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Review Article

Non-invasive imaging techniques for the differentiation of acute and chronic thrombosis

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ABSTRACT

Thrombosis is the localized clotting of blood that can occur in both the arterial and venous circulation. It is a key factor in the pathogenesis of acute coronary syndrome, myocardial infarction and stroke and the primary cause of deep vein thrombosis and pulmonary embolism. Rapid and accurate diagnosis of thrombotic episodes is crucial in reducing the morbidity and potential mortality associated with arterial and venous thrombotic disorders by allowing early targeted therapeutic interventions. From a clinical perspective the ability to accurately assess the age and composition of thrombus is highly desirable given that anticoagulation and, in particular, fibrinolytic therapies are more effective in treating acute rather than chronic thrombosis. While there are no imaging tests used in routine clinical practice that can reliably determine the age of thrombus and differentiate between acute and chronic thrombosis there are several emerging non-invasive techniques that can provide an indication of the age of a thrombus depending on its location in the body. Examples of techniques developed for venous thrombosis include Doppler imaging with venous duplex ultrasonography, ultrasound B-mode imaging integrated with IER (intrinsic mode functions-based echogenicity ratio), elastography, scintigraphy imaging with ^{99m}Tc-recombinant tissue plasminogen activator (^{99m}Tc-rt-PA), and magnetic resonance direct thrombus imaging (MDRTI). Magnetic resonance imaging (MRI) has been used to noninvasively detect and differentiate acute and chronic arterial and venous thrombosis. These methods have limitations that need further investigation to enable cost-effective and clinically relevant treatment practices to be established in the future. This review will discuss the difference between acute and chronic thrombosis and the role of non-invasive imaging techniques in discriminating between the two.

1. Introduction

Thrombosis is the localized clotting of blood that can appear in the arterial and venous circulation [1]. Venous or arterial thrombosis risk increases exponentially in men and women with age, due to increased comorbidity as well as the effects of aging as a process [2]. The composition of thrombi changes over time resulting from a complex interplay between coagulation factors, proteases, cytokines, leukocytes, and other factors. Thrombi undergo alteration through four stages, which are induction, acute fibrin dominant, intermediate, and chronic connective tissue dominant stages. All four phases are likely to be present at varying degrees although some phases may dominate [3].

Arterial thrombosis may occur as a complication of atherosclerotic disease or *via* thromboembolism – such as cardiac source embolism from atrial fibrillation and or paradoxical embolisation [4]. Atrial fibrillation is a type of abnormal heart rhythm of the heart, which can cause thrombosis that can migrate and block blood supply to vital organs, for example in the cerebral circulation leading to a stroke. Paradoxical embolism is a clinical phenomenon of a thrombus originating from the venous system and travelling into the systemic arterial circulation through a right to left cardiac shunt [4].

Arterial atherosclerotic thrombosis usually occurs after the erosion or rupture of a vulnerable atherosclerotic plaque leading to the formation of platelet-mediated or platelet-rich thrombi (white clots), which can occlude blood vessels [5] resulting in tissue ischemia [6] Acute arterial thrombosis is one of the focal causes of most myocardial infarctions and is the cause of most strokes [1]. Platelets circulate in the blood and quickly form a primary haemostatic plaque at the sites of vascular injury [7]. Platelets that are rapidly conscripted to the site become activated and release granules that further support platelet recruitment, adhesion, aggregation, and activation [1]. Exposure of blood to tissue factor (*i.e.* factor III), a protein which is present at high

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concentration in atherosclerotic plaques ultimately generates fibrin, the main protein component of the thrombus [8], initiates the coagulation cascade, which can happen as quickly as 3 min after the unstable atherosclerotic plaque rupture [9].

Arterial atherosclerotic thrombosis is a process that can take hours or days to form singularly within the vessel lumen [10]. The spatial association between the onset of acute coronary events and the arterial thrombi maturation is not well-known even though the pathophysiology of the thrombosis has been comprehensively investigated [11,12]. Thrombi formed at the site of the plaque can undergo various stage of healing process and organize to form thrombi that may be asymptomatic. This process can take place 7 days after the thrombus formation [12]. However, multiple small and non-occlusive organizing arterial thrombi that persist for days or even weeks can lead to acute coronary occlusion if not being treated immediately [12]. This is because fresh arterial thrombi may latch on the already formed older/organizing non-occlusive thrombi in the vessel lumen and become occlusive [13]. Most chronic/organizing arterial thrombi are resistant to lysis by standard fibrinolytic therapy due to intensive fibrin formation [14]. Approximately two-thirds of coronary thrombi in sudden coronary deaths are organizing, particularly in young individuals—especially women, who perhaps might require a different strategy of treatment.

Venous thromboembolism which consists of deep vein thrombosis (DVT) and pulmonary embolism (PE) is the third most common cause of cardiovascular-associated death, after myocardial infarction and stroke [1]. Venous thrombosis can affect people of various ages [15]. DVT commonly originates in the large veins of legs. Pulmonary embolism (PE) may occur when a deep vein thrombus breaks away, moves in the pulmonary circulation and lodges in a pulmonary artery causing obstruction to blood flow and distal ischaemia and tissue necrosis in the pulmonary parenchyma. Venous thrombi are rich in fibrin and erythrocytes [1]. Venous thrombosis typically occurs at venous valvular sinuses, where changes in blood composition and/or changes to the blood vessel promote thrombosis, thus reducing or blocking blood flow [16].

An acute venous thrombus, or a fresh thrombus, is composed primarily of a dense fibrin mesh that persists for about 5 to 7 days and cell layers following the thrombus induction (Fig. 1A). Red blood cells and platelets are trapped within the thrombus during fibrin polymerization and crosslinking. Importantly, collagen is rarely seen in an acute thrombus. [3]. Thus, acute venous thrombus is highly sensitive and susceptible to anticoagulant and fibrinolytic therapy [17,18]. Nevertheless, fibrinolysis does not disintegrate the cellular component of a thrombus. Red blood cells may continue to build up the bulk of acute thrombi while platelets, activated by tissue factor and other inflammatory signals, express P-selectin that facilitates the infiltration of leukocytes such as neutrophils and monocytes [19]. Venous thrombi undergo alteration through four stages: induction, acute fibrin dominant, intermediate, and chronic connective tissue dominant stages. Histological observation of *in vitro* thrombus structure indicates that the number of red blood cells (RBC) decreases with thrombus age [20] - a finding also demonstrated with *in vivo* thrombus [11,22–24].

Older or organized venous thrombi, appear at 7–10 days after thrombus initiation; they are initially observed at the periphery of a thrombus and this progressively moves inward but with great disparity [3]. As a venous thrombus undergoes continued organization over time, collagen deposition becomes more pronounced forming a fibrotic collagenous framework that is more resistant to fibrinolytic treatments (Fig. 1B). Infiltration of macrophages and monocytes after leukocytes creates a well-defined microvessel network [19]. The rapidly accumulated fibrin that is trapped inside a thrombus [25] stabilizes *via* crosslinking processes leading to the formation of mature venous thrombus. Neutrophil extracellular traps (NET) in aging thrombus may bind to red blood cells and promote platelet aggregation *in vivo* [26] that contributes to the thrombus stability, rendering older venous thrombosis highly resistant to thrombolytic treatment [27].

Pharmacological agents used to treat thrombosis include antiplatelet therapies, anticoagulants and thrombolytic (fibrinolytic) therapies. Antiplatelet drugs are the cornerstone therapy used in atherosclerotic cardiovascular disease where they reduce the rates of subsequent or incident myocardial infarction and stroke by inhibiting the activation and aggregation of platelet rich thrombi. Anticoagulant therapies are the mainstay of therapy for venous thrombosis and arterial thromboembolism patients. In patients with DVT and or PE, anticoagulant therapy significantly reduces the subsequent risk of pulmonary embolism or further deep vein thrombosis. Anticoagulants also significantly reduce the incidence of arterial thromboembolism and ischaemic stroke in patients with atrial fibrillation. Thrombolytic drugs, such as tissue plasminogen activator, streptokinase, and urokinase are used to dissolve thrombi in selected patients with myocardial infarction, ischaemic stroke, pulmonary embolism and extensive deep vein

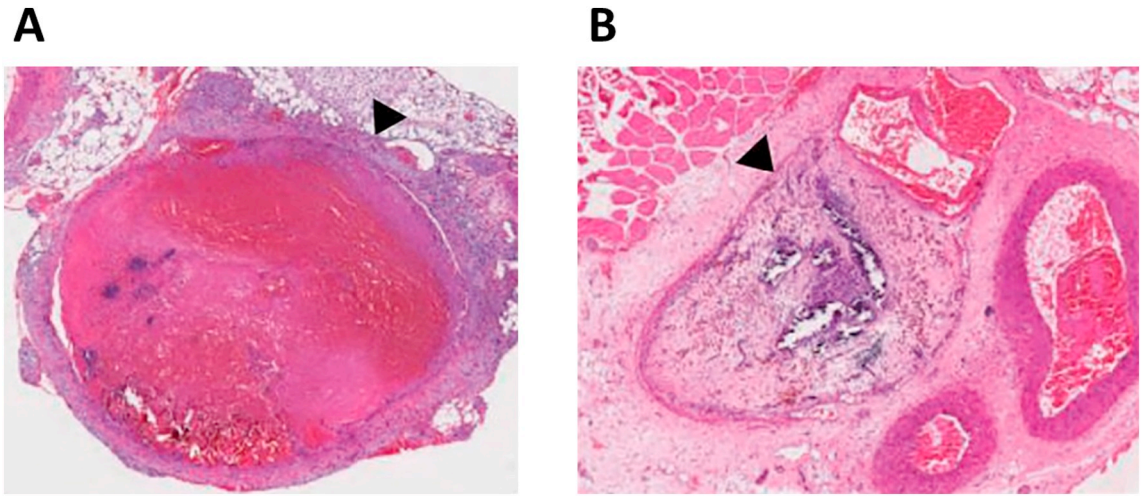

Fig. 1. Hematoxylin and eosin staining of 5-micron sections of human femoral veins. (A) Vein containing a 3 days old thrombus. Black arrowhead indicates the vein wall. (B) Vein containing a 30 days old thrombus with significant fibrosis. Black arrow indicates the chronic fibrotic thrombus. Images are 100× in magnification. Reproduced with permission [3].

thrombosis *via* catheter-directed therapy [28] but carry risks of potentially life-threatening hemorrhage [29]. Importantly, the effectiveness of anti-coagulant and thrombolytic therapies decreases over time (post the formation of the thrombi) due to dynamic changes in the composition of a thrombus [3].

Given the time dependent nature of thrombosis and the potential risks of pharmacological treatment, distinguishing between acute and chronic thrombus may allow for more personalized therapy, for instance by providing more intensive treatments including fibrinolysis in cases of acute thrombosis and withholding therapy in chronic thrombosis where lysis is likely to be relatively ineffective.

This review will focus on different types of non-invasive diagnostic techniques that have been developed to distinguish the acute and chronic nature of both arterial and venous thrombosis.

2. Non-invasive diagnostic techniques developed to distinguish between old and new thrombosis

Clinicians currently use a combination of clinical history and examination, laboratory markers, risk scores and imaging methods to estimate the age of a thrombus. Patient history alone is unreliable and clinical findings typically vary from patient to patient due to differences in collateral circulation and other patient factors including prior episodes of thrombosis. There are no validated or standardized algorithms to evaluate thrombosis age on clinical grounds [3]. However, several promising techniques to distinguish the thrombosis according to its age and location have been developed and are under investigation and evaluation.

2.1. Ultrasound techniques

Ultrasound imaging is a first-line diagnostic technique for screening thrombus as it is cost-efficient for routine clinical evaluation of thrombus age [20]. Doppler ultrasound is widely used to diagnose peripheral venous thrombosis as it is inexpensive, noninvasive, and does not use ionizing radiation [31,32]. Doppler ultrasound has the potential to distinguish between acute and chronic venous thrombosis by utilizing color-flow Doppler imaging and spectral Doppler waveform analysis but the results may be affected by confounding conditions such as obesity, edema and hip or knee arthroplasty [33]. Venous duplex ultrasonography (VDU) that combines color flow Doppler imaging with compression ultrasonography [25] is the current gold standard for DVT. The duplex ultrasound imaging is the combination of traditional ultrasound that uses sound waves that bounce off blood vessels to create pictures; and Doppler that records sound waves reflecting off moving objects, such as blood, to measure their speed and other aspects of how they flow. VDU includes two distinct diagnostic criteria to distinguish between acute and chronic thrombosis that are the spectral/color Doppler signal and the incompressibility of the vein. VDU has a range of sensitivity around 56% to 100% and specificity ranged from 94% to 100% [35].

Features favoring acute rather than chronic venous thrombosis by VDU include occlusive rather than non-occlusive hypoechoic thrombus in a distended vein. The presence of non-occlusive thrombus, a small contracted vein, hyperechoic thrombus and the presence of well-established collaterals are more suggestive of chronic venous thrombosis. Veins with acute thrombi normally show partial or no compressibility without indemnities and are inflated in the presence of a hypoechoic thrombus in the vessel lumen [33]. In VDU, the primary diagnostic criteria for acute venous thrombi is non-compressibility of the vein with the complete absence of spectral or color Doppler signal (Fig. 2A). In chronic thrombus area, the vein is usually incompressible, irregular and has echogenic thrombus appended to venous wall with the development of indemnities. However, chronic venous thrombus ultrasound shows compressible vein in the presence of Doppler spectral or color flow (Fig. 2B) [33]. VDU replaced phlebography and computed tomography venography (CTV) that were previously used to diagnose several forms of venous thrombosis. They have been used to diagnose several forms of venous thrombosis. Findings such as venous obliteration and fibrotic bands indicate a chronic venous thrombus [36]. However, phlebography and CTV are expensive, invasive, not sensitive, involve radiation exposure and have risks of contrast-related complications, therefore are less frequently used clinically [3]. VDU is readily available, quick, cost effective and noninvasive, however, it is difficult and less sensitive in patients with obesity, edema, tenderness, recent hip or knee arthroplasty, overlying bandages and immobilization devices [33].

Ultrasonography (B-mode image) is the combination of duplex ultrasound imaging with the venous compression technique that was developed and clinically used to examine thrombi [37]. B-mode refers to brightness mode ultrasound that is made by a linear array of transducers simultaneously scanning a plane through the body that can be viewed as a two-dimensional image on screen. B-mode ultrasonography consists of traditional and Doppler ultrasound that allows the recording and measurement of blood flow in the vein; and compression technique that enables the measure of vein incompressibility in the presence of thrombosis [38,39]. The signal intensity analysis of B-scan enables the evaluation of the thrombus structure and age as the backscattered ultrasounds signals from the red blood cell (RBC) and the corresponding signal intensity may be affected by the change of RBC content in thrombi over time. This technique was proposed based on the histological observation of *in vitro* thrombus structure in which the number of RBCs decreased with thrombus age [20] which was consistent with *in vivo* thrombus [11,22–24]. Nevertheless, B-scan is highly dependent on system gain that determines amplitude signal gains and corresponding image brightness. Every user in different clinical settings may use different gains for screening that gives diverse interpretations for tissue echogenicity. A conventional ultrasound B-scan that can describe the signal intensity without significant effect of system gain is desirable to effectively assess thrombus age [20].

Fang et al. [20] proposed a conventional ultrasound B-mode image integrated with intrinsic mode function-based echogenicity ratio (IER) approach that could establish personalized thrombolytic therapies. The study showed that the envelope amplitude value of aged thrombus was smaller than that of fresh thrombus (Fig. 3). Empirical mode decomposition (EMD) is a key component of Hilbert-Huang transform (HHT) which is an adaptive time-frequency analysis method for non-linear and non-stationary data [40,41]. EMD of the ultrasound signal was used to decompose the signal into a set of intrinsic mode functions (IMF) that was further used to calculate IER *via* Hilbert transform. EMD does not require a signal base for decomposition and all signals would have a similar gain effect since it arises from the same measurement system. In the range of 15 to 30 dB of system gain, the proposed average IER varies between 8 and 10 for newly formed thrombi and around 3 for aged thrombi (P value < 0.05) [20]. This proposed IER is less affected by system gain in describing the change in thrombus echogenicity during thrombus maturation. The IER defined by EMD may provide an objective evaluation of thrombus age with the conventional B-mode image in clinical application, however the sensitivity of IER in detecting different tissue echogenicity needs to be improved before this method can be applied in clinical setting.

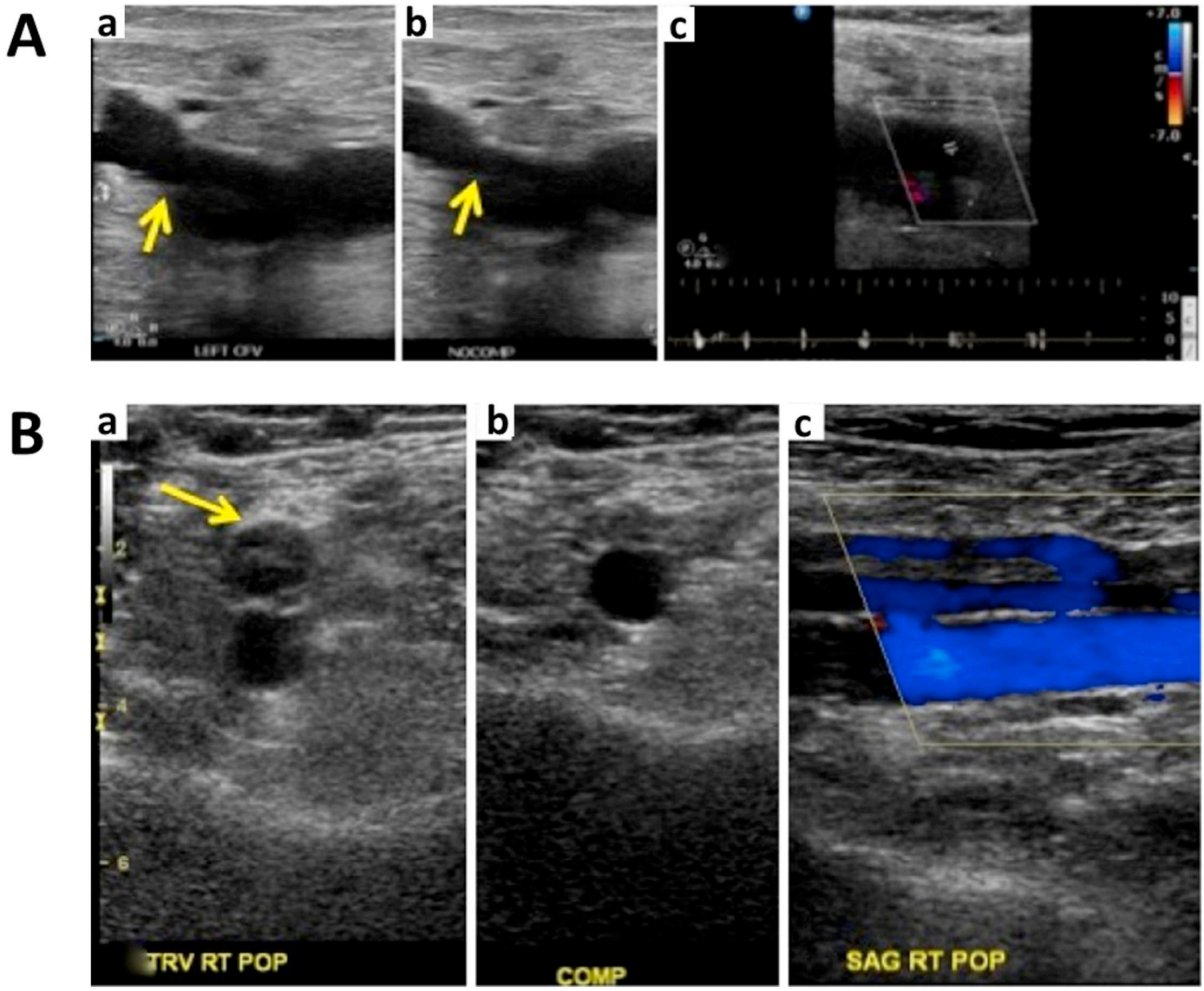

Fig. 2. Venous duplex ultrasound of deep vein thrombosis. (A) Acute deep vein thrombosis. Gray scale ultrasound examination of a patient's left common femoral vein demonstrates an enlarged (arrow in a), non-compressible vein (arrow in b) with low-level intraluminal echoes. Corresponding color flow and spectral Doppler (c) suggest no flow within the vein. (B) Chronic recanalized vein thrombosis. Gray scale ultrasound examination of the right popliteal vein demonstrates echogenic venous wall and a compressible lumen with an eccentric linear area of echogenic material (arrow in a). Doppler flow is noted around this linear area of echogenic material. Reproduced with permission [33].

2.2. Elastography

Ultrasound, normal magnetic resonance imaging or CTV cannot give direct information on tissue rigidity, a characteristic that evolves as a thrombus matures [25]. Elasticity imaging techniques such as elastography can non-invasively estimate the stiffness and tissue resistance to the applied force by using Young Modulus which is a ratio of stress to strain measurement, expressed in units of pressure, computes the amount of deformation of an entity provided with an external force [25]. Elastography techniques have been widely used in liver imaging, particularly for cirrhosis assessment. Physicians have recently adopted the technique to characterize a variety of other tissues such as brain, breast, prostate, thyroid, muscles and thrombus. Few studies have proved that the techniques are promising accurate and cost-effective, which would be feasible in a clinical setting. Elastography allows assessment of both quantitative and semi-quantitative elastic properties of tissues which is helpful to ascertain the thrombus pathological age [3]. Mature thrombus toughens overtime as it undergoes progressive collagen deposition and steady fibrin cross-linking [32,42], creating a gradual reduction in thrombus elasticity. Strain (SE) and shear wave (SWEI) elastography techniques are commonly used to investigate venous thrombosis. Yi et al. [43] used SE technique to assess clot age differentiation in DVT patients. The strain ratio of the chronic and subacute thrombosis group was greater than the acute thrombosis group. SWEI technique was first used to evaluate the induced thrombosis in animal models by Mfoumou et al. [44] (Fig. 4). Their results demonstrated that Young's modulus increased with thrombus maturity and this finding was further supported by the work of Liu et al. [45]. Both techniques still require further studies with a large cohort of human participants to verify the accuracy and reproducibility of the data generated in the previous studies and to establish a cost-effective procedure for clinical practice [25].

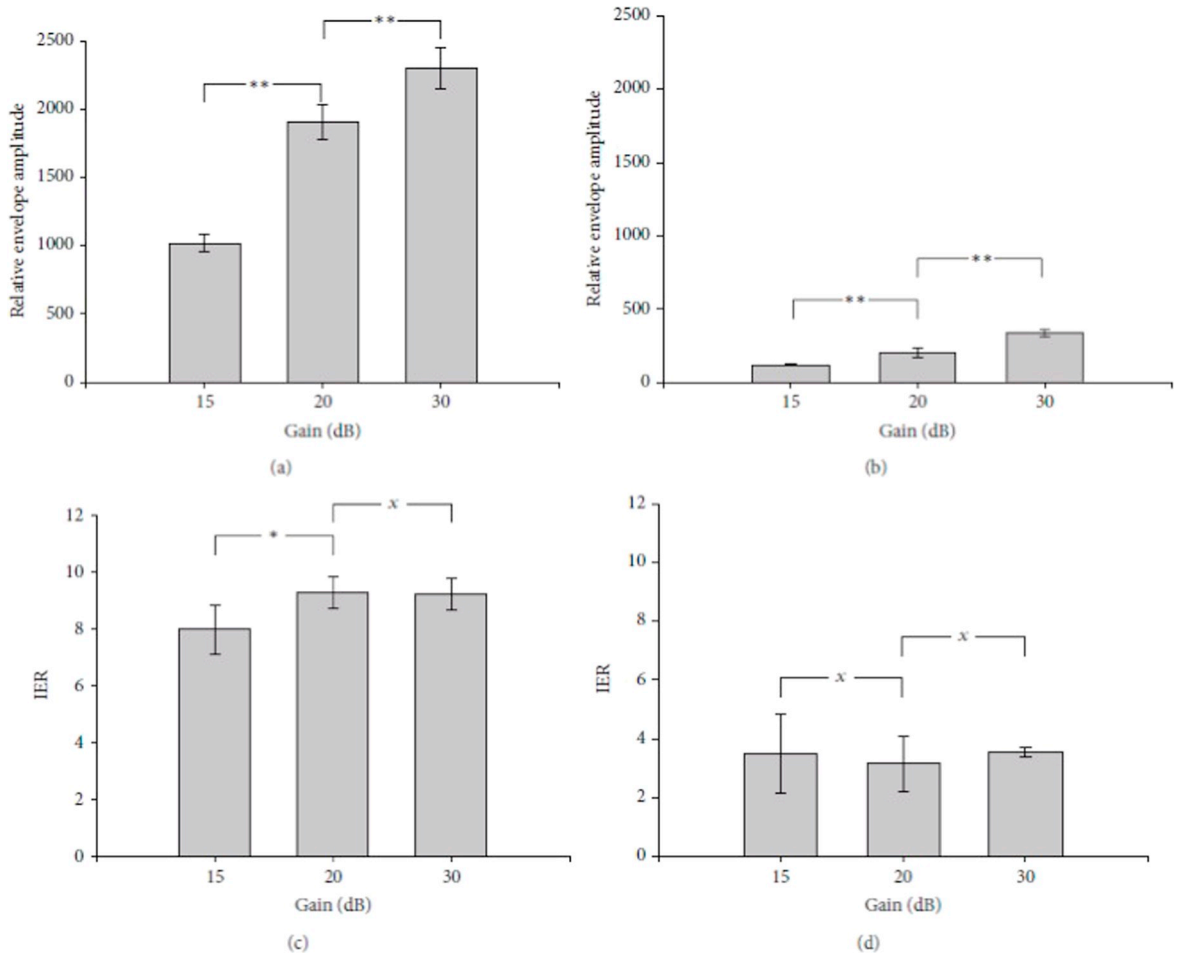

Fig. 3. B-mode ultrasound technique developed to differentiate thrombosis: (a) and (b) The envelope amplitude of B-mode image obtained from different system gains for *in vitro* porcine fresh and aged thrombi, respectively; (c) and (d) The IER obtained from different gains for fresh thrombi (taken at 3 h) and aged thrombi (taken after 5 days), respectively. The signal amplitude values for B-mode and the IER decreases with increasing thrombus age due to decrease in the number of red blood cells in thrombus ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{x}P > 0.05$). Reproduced with permission [20].

2.3. Radionuclide techniques

Radionuclide techniques were proposed twenty years ago as alternatives to contrast venography and duplex sonography [33]. Butler et al. [46] developed scintigraphy imaging with ^{99m}Tc-recombinant tissue plasminogen activator (^{99m}Tc-rt-PA) technique to detect proximal and calf vein thrombosis. Tissue plasminogen activator (TPA) is a serine protease enzyme naturally produced in vascular endothelium and released into the circulation. TPA contains a fibrin binding site and a catalytic site that converts plasminogen to plasmin. The rt-PA is a fibrin-selective agent as it binds directly to fibrin causing a conformational change that brings about a significant increase in its plasminogen catalytic activity. In Butler et al. study [46], a rt-PA with permanently inhibited plasminogen active site was employed, that enabled rt-PA to bind to fibrin only and not causing thrombus lysis. The rt-PA was attached to the 99m technetium radiotracer to allow the scintigraphical imaging of thrombi. Butler et al. [46] demonstrated that the technique had sensitivity of 86% and specificity of 93% in detecting isolated calf vein thrombus of variable ages in humans.

Brighton et al. [47] adapted the technique previously developed by Butler et al. [46] for their research on the uptake of ^{99m}Tc-rt-PA in acute DVT over the first 30 days after diagnosis. The hypothesis of the study was that less fibrin sites became available for ^{99m}Tc-rt-PA as fibrin became inactive due to cross-linking of fibrin bulk within the aged thrombus. There should be a dynamic decline in the uptake of ^{99m}Tc-rt-PA in old thrombus compared to the fresh thrombus. Brighton et al. [47] proposed that the newly formed thrombus rich in active fibrin would be a ^{99m}Tc-rt-PA-avid material whereas the mature thrombi would not take up the radiotracer and becomes ^{99m}Tc-rt-PA-cold. Their study did support the proposal that scintigraphy with ^{99m}Tc-rt-PA was able to detect fresh thrombi (Fig. 5) and hence could provide an easy imaging technique to differentiate old and new thrombi. This finding might have a practical clinical indication in recurrent ipsilateral DVT diagnosis. Unfortunately, there is no commercially available preparation of this tracer currently available. The uptake of ^{99m}Tc-rt-PA by thrombi technique needs to be assessed for its reproducibility as well. [47].

2.4. Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a promising alternative technique that can non-invasively detect and distinguish acute and chronic thrombosis *in vivo* [48]. MRI enables accurate quantification and characterization of atherosclerotic lesions in various arterial beds [49–51]. Generally, in gradient-echo (GRE) magnetic resonance (MR) angiography ("bright blood" imaging), flowing blood appears bright; while in presaturated spin-echo (SE) MR angiography ("black blood" imaging), flowing blood appears dark. Corti et al. [23] tested multi-contrast fast spin echo sequences that are commonly used for high-resolution MRI of atherosclerotic plaques to visualize and characterize the

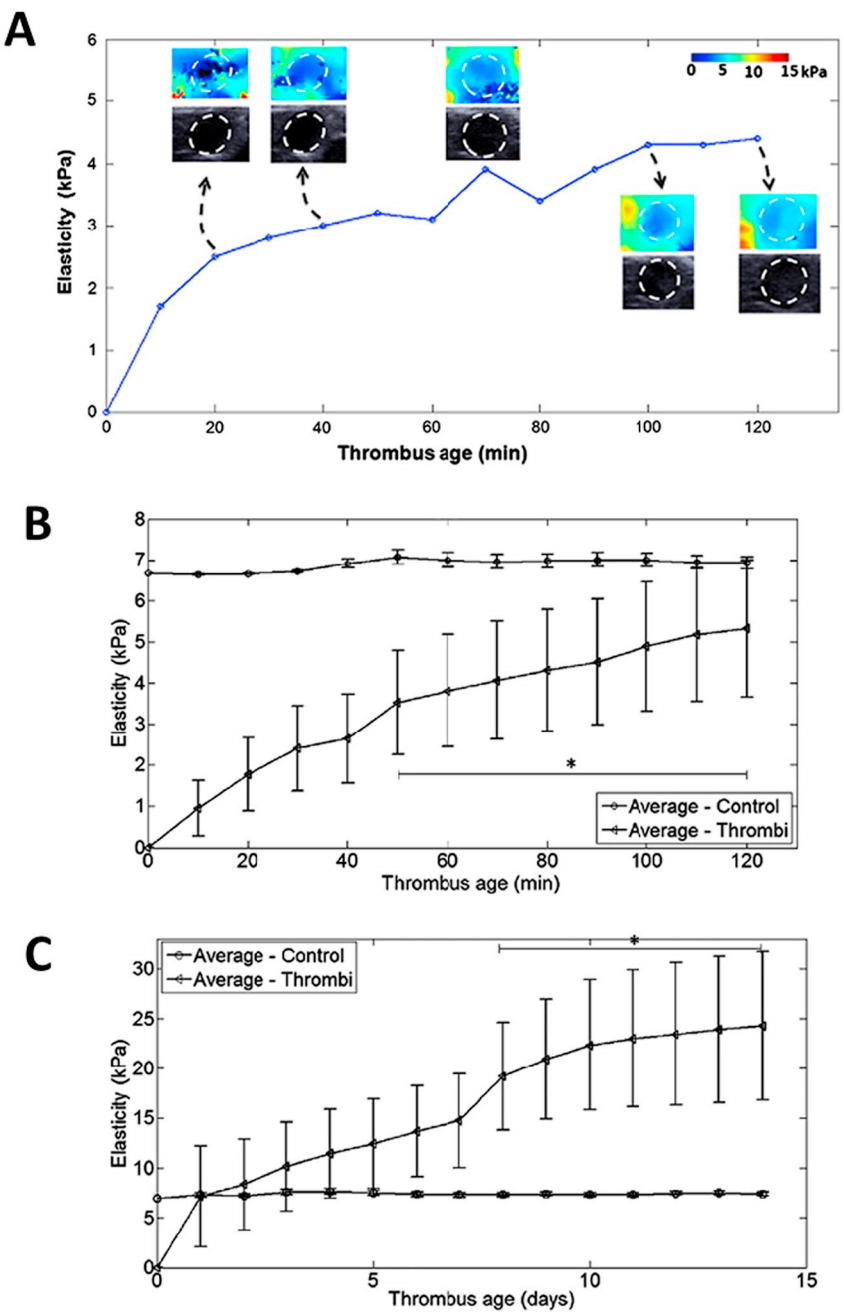

Fig. 4. Ultrasound elasticity imaging of rabbit jugular venous thrombi. (A) Typical correspondence of ultrasound elasticity imaging to determine the mechanical property of a thrombus. The Young's modulus in the ROI increases with time. B-mode (bottom boxes, dynamic range 50 dB) and elasticity (upper boxes, on a scale 0 to 15 kPa) maps over 2 mm × 1 mm ROI are displayed on the graph at 20, 40, 70, 100 and 120 min after thrombus induction. The clot boundary is approximately outlined by a white dashed-lined ellipse in each image. The thrombus hardening correlates with the gradual color change from dark blue to light blue within the thrombus in the elasticity map. (B & C) Average elasticity modulus variation within 2 h and 2 weeks, respectively after thrombus induction. Reproduced with permission [44]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

arterial thrombus age of porcine carotid artery. In this study, black-blood MR imaging technique was created by nullification of the signal coming from flowing blood caused by the double inversion recovery fast spin echo sequences. T1-weighted (T1W) and T2-weighted (T2W) sequences of this technique were used to obtain serial high resolution *in vivo* MR images. Black blood MR imaging is predominately used in cardiac imaging and vessel wall dissection due to its inability to generate pulsation artifacts, therefore, producing a better image quality than the bright blood method [52]. The study described characteristic temporal changes of thrombus appearance and relative signal intensity in multi contrast-weighted MRI images that mirrored the histological changes in thrombus composition. The relative signal intensity in T2W images was higher than T1W images during the first three weeks after thrombus induction supporting the potential use of T2W sequences to detect acute thrombosis. After 6 weeks, the complete thrombus organization was verified histologically and T2W images had similar signal intensity to T1W images. A study by Corti et al. [23] reported the adequate precision of MRI in distinguishing old and new thrombus *in vivo* by using T1W and T2W characteristic images (Fig. 6).

MRI is also known for its capacity to differentiate thrombus stage and hemorrhage formation [53]. Formation of paramagnetic methemoglobin in a subacute/acute phase of thrombus stage and hemorrhage formation showed an increase in signal on T1-weighted sequence MRI. This sequence has been used in the clinical setting to detect lower limb

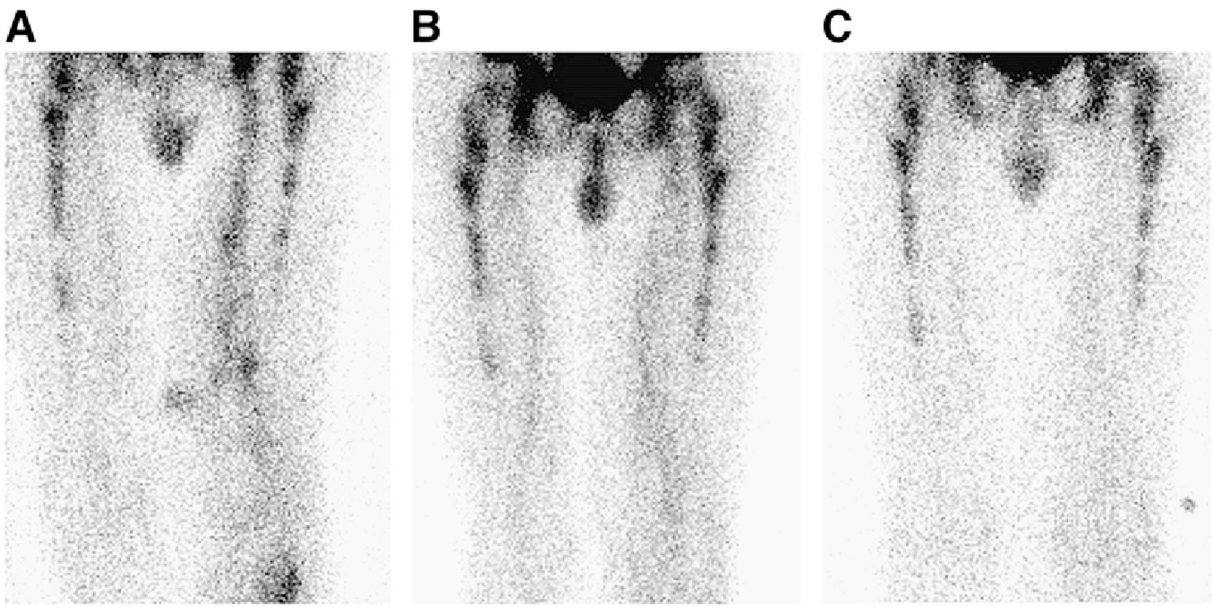

Fig. 5. Radionuclide scanning of ^{99m}Tc-rt-PA uptake over patient's anterior thighs and posterior calves: Sequential radionuclide images obtained on days 1 (A), 7 (B), and 30 (C) in patients with left proximal vein thrombosis. Day 1 showed marked accumulation of ^{99m}Tc-rt-PA in left proximal vein, with urinary contamination over medial left calf being noted. Day 7 scan showed appreciably less uptake of ^{99m}Tc-rt-PA by thrombi in the calves. Day 30 showed no significant uptake. Ultrasound was positive for proximal vein thrombosis on Day 1, 7, and 30. Reproduced with permission [47].

DVT [54] and pulmonary embolism [55]. The technique, also called magnetic resonance direct thrombus imaging (MDRTI), had a sensitivity of 95% and specificity of 100% for a first venography proven symptomatic DVT in legs. Tan et al. [56] demonstrated the high diagnostic accuracy of MDRTI in detecting acute proximal ipsilateral recurrent DVT (Fig. 7) and chronic residual thrombosis of at least 6 months old with a normal D-dimer level in the leg veins where recurrent DVT was not suspected with a sensitivity of 95% (95% confidence interval, 83% to 89%) and specificity of 100% (95% confidence interval, 92% to 100%). [56] D-dimer is a fibrin degradation product generated after a thrombus is degraded by fibrinolysis. Nevertheless, more data is required to ensure the safety of patients withholding anticoagulants with a single normal MDRTI to enable this technique to be applied in clinical practice [56].

MDRTI was also used by Moody et al. [53] to characterize complicated carotid plaques in patients that suffered from anterior cerebral circulation ischemia. Histopathology is used as a gold standard comparison to assess the accuracy of MDRTI in identifying the complicated plaque retrieved from the patients. The complicated plaque was defined as intraplaque associated with high risk of thromboembolism. Moody et al. [53] demonstrated that the 3D T1-weighted imaging of carotid vessels in patients with cerebral ischemia could accurately recognize histologically verified complicated plaque. This method will be highly favorable in research and clinical settings for carotid atherosclerotic disease examination since the sharp contrast of short T1-species within the plaque makes it easier for interpretation.

Cardiac magnetic resonance (CMR) T1-weighted imaging technique has also been developed to detect methemoglobin as a marker of acute coronary thrombi in situations such as coronary plaque disruption and intraplaque hemorrhage [57]. This technique detected patient's thrombi at the site of the causative coronary plaque with a sensitivity and specificity of ~90% in post-myocardial infarction patients [58]. It also provided important prognostic information as high-intensity plaques were often observed in patients with known or suspected coronary disease. CMR imaging consisted of MR angiography and T1-weighted imaging of plaque as illustrated in Fig. 8. MDRTI and CMR have not been reported to detect chronic thrombi, probably due to the lack of methemoglobin in chronic thrombosis.

Iron oxide nanoparticles have been developed as effective contrast agents for molecular MRI of cardiovascular diseases, especially thrombosis [59–68]. These nanomaterials provide either negative [69] or dual positive/negative contrast effects [66,70]. Recently, an activatable magnetic resonance nanosensor based on iron oxide and gadolinium has been developed as a potential imaging agent for detecting and discriminating thrombosis [59]. This nanosensor (TF) was able to switch between T_1-weighted and T_2-weighted MRI signal depending on thrombus age or the presence or absence of thrombin at the thrombus site (Fig. 9). TF nanosensors were activated when incubated with a fresh thrombus and showed T_1-signal (bright) and also T_2-signal (dark) on the surface of the thrombus (arrow). When TFs were incubated with an aged thrombus, they were not activated and only showed T_2-signal. T_CF, TF_C, and T_CF_C are control nanoparticles. They did not exhibit any T_1-signal on both fresh and aged thrombi. The study demonstrated the successful synthesis of an activatable nanosensor for imaging and aging thrombi. However, the efficiency of this nanosensor has not yet been validated *in vivo*.

3. Conclusions

Knowledge of the age and composition of a thrombus is critical to the management of patients with acute or chronic thrombotic conditions. Non-invasive and cost-efficient diagnostic techniques with

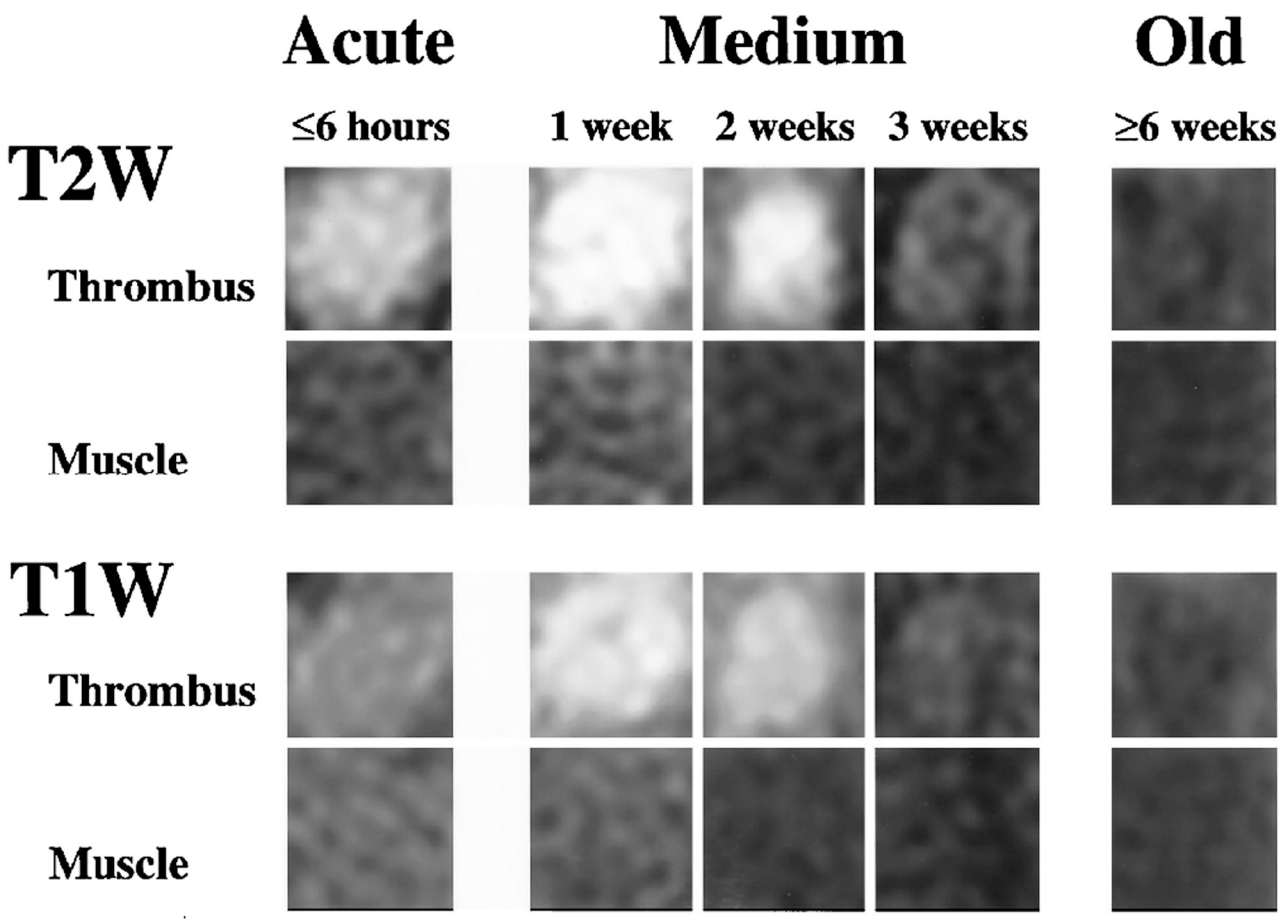

Fig. 6. Representative T2-weighted (T2W) and T1-weighted (T1W) axial magnetic resonance images of the porcine carotid arterial thrombus and adjacent muscle (used as reference) at different time points after thrombus induction were selected to create a reference table for visual comparison. This figure was used as reference for signal intensity analysis by the two independent observers. Reproduced with permission [23].

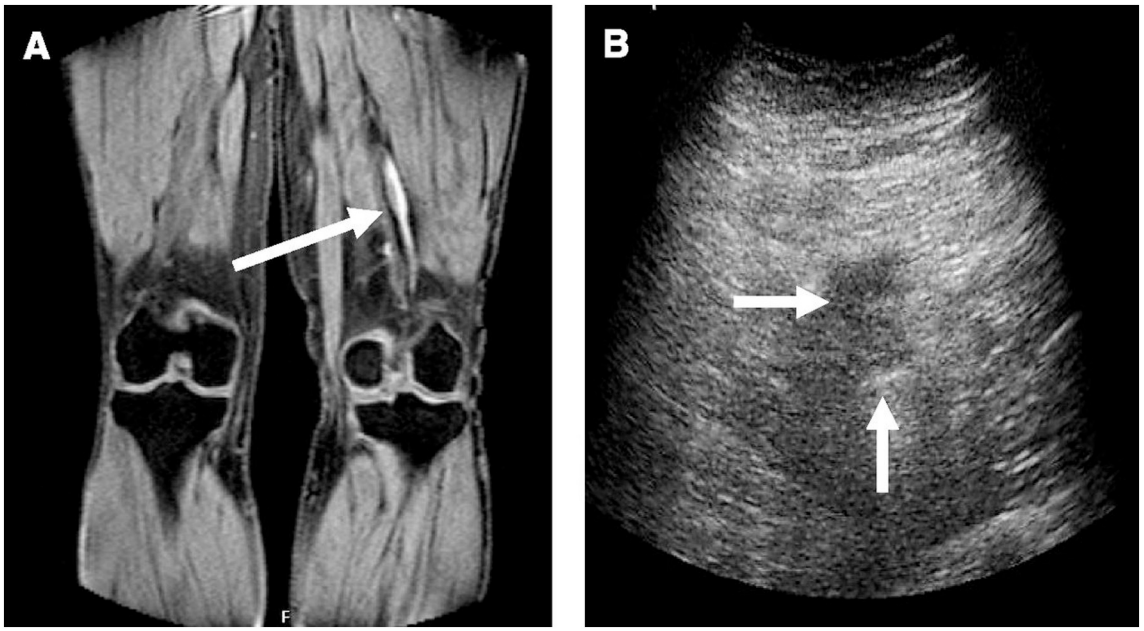

Fig. 7. Abnormal signal on MRDTI in the popliteal vein of a patient's left leg. (A) Arrow indicates positive MRDTI signal in a patient with symptomatic and compression ultrasonography (CUS)-proven ipsilateral recurrent DVT in the popliteal vein; (B) Arrows indicate incompressibility of corresponding vein on ultrasonography. Reproduced with permission [56].

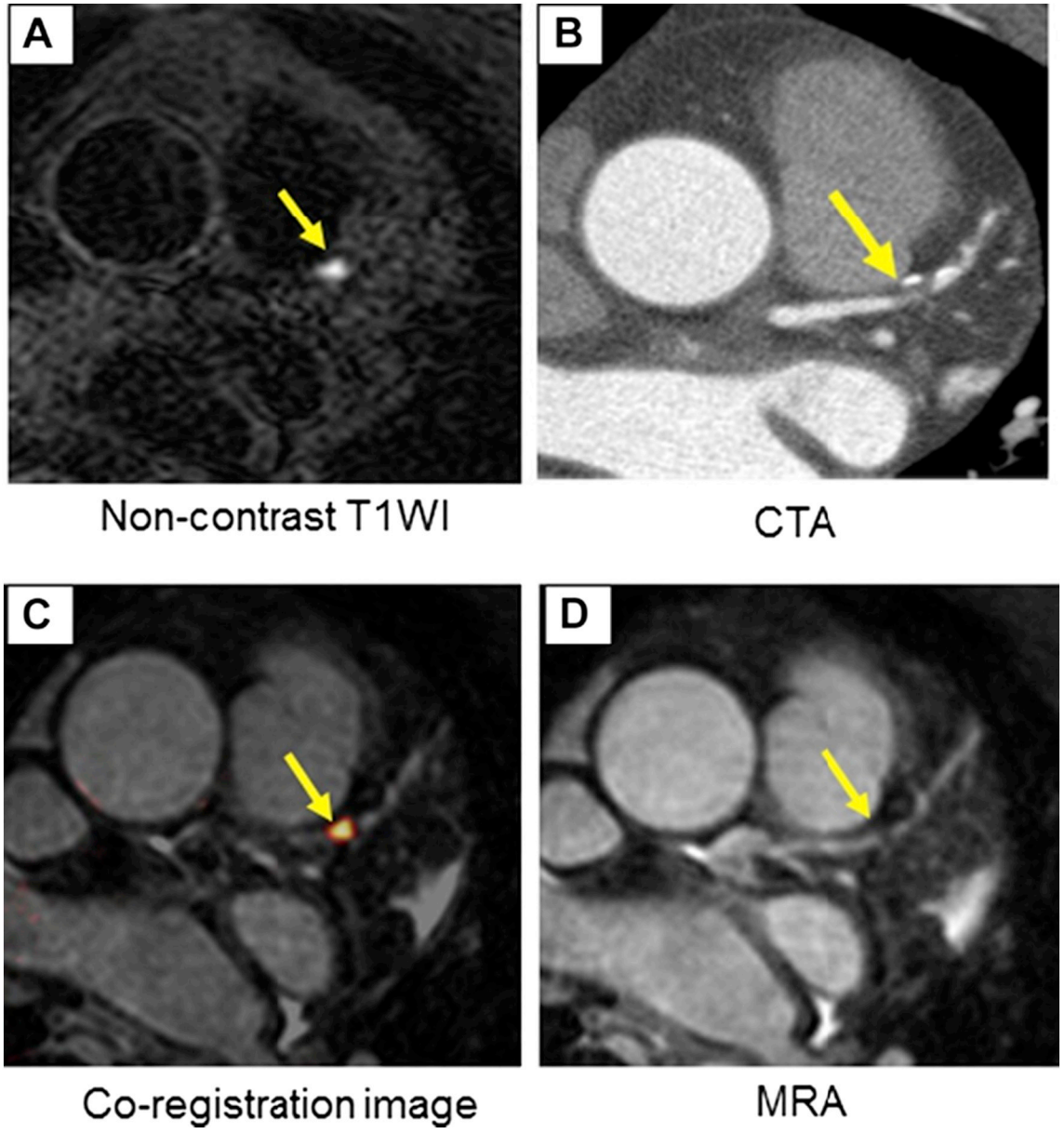

Fig. 8. Representative images of high-intensity plaques on CMR. The high-intensity signal on T1-weighted imaging (T1WI) (A) corresponds to the patient's coronary plaque defined by computed tomographic angiography (CTA) (B). Magnetic resonance images of coronary plaque (yellow arrow) in the proximal left anterior descending coronary artery co-registered (C) with noncontrast T1W1 (A) and magnetic resonance angiography (MRA) (D) showing that a high-intensity signal on T1WI (A) corresponds to the coronary artery wall on MRA (D). Reproduced with permission [57]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

superior clinical markers that can assess thrombus age based on its location and composition are essential to improve the efficacy of anticoagulation treatment and thrombolytic therapies; and reduce the adverse effect rate. These techniques would critically assist in the clinical decision as to whether pharmacological and/or mechanical and thrombolytic treatment is required to dissolve the thrombi. There are diagnostic techniques currently used in clinical practice to examine thrombosis such as venous duplex ultrasonography and ultrasound imaging. However, both techniques have limitations that require further developments to improve their efficacy in differentiating between old and new thrombosis. MRI, elastic imaging, and radionuclides techniques are potential diagnostic tools presently undergoing research and development as they need additional studies to support their utility in assessing thrombosis age and to establish their usefulness and cost-effectiveness before they are placed in clinical setting. In addition, the sensitivity and specificity of the some reviewed techniques have not been reported and need to be investigated to facilitate their clinical translation.

Conflict of interest

The authors declare that the research was conducted in the absence

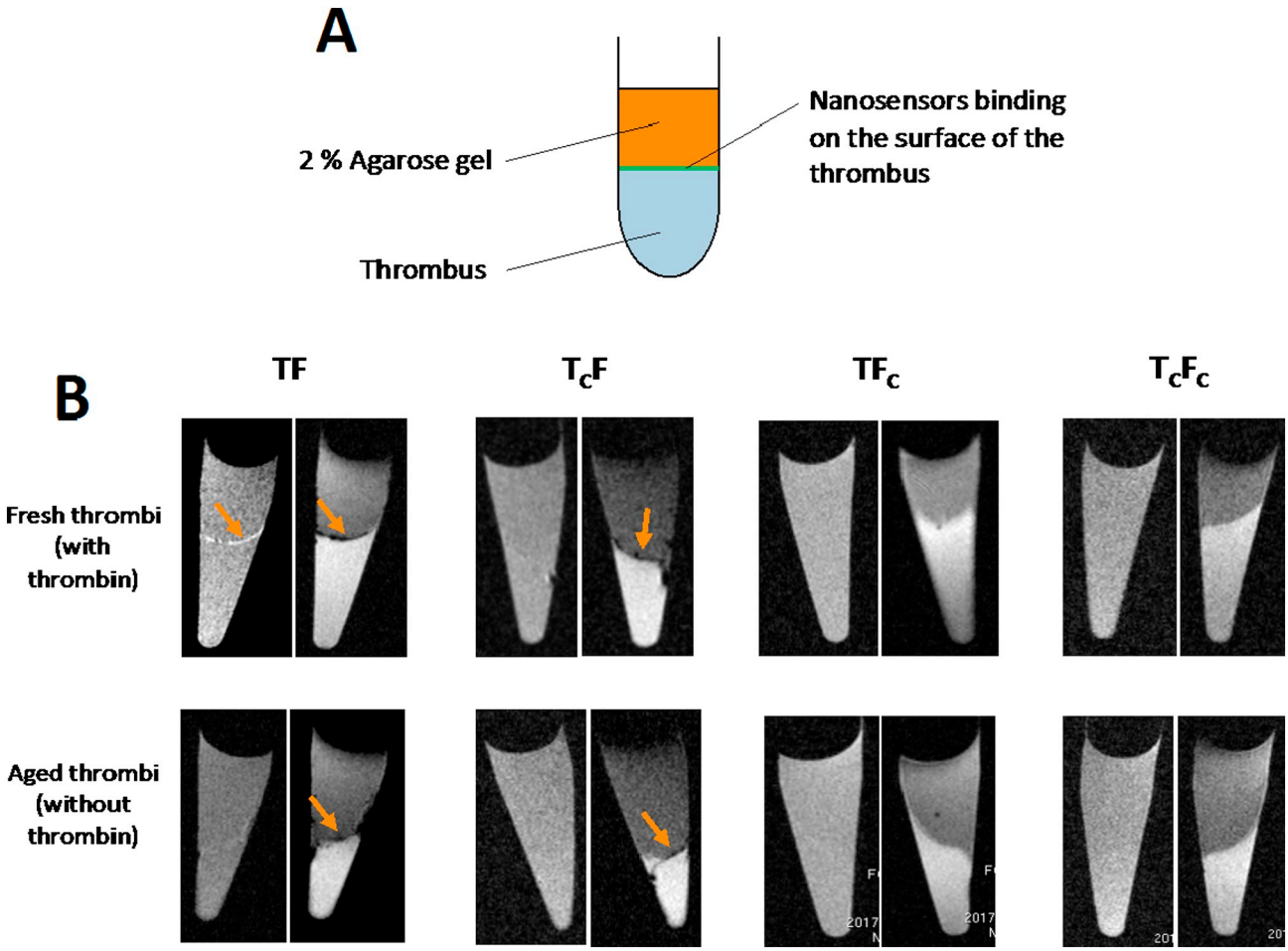

Fig. 9. *In vitro* human thrombus binding assay with different nanoparticles. (A) Illustration of the 0.6 mL tube containing the thrombus in this experiment. (B) MR images of thrombi incubated with different nanoparticles. TF is a targeting and activatable nanosensor. T_CF is a targeting and non-activatable nanoparticle. TF_C is a non-targeting but activatable nanosensor. T_CF_C is a non-targeting and non-activatable nanoparticle. For each nanoparticle, the left image is T_1-weighted image and the right one is T_2-weighted. Reproduced with permission [59].

of any commercial or financial relationships that could be construed as a potential conflict of interest.

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