathogenesis of Thrombo-Embolic Ischaemic Stroke

Thrombo-embolic ischaemic stroke occurs when a vessel becomes occluded resulting in the loss of circulation, often to a vascular region of the brain. The affected region of the brain corresponds to deficit in neurological function after the ictus[8]. Thrombo-embolic ischaemic stroke is classified as a large artery, small vessel (lacunar), or cardioembolic. When atrial fibrillation or a source of embolism can be detected during the examination of a patient, the stroke is said to be cardioembolic, and these strokes cause the greatest mortality and are most often recurring. When no such source can be detected, atherothrombotic ischaemic stroke is diagnosed. Large artery strokes may involve thrombosis in the carotid and vertebrobasilar or cerebral arteries, but can also be cardioembolic[8, 9].

Central to this type of stroke is the role of platelets, their adhesion, activation, and aggregation, together with abnormal coagulation of plasma proteins, eventually resulting in a systemic pathophysiology of haemostasis. Platelet adhesion to the sites of vascular damage and activation and aggregation into a platelet plug are processes of the normal intrinsic pathway of the coagulation cascade and haemostasis, but they also play an integral role in the pathophysiology of atherosclerotic and thrombotic disease[10, 11]. Systemic platelet activation and aggregation are enhanced in the acute phase of thrombo-embolic ischaemic stroke. The greater degrees of enhancement are associated with increased infarct size and poorer prognosis, with decreased response to anti-platelet therapy. Patients whose platelet activation and aggregability do not return to baseline or normal (where baseline values are not available) levels within 3 months after the initial infarction are considered to be at risk of recurrent stroke[11].

## The Role of Platelet Activation, Adhesion, and Aggregation

Studies have shown that thrombo-embolic ischaemic stroke is the result of a thrombo-inflammatory cascade initiated by platelet adhesion and activation, with aggregation and eventual thrombus formation occurring as a side event. Before the exact mechanisms of this cascade can be investigated, it is first necessary to understand the normal platelet activation and adhesion *in vivo*, a process driven by receptor-ligand interactions[10, 12-14]. When a vessel wall is damaged, an interaction between glycoprotein (GP) Ib-V-IX complex and von Willebrand factor (bound to the now exposed collagen) causes circulating platelets to decelerate. Thus, platelets move much closer to the vessel walls, which enable platelet GPVI contact with the exposed collagen. This interaction in turn activates an intracellular signaling cascade that ends with the synthesis and release of platelet agonists. The most significant of these are thromboxane A2 (TXA2) and adenosine diphosphate (ADP). These two factors together with thrombin activate G-protein coupled receptors (GPCRs) on platelet membranes to induce other cellular cascades with the aim of activating integrins, releasing granule contents, and promoting coagulation. Integrins mediate firm platelet adhesion to the extracellular matrix of the vessel wall and promote platelet aggregation. They are present on the surfaces of resting platelets, but at a very low affinity, in order to prevent platelet adhesion to healthy vascular walls.

Once platelets have adhered and are activated, additional platelets must aggregate to form a thrombus. TXA2 and ADP play an additional role in mediating thrombus growth and activating additional platelets. C-type lectin-like type II (CLEC-2) is a transmembrane receptor expressed by platelets and is active

in aggregate stabilization and thrombus growth. CLEC-2 appears to adopt the role of GPVI in platelets recruited to thrombus but that will not bind to collagen. The identity of the CLEC-2 ligand in platelet–platelet adhesion is currently unknown[9, 10].

In case of platelet adhesion during a thrombo-embolic ischaemic stroke, activation and aggregation occur in an abnormal manner. Owing to the pertinent role of platelets in thrombus formation, anti-platelet drugs are of interest for investigation in stroke prevention. Various trials have found that the inhibition of early platelet adhesion processes limits infarct progression, but the inhibition of aggregation has no such effect. Thus, where thrombo-embolic ischaemic stroke progression is concerned, it appears that platelet mechanisms differ from normal thrombus formation[10].

The following points have come to light based on the results of various trials, including those using knock-out mouse models[10]:

- GPVI-depleted individuals show reduced stroke volumes without increased bleeding complications
- GPVI-elevated individuals have increased risk of stroke development
- GPIb and GPVI are important in regulating microvascular thrombus formation and serve as pro-inflammatory molecules, advancing infarct progression
- GPIb binds to factor XII, and this couples platelet activation to the intrinsic pathway of coagulation as well as signaling for inflammation by bradykinin synthesis, a pro-inflammatory peptide hormone
- GPVI induced PolyP (an inorganic polyphosphate) released from platelets. PolyP activates the intrinsic pathway and induces inflammation through factor XII.

Research has confirmed that systemic platelet activation is enhanced in thrombo-embolic ischaemic stroke by performing whole blood (WB) flow cytometry to detect platelet membrane glycoproteins. Of particular interest was the glycoprotein PAC-1. Although the other glycoprotein studies returned to baseline levels (indicating return to baseline of platelet activation) after 3 months, only PAC-1 could be persistently elevated and be used to measure the persistent platelet activation, which was found to be a predictor of recurrent stroke[11]. Thus, these results substantiate that platelet adhesion and activation are of vital significance in ischaemic stroke pathology rather than aggregation and thrombus formation. **Fig. 1A** shows the receptor-ligand interactions in platelet activation, adhesion and aggregation, and the diverging mechanisms in thrombus formation versus thrombo-inflammation.

Haemostasis cannot be achieved without thrombus stability. The formed thrombus can either become embedded in a fibrin network (which forms after tissue factor release has activated the extrinsic coagulation pathway) or break away because of high shear forces, becoming an embolus. Central to the involvement of platelets is an aberrant coagulation pathway physiology, leading to a matted clot consisting of dense fibrin fibers[15-17]. **Fig. 1B** shows the pathway, coagulation pathway, and hypercoagulation events in thrombo-embolic stroke and areas where pathophysiology may result in ultimate hypercoagulation of fibrin fibers and also indicates where typical treatment regimens have an effect. Typical prevention regimes involve antiplatelet treatment using the thromboxane inhibitor Asprin[18] or Clopidogrel (ADP P2Y12 Receptor antagonist[18]) and anticoagulant treatment using Warfarin (target Vit K-dependent coagulation factor[19]). Treatment regimes involve thrombolytic actions (e.g., r-tPA that activates the fibrinolytic cascade[20]). One easy point-of-care technique is the VerifyNow® System (http://www.itcmed.com/products/verifynow-system-platelet-reactivity-test) that assesses platelet reactivity to antiplatelet medications such as aspirin, clopidogrel, and GP IIb/IIIa inhibitors[21-23]. However, in a recent study, it was noted that large, adequately-sized, prospective multicentre collaborative studies are urgently needed to determine whether the comprehensive assessment of antiplatelet therapy improves the ability to predict the risk of recurrent vascular events in cardiovascular patients, including ischaemic stroke patients[22]. Although this technique may be employed in an individualized patient-orientated approach, we propose a more comprehensive approach, which focuses not only on platelet function.

Stroke diagnoses and patient treatment is of great importance to save lives and improve recovery rates. The benefits of treatment are also highly time dependent; thus, effective and accurate diagnostic procedures as well as adherence to inclusion and exclusion criteria are of utmost importance. One of the clinical techniques used is TEG® to assess viscoelastic properties of the coagulation system. Another purely research orientated approach is to study clot formation using scanning electron microscopy (SEM) that may also provide great insights regarding the actual clot ultrastructure. The next paragraphs will discuss these two seemingly diverse techniques and show how they complement each other and how they both may add clinical relevant information in the treatment regime of thrombo-embolic ischaemic stroke in a truly individualized patient-orientated approach.

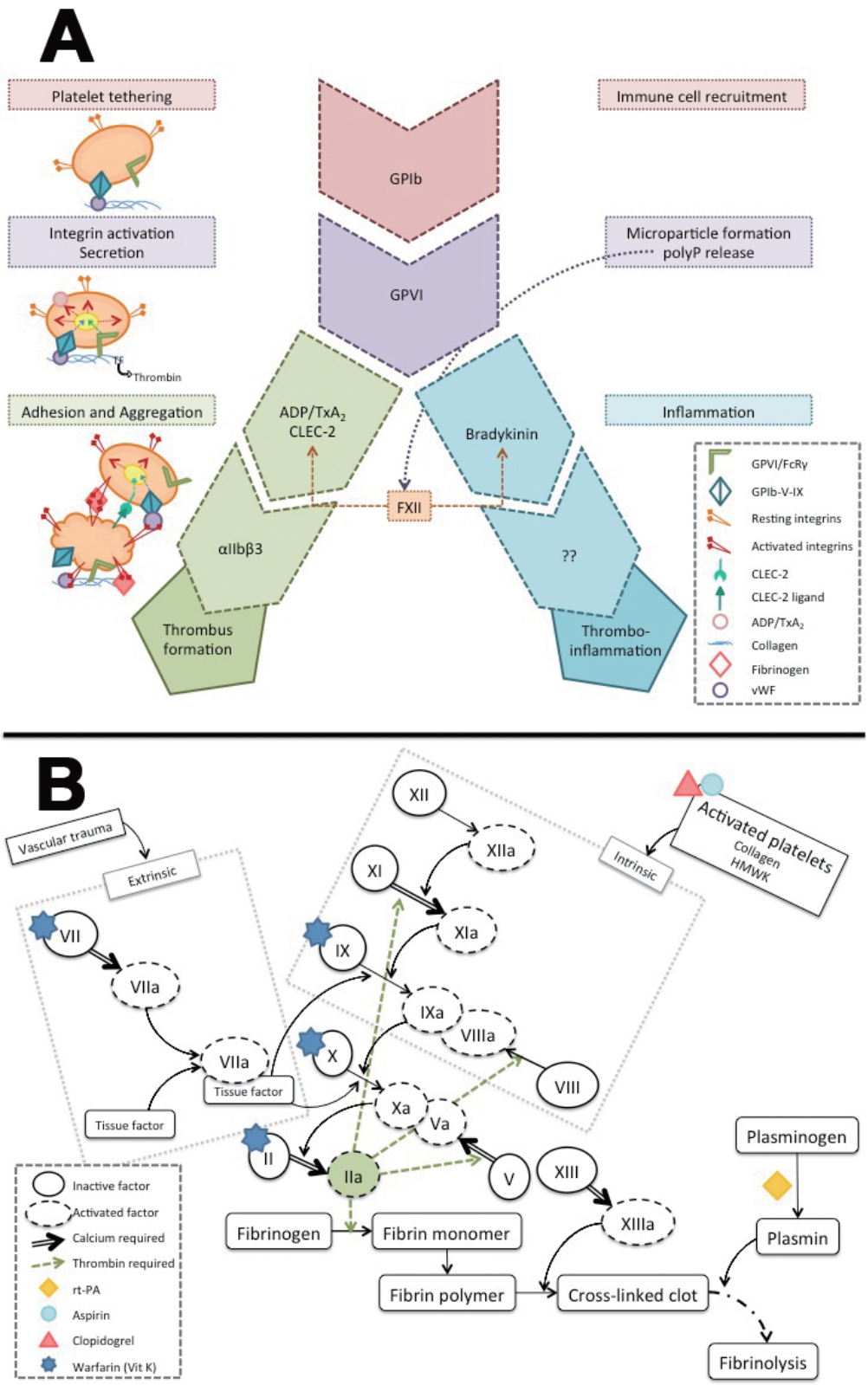


**Fig. 1.** A) Platelet activation and the receptor–ligand interactions in platelet activation, adhesion, and aggregation as well as the diverging mechanisms in thrombus formation versus thrombo-inflammation. B) The coagulation pathway and hypercoagulation events in thrombo-embolic stroke.

**Table 1.** TEG® parameters typically generated for WB and platelet poor plasma

| Whole blood | | | |
|---|---|---|---|
| Parameter | | | Description |
| R | Reaction time | min | Rate of initial fibrin formation |
| K | Clotting time | min | Time until fixed level of clot firmness achieved |
| $\alpha$ | Angle | degrees | Rate of clot growth |
| MA | Maximal Amplitude | mm | Maximum strength/stiffness of the developed clot (platelets play a pertinent role here) |
| G | Shear elastic modulus strength | dynes/cm$^2$ | 5000 MA/(100-MA) |
| LY30* | Lysis 30 | percent | Percentage lysis obtained 30 min after MA |
| Platelet Poor Plasma | | | |
| TMRTG | Time to maximum rate of thrombus generation | min | Time to onset of coagulation |
| MRTG | Maximum rate of thrombus generation | dynes/cm$^2$/s | Velocity of thrombus formation |
| TTG | Clot strength | dynes/cm$^2$ | Thrombus strength |

*LY30 can only be determined with uncitrated whole blood

## TEG®

Traditional coagulation tests, such as prothrombin time (PT), partial thromboplastin time (PTT), and the international normalised ratio (INR), platelet count, and bleeding time are limited to the time of fibrin formation via the intrinsic or extrinsic pathway. These tests provide a measure of a patient's bleeding risk, but fail to assess the risk of thrombus formation[24, 25]. An alternative analysis, i.e., the TEG® analysis describes coagulation, clot formation, and structure in various dimensions. TEG® provides a global assessment of the viscoelastic properties of blood under low shear conditions. Therefore, this technique is a dynamic measure of clot formation, strength, and lysis. In clinical setting, WB is typically used in TEG®[24-28]. It can be citrated or naïve, uncitrated blood. Here we only focus on citrated WB because uncitrated naïve blood requires the patient to be near the machine to prevent coagulation of uncitrated blood before analysis.

Recently, platelet poor plasma (PPP) (prepared from citrated blood) has also been used, albeit only in a research setting. Through modification of the TEG® protocol for WB, it becomes possible to evaluate clot strength as a function of fibrinogen and coagulation factors. This requires centrifugation of WB samples so that platelets (and other cellular components) are removed, but the coagulation-activating factors they produce are left behind. This allows the measurement of thrombus generation parameters with greater accuracy than in a WB sample. The thrombus generation parameters provide a stronger evaluation of coagulation kinetics[29-32].

Tracings are typically obtained by performing WB TEG® and modified TEG® PPP assay on citrated blood. For WB TEG®, 340-µL citrated WB is exposed to 20 µL of calcium chloride ($CaCl_2$) in disposable cups of a TEG® 5000 computer-controlled device (Haemoscope Corp., Niles, IL, USA). To prepare PPP, citrated WB samples are centrifuged for 15 min at 227.5 × g, plasma is aspirated into Eppendorf tubes, which are once again centrifuged at 5600 × g for 10 min. PPP techniques are described in detail in selected references[33-36]. PPP is typically frozen for at least 24 h (or longer until enough samples for a particular study have been collected), thawed, and then exposed to calcium as described above. **Table 1** shows a comparison between citrated WB and PPP parameters generated from the TEG® adapted from Nielsen and co-workers, 2007[37]. TEG® is not the only test capable of quantifying the clotting process in WB, although it is more widely used and results gained have a greater degree of reproducibility.

Alternatives to TEG® are ROTEM (rotation thromboelastometry) and Sonoclot®[24]. TEG® and ROTEM are near interchangeable technologies; however, the mechanical differences in these technologies

prevent comparison of tracings produced because the range values are different. Sonoclot® is more related to overall changes in viscosity during the coagulation process, and the tracing produced by this technology, the "Sonoclot Signature," is considered secondary to TEG® in precision[24].

## TEG® in Thrombo-Embolic Ischaemic Stroke Diagnosis

One of the earliest TEG® studies in stroke was published by Ettinger in 1974. Milton recruited 203 stroke patients and performed TEG® of WB to create frequency distribution curves based on the ratio $\frac{MA}{(R+K)}$, which relates strength of the final clot to the speed of coagulation. Based on an age-matched control population, a normal frequency distribution was defined between 1.6 and 4.0. Hypercoagulability was defined at a ratio of $>4.0$ and was observed after cerebral infaction in 29%−38% of the patients studied[27].

In 1999, the British Journal of Surgery published the results of a study by Handa *et al* where they found that TEG® of WB can be used to differentiate ischaemic from haemorrhagic stroke. The researchers found that the 15−30 min test produced normal TEG®s for haemorrhagic patients, whereas ischaemic patients produced hypercoagulable tracings[38]. The results of these two early studies substantiate the efficacy of TEG® as a diagnostic tool in the critical stage of early stroke diagnosis. As previously discussed, primary cerebral infarction treatment is extremely time sensitive. A TEG® tracing takes 15−30 min to produce, thus providing rapid results in the form of a full hypercoagulation screening of WB currently suggested in the International guidelines for the management of stroke and transient ischaemic attack[39, 40].

## TEG® in Monitoring Treatment Efficacy

The only currently accepted treatment for acute thrombo-embolic ischaemic stroke is recombinant tissue plasminogen activator (tPA), an intravenous thrombolytic agent. IV tPA must be administered within 4.5 h of thrombo-embolic ischaemic stroke onset at a dose of 0.9 mg/kg body weight and at a maximum of 90 mg dosage, of which 10% is administered as a bolus and the remaining is administered as an infusion over 60 min. The benefits of treatment are highly time dependent; thus, effective and accurate diagnostic procedures as well as adherence to inclusion and exclusion criteria are of great importance[39-42].

Elliott *et al* showed pre- and post-tPA TEG® tracings performed on WB of thrombo-embolic ischaemic stroke patients, with the R, K, $\alpha$, MA, G and LY30 values being compared[25]. The R and K times were shorter and $\alpha$ angles were greater in thrombo-embolic ischaemic stroke patients than in controls for the pre-tPA tracings, confirming hypercoagulability in thrombo-embolic ischaemic stroke. The post-tPA tracings showed vast reductions in MA, G, and LY30 compared with pre-tPA tracings, indicating reductions in clot strength and efficacy of tPA for intravenous thrombolysis. However, the post-tPA parameters showed significant variation between patients, especially in lysis (LY30). This indicates that tPA efficacy is not uniform at the currently approved weight-dependent dosing regimen after initial bolus[25]. Despite the obvious significance of platelet activation in ischaemic stroke, very few antiplatelet treatment strategies have been approved for secondary stroke prevention because of the low efficacy and severe side effects. Approved treatments as per South African, United States, and European guidelines are Aspirin and Clopidogrel[40].

TEG® has been used to investigate the efficacy of this same treatment regimen in secondary ischaemia prevention. The results showed that the current "one size fits all" dual treatment regimen of clopidogrel and aspirin fails to prevent bleeding and ischaemia as it assumes that all patients experience the same degree of platelet activation and haemostasis. In fact, several studies on the pharmacodynamics of clopidogrel have shown vast variability in responsiveness across patients[26, 28, 43]. The results obtained by Elliott *et al* discussed above also indicated that TEG® of WB could be used as a screening tool in patients at risk of vascular disease. Based on the pre-tPA tracings of thrombo-embolic ischaemic stroke patients, the researchers could conclude the following: coronary artery disease, blood glucose, and platelet count were positively correlated with higher G values; coronary artery disease was associated with a longer R time; male gender, INR, and platelet count were negatively correlated with MA; and small vessel occlusion, INR, and hemoglobin were positively correlated with $\alpha$ angle[25]. These results suggest that monitoring the parameters G, R, MA, and $\alpha$ would be of interest in vascular and metabolic disease patients. By performing regular TEG® tests it may be possible to detect increasing risk of ischaemia and hemorrhage, either as first time events, recurrences, or adverse effects of procedures related to their conditions[25, 28, 43]. TEG® can also be used to detect tissue factor (TF) pathology[37], typically observed in atherosclerosis patients[44].

However, there are cases where TEG® analysis is not suitable, e.g., when kaolin and tissue factor activa-

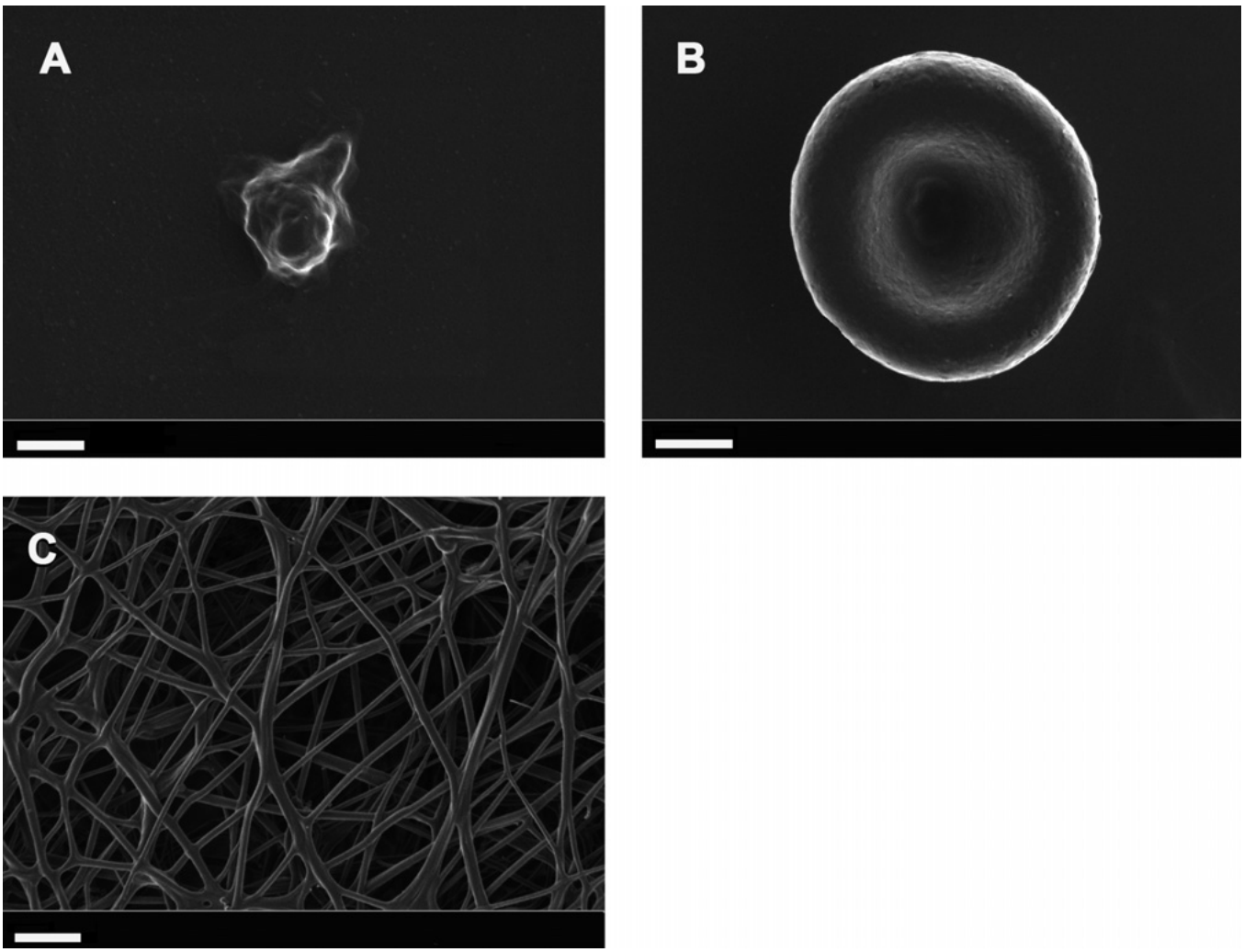


**Fig. 2.** A–C) SEM of WB and platelet poor plasma from a healthy individual. A) Platelet in WB B) Erythrocyte in WB C) Extensive fibrin fiber network created with platelet poor plasma and added thrombin. Scale bar: 1 µm

tion is used in patients using Warfarin[45, 46]. The mechanism of action of Warfarin is well characterized. Warfarin inhibits the synthesis of vitamin K-dependent coagulation enzymes Factors II (FII), VII (FVII), IX (FIX), and X (FX)[47] (see **Fig. 2**). Nielsen *et al* discussed in detail the mechanism responsible for the inadequacies of Warfarin-based therapy in the setting of blood–biomaterial interfaces and mentioned that it is likely the lack of enzyme-specific inhibition of key proteins that is involved with contact activation[47].

In some experiments, kaolin is used to reduce the time to trace generation. When kaolin and TF were used, patients on Warfarin showed normal TEG® tracings. Therefore, blood tested using kaolin activation in TEG® research is not necessarily useful, as poor correlations between kaolin activated and blood have been found using the TEG®[48]. However, if thrombin-activated coagulation in the TEG® together with added $CaCl_2$ is used, it will result in the bypass of all factors (especially FII) affected by Warfarin. Here we only propose the use of $CaCl_2$ and not kaolin or TF activation. However, the use of TEG® using naïve (uncitrated) WB from patients using Warfarin needs to be further investigated.

Both WB and PPP analysis can provide valuable additional information during initial diagnoses and also after treatment to follow the effects of the treatment regimes.

## SEM in Thrombo-Embolic Ischaemic Stroke

SEM is a specialised, high resolution method that is used to examine surfaces on high magnification. SEM analysis has shown the fibrin network and plate-

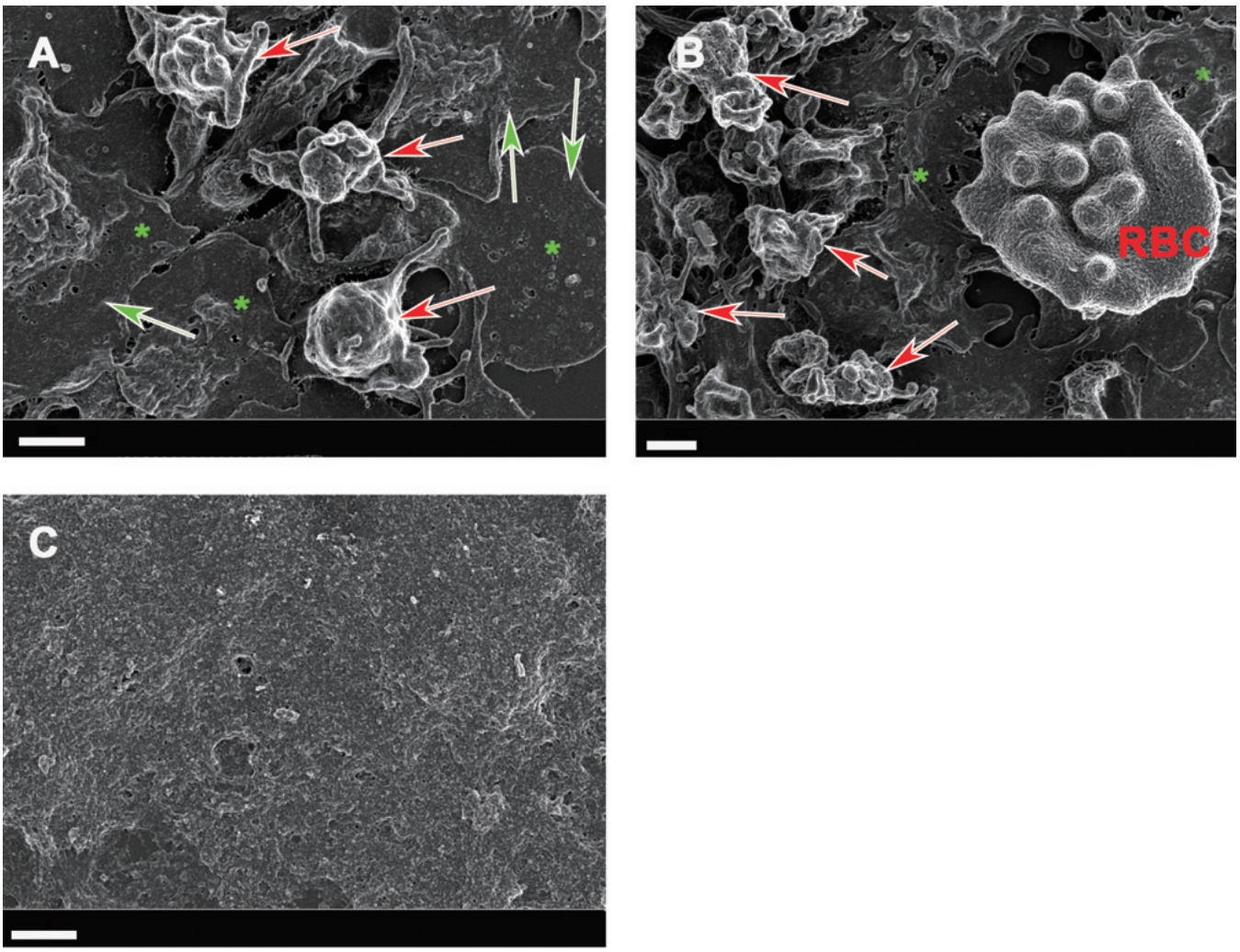


**Fig. 3.** A–C) SEM of WB and platelet poor plasma from a diagnosed thrombo-embolic stroke patient. A) Hyperactivated platelets in WB, showing pseudopodia and extensive spreading B) Typical eryptotic erythrocyte in WB, surrounded with hyperactivated platelets C) Extensive fibrin fiber network created with platelet poor plasma and added thrombin where matted dense layer is visible with little individual fibrin fibers noted. Scale bar: 1 µm. Eryptotic erythrocytes are shown with label RBC; Red arrows: activated platelets, with pseudopodia; Green arrows and green asterisk: hyper-coagulated platelets with extensive spreading.

let and erythrocyte morphology to be drastically altered in stroke[15, 16, 49-52], showing thrombo-embolic ischaemic stroke to be a hypercoagulable or prothrombotic condition[16]. This technique provides detailed, high resolution images that can show specific changes to ultrastructure. This includes the following:

• SEM of WB, where detail platelet and erythrocyte structure is observed

• SEM of WB clot structure when thrombin is added to observe the fibrin fiber with cellular interactions

• SEM of PPP with added thrombin, where only fibrin fibers are observed in the absence of platelets or other cellular components

Therefore, SEM complements TEG®, thus making it a useful tool in comparative visco-elastic analysis. Therefore, SEM and TEG® in tandem provide a holistic approach to investigate the coagulation profile in thrombo-embolic ischaemic stroke. Examples of the two techniques are presented in the figures. **Fig. 2A** shows a typical health platelet with little spreading, open canicular pores, and no pseudopodia. **Fig. 2B** shows a typical healthy erythrocyte, and **Fig. 2C** shows an extensive fibrin network created with added human thrombin. These figures are representative micrographs chosen from our database of hundreds of micrographs

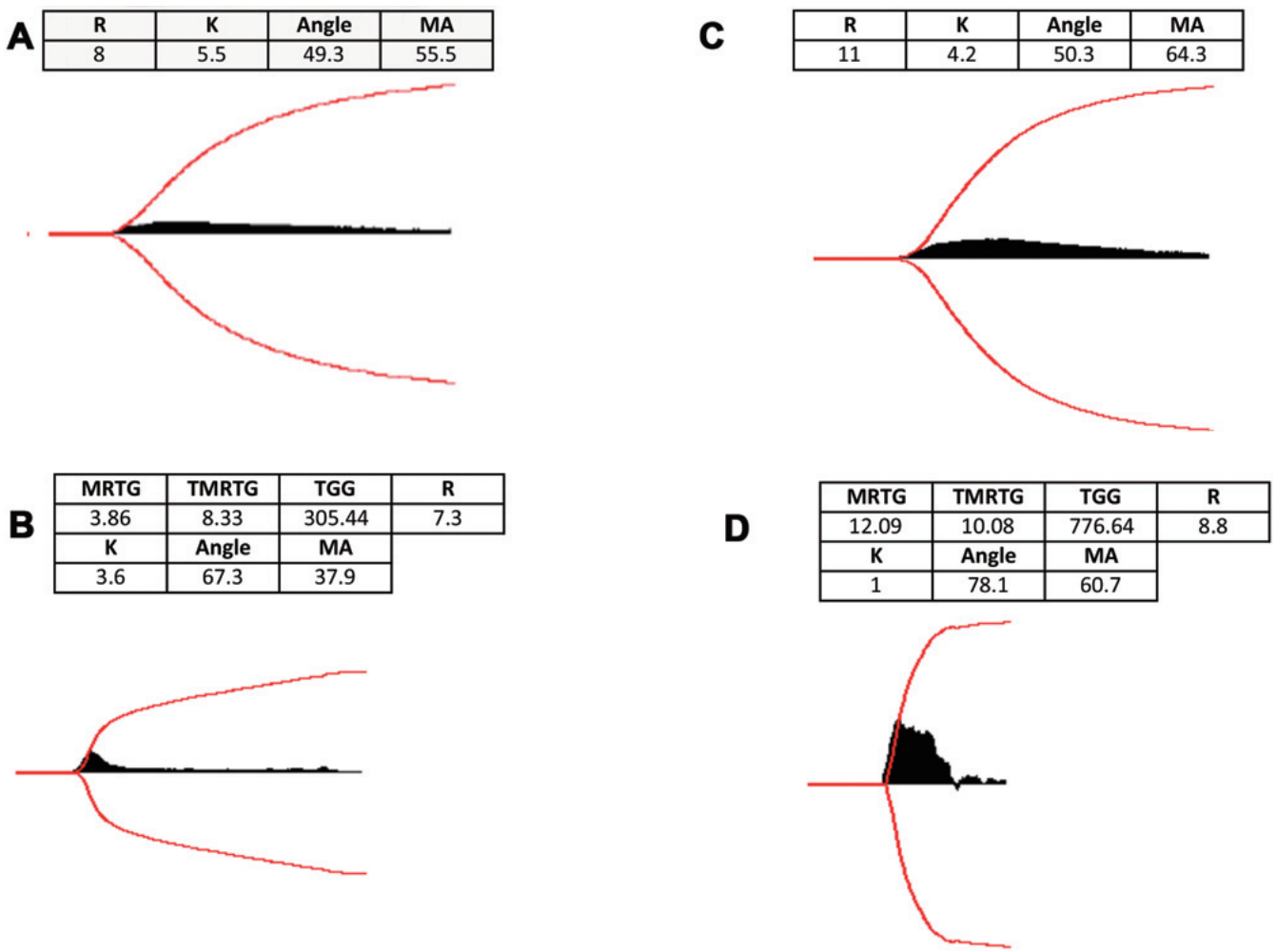


**Fig. 4.** TEG® of WB (A) and PPP (B) from healthy individual and a stroke patient; WB (C) and PPP (D).

from healthy individuals. **Fig. 3A–C** shows micrographs of typical morphology from thrombo-embolic stroke and is representative of our stroke patient database created since 2009. **Fig. 3A** is a micrograph from WB smears where over-activated, spreaded platelets are seen. In **Fig. 3B**, WB smear shows an eryptotic erythrocyte with overactivated platelets, and **Fig. 3C** shows a matted thickened dense fibrin fiber network, created from PPP and added thrombin. The term eryptosis is newly described and recently discovered phenomenon; it is a type of suicidal death of erythrocytes characterized by erythrocyte shrinkage, blebbing, and phospholipid scrambling of the cell membrane. Adhesion of eryptotic erythrocytes to the vascular wall may also lead to impairment of microcirculation. For a detailed discussion on eryptosis refer to various publications by Lang and Quadri[53-56]. The additional information that SEM analysis can add to diagnosis and follow-up of patients includes ultrastructural details regarding cellular structure or erythrocytes, platelet activity as well as fibrinogen coagulation patterns, and most importantly, that Warfarin therapy does not impact SEM methods.

**Fig. 4A** and **B** show a typical healthy WB and PPP TEG® traces, and **Fig. 4C** and **D** show TEG® traces from WB and PPP of a stroke patient. The PPP TEG® analysis focuses on only the coagulation-activating factors, and therefore shows only the viscoelastic properties of the clot structure. Therefore, the emphasis is particularly on the pathology in these coagulation-activating factors and correlates directly with the SEM micrographs produced with PPP and added thrombin. **Fig. 3C** shows a dense matted fibrin layer, and this correlates well with the much increased MRTG, TMRTG, and TGG values of the TEG® in thrombo-embolic ischemic stroke. WB TEG® analy-

sis focuses on the entire blood hemostatic activity, and this correlates with the WB SEM smears, where both platelets and erythrocytes structure and pathologies can be seen. Eryptotic and hyperactivated platelets are central to the hypercoagulation and pathology noted in thrombo-embolic ischemic stroke. The significantly increased MA in both the stroke WB and PPP TEG® analysis (**Fig. 4C** and **D**) is indicative of platelet hyperactivity, and this correlates perfectly with the SEM analysis of WB (**Fig. 3A** and **B**).

## Conclusion

Clinicians and researchers both agree that because of the delicate balance between thrombotic and bleeding events, it is important to comprehensively understand the associations between the patient's baseline risk factors and vascular complications for effective clinical management[57]. TEG® is capable of measuring the viscoelastic properties of a thrombus from WB and PPP by dynamically measuring thrombus formation, strength, and lysis; therefore, TEG® is a very useful technique to show the kinetic visco-elastic properties. Kaolin activation is not suitable for TEG® studies of patients on Warfarin; however, here we do not suggest the use of kaolin in activation of the coagulation system. The usefulness of the TEG® using naïve and $CaCl_2$ activated WB and PPP of patients on Warfarin needs to be investigated before a final decision can be made. However, except for the (possible) exclusion of patients on Warfarin (until the exact TEG® methodology is further researched and refined), both TEG® and SEM can be used to study any patient profile.

SEM is useful to show the extent of platelet structural damage and interactions between erythrocytes/platelets/white blood cells and fibrin fibers. Platelets are hyperactivated in the acute phase of thrombo-embolic ischaemic stroke. Treatment typically is focused to reduce the activation, and there are very few options available to the practitioner to determine how effective it actually is in reducing apoptotic/eryptotic and necrotic activity of platelets and erythrocytes. Thus, SEM is a direct visual diagnostic method. When thrombin is added to both WB and PPP, it visually shows how the clot looks like and it can directly be compared to the kinetic results obtained from TEG®. In conclusion, both TEG® and SEM results can be used in combination and as a part of the current diagnoses regimes, but more importantly, to follow the patient's recovery in the presence of a specialized treatment regime. This may be one step closer to an optimal health care system, where an individualized patient-centered approach is followed for each patient with precision medicine being the ultimate goal.

## Ethical Considerations

Ethical clearance was obtained from the University of Pretoria Human Ethics Committee for the use of blood from thrombo-embolic ischaemic stroke patients and healthy volunteers. All participants completed informed consent forms.

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## Conflicts of Interest Statement

There are no conflicts of interest to declare.

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