Association of thrombus histopathology with mechanical thrombectomy: an in vitro study tested by thromboelastography and magnetic resonance imaging

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Research Article

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Abstract

Objective:

To detect the association between the content of red blood cell (RBC) within thrombi and thromboelastography, magnetic resonance imaging and retrieving strength during thrombectomy in vitro.

Methods:

Arterial and venous blood samples, considered as RBC-rich groups, were collected from twenty healthy beagle dogs under general anesthesia. RBCs were diminished by gathering the plasma and buffy coat layer after centrifugation. Then samples were grouped as: arterial whole blood group, venous whole blood group, arterial plasma group and venous plasma group. Blood routine test and coagulation test were used to quantify the content of RBC, platelet and fibrin. After tested by thromboelastography, these four groups of blood samples were proceed to clots and compared by magnetic resonance imaging (MRI), HE staining and mechanical thrombectomy in vitro.

Results:

TEG assay showed MA of whole blood groups with high RBCs was significantly higher than that of plasma groups. MRI demonstrated the plasma clots were much more homogeneous than whole blood samples. T2 value of plasma clots on GRE T2*MR imaging sequence were higher than that of whole blood groups ($P<0.001$). The whole blood clots showed significant higher tension, but were more fragile compared with that of plasma clots. Moreover, clot tension was found positively correlated with MA.

Conclusion:

RBC-rich clots showed lower T2 value, higher retrieving strength but more fragile during mechanical thrombectomy in vitro compared with those of plasma clots. Further, the retrieving strength was positively correlated with MA.

Introduction

Acute ischemic stroke (AIS) is one of the leading causes of neurologic morbidity and mortality. The several large-scale, randomized trials have revolutionized the present stroke treatment. Nowadays, mechanical thrombectomy with stent-retrievers or by catheter aspiration with or without thrombolysis has become the first-line treatment for AIS with large vessel occlusion [1–5]. However, up to 20% of AIS still do not have successful recanalization [6].

Among the potential reasons, thrombus characteristics have been recognized as the key factor that affecting the revisualization rate and clinical prognosis. A better understanding of the thrombus properties could lead to the design of strategies aimed to increase efficacy of mechanical thrombectomy. Most of the studies were based on image findings or component analysis of the retrieved clot

characteristics such as thrombus location, thrombus burden (length), thrombus perviousness scores or thrombus composition [6–10]. However, the physical properties of the clots could be interacted with each other, and few studies could control other variables to one key factor due to individual nonuniformity.

Meanwhile, thrombelastography (TEG) is a whole blood viscolastic assay that can be used to qualitively and quantitatively monitor the whole process, from initiation of coagulation systems to blood clot dissolution [11]. Maximum amplitude (MA) is the most important information provided by TEG, which represents the maximal clot strength. Previous studies have suggested that MA could predict risk for ischemic stroke such as early diagnose or long-term prognosis as well as post-procedural ischemic events after cerebral artery stenting [12, 13]. The relationship between MA and the difficulty of mechanical thrombectomy, however, has not yet been evaluated.

In this study, we aimed to test the differences in TEG, MRI signal and clot properties between RBC-rich and RBC-deficiency autologous thrombus originated whether from arterial blood or venous blood. The tensile test was used to evaluate the clot strength during mechanical thrombectomy in vitro.

Materials And Methods

Animal preparation

The experiments were performed according to the protocol approved by Animal Experiment Ethical Committee (Nanjing Medical University, Nanjing, China) and this study was in accordance with ARRIVE guidelines. Twenty healthy adult beagle dogs (Laboratory Animal Centre, Anhui, China) of either gender were used. Dogs were fed with commercial dry food twice a day and housed in a single cage at a well-ventilated facility.

Before procedure, dogs were acclimatized to our animal facility for one day. The methods of anesthesia and physiological monitoring were described in our previously study [14]. In brief, 3% pentobarbital sodium (1ml/kg) was given by muscle injection at either side of lower limb. The depth of anesthesia was controlled according to the corneal reflection and respiratory rhythm. The heart rate, blood pressure and oxygen saturation were monitored during procedure. Additionally, a dose of 5mg diazepam and 0.4mg atropine were given by muscle injuction to reduce gland secretion. Oxygen was maintaned at 2l/min. Right femoral artery and left femoral vein accesses were obtained by using 5-French sheaths (Terumo Medical Corporation, Tokyo, Japan) for blood sample collection and subsequent anesthesia drug administration.

Blood collection and centrifugation

A 4ml disposable anticoagulant tube (Insepack, Beijing, China) was used for blood sample collection. A total of four tubes which contained 8ml arterial and venous whole blood were extracted by 10ml syringe from femoral artery or vein sheath. One tube of arterial or venous whole blood samples was centrifuged

at 4000 rpm for 10 min at room temperature. Whole blood samples were centrifuged into three layers, the plasma layer, the middle buffy coat layer and red blood cell layer from up to bottom, respectively.

Sample grouping and component analysis

After centrifugation of the arterial or venous whole blood, the plasma layer and the buffy coat layer were extracted and mixed homogeneously. The arterial and venous mixture (plasma + buffy coat layer) were grouped as AP and VP, respectively. Blood samples were then divided four groups: arterial whole blood (AW), venous whole blood (VW), AP and VP. Blood routine examination, blood coagulation examination and thromboelastogram (TEG) analysis were performed.

Blood routine test and coagulation function test

Blood samples were coagulated with EDTA-2K (1.5-2.2mg/ml) and mixed homogeneously for blood routine test according to the manufacture's (Sysmex) illustration. Before coagulation function test (Sysmex), blood samples were coagulated with sodium citrate at a ratio of 1:9 followed by centrifuging at 1500 RPM for 10 minutes.

TEG analysis

A TEG 5000 machine (Haemoscope, Massachusetts, USA) was used for TEG analysis. According to the operational instruction, a total of 0.36ml of each sample was collected and rotated in a small beaker with a 4°45' angle at 37℃, each rotation lasted for 10 seconds. During rotation, polymer reaction of fibrin (FIB) occurred between the beaker wall and torsion viscosimeter, which resulted in blood coagulation. The mechanical impedance of blood clots was recorded by the probe and torsion viscosimeter during the coagulation. The TEG was reflected as the curve of mechanical impedance changed with time. Five main parameters were recorded including R value (recation time), MA (maximal amplitude), K value, α angle and CI (coagulation index).

MRI and histological examination for clots

A total of 1.5ml blood samples were transferred into 1.8ml frozen pipe (Corning, New York, USA) and mixed with 100u thrombin (Zhuhai, China) homogeneously. Blood clots were generated after 2 hours at room temperature. As is shown in Fig. 1, four sample groups settled in self-made container in row (aw, vw, ap and vp, respectively), and doubled for each group. Lipiodol and water were used as control groups (left and right hole, respectively). Before MRI, the self-made container was merged with water. The T2 weighted imaging (T2WI) and gradient-echo (GRE) was used for analysis. Clots were embedded into paraffin and cut into 5-μm-thick sections followed by hematoxylin-eosin (HE) staining.

Tension test for clots

The clots were modified 40mm in length and 5mm in diameter, and were transferred into self-made container containing normal saline (Fig. 2). As is shown in Fig. 2, the container was consisted of two parts, the cylinder-shaped part and the cone-shaped part. The inner diameters were 7mm of the cylinder-shaped part and 2mm at the tip of the cone-shaped part, respectively. A 6mm*30mm solitare FR stent

(Medtrinic, Irvine, CA, USA) and Headway 21 micro-catheter (Micro Vention, Tustin, California, USA) were used to simulate the mechanical thrombectomy in vitro. Briefly, after a traxcess-14 micro-guidewire (Micro Vention, Tustin, California, USA) passing through the clot via the tip of the cone-shaped part, the micro-catheter was advanced and the stent was exchanged to the clot body. By withdrawing the micro-catheter, the stent was deployed and maintained for 5 minutes. Then the stent was drawn out from the tip with a uniform speed. The maximum strength and whether the clot was fractured (fragility) were recorded.

Statistical analysis

Continuous variables are reported as the mean ± standard deviation. Categorical variables are reported as a frequency/percentage. One-way ANOVA test or $\chi 2$ test were used for comparison of different groups. Pearson correlational analysis was used to test the factors associated with the maximum strength of the clots. Statistical analysis was performed with IBM SPSS Statistic 26 (IBM-Armonk, New York, USA). A 2-tailed $P < 0.05$ was considered statistically significant.

Results

Blood routine test and coagulation function test

As is shown in Table 1, the red blood cell (RBC) content in each groups were: aw: 6.00 ± 0.59 10^{12}/l, vw: 6.06 ± 0.45 10^{12}/l, ap: 0.24 ± 0.08 10^{12}/l and vp: 0.19 ± 0.06 10^{12}/l, respectively. A significant higher RBC content were found in whole blood groups ($p < 0.001$). No significant differences were found in platelet (PLT) or FIB in each group ($P = 0.083$ and 0.386, respectively).

Table 1
Comparison of clot properties in different groups

	Aw	Vw	Ap	Vp	p
RBC (10^{12}/l)	6.00 ± 0.60	6.06 ± 0.45	0.24 ± 0.08	0.19 ± 0.06	0.000
PLT (10^{9}/l)	322.3 ± 25.2	323.2 ± 36.5	304.9 ± 24.5	304.6 ± 34.4	0.083
FIB(g/l)	2.29 ± 0.57	2.32 ± 0.56	2.05 ± 0.56	2.13 ± 0.58	0.386
MA	65.06 ± 8.03	65.74 ± 6.55	54.07 ± 6.61	58.12 ± 8.17	0.000
T2WI	360.2 ± 5.1	362.6 ± 5.2	89.3 ± 2.7	91.4 ± 1.9	0.000
Tension(N)	0.39 ± 0.04	0.39 ± 0.03	0.35 ± 0.02	0.36 ± 0.02	0.000
Fracture (%)	20%(4/20)	25%(5/20)	0%(0/20)	0%(0/20)	0.016

Aw: arterial whole blood group, Vw: venous whole blood group, Ap: arterial plasma group, Vp: venous plasma group, RBC: red blood cell, PLT: platelet, FIB: fibrin, MA, maximal amplitude, T2WI, T2 weighted imaging

The RBC ratio of ap/aw and vp/vw were 4.00 ± 1.63% and 3.15 ± 0.98%, respectively. The PLT ratio of ap/aw and vp/vw were 94.6 ± 1.86% and 94.3 ± 1.71%, respectively. The FIB ratio of ap/aw and vp/vw were 89.67 ± 9.43% and 92.18 ± 7.76%, respectively. These results reflected that during sample extraction, few RBCs (< 5%) were mixed in ap or vp groups. Meanwhile, most of the buffy coat layer which contained mostly of PLT and FIB was extracted in ap or vp groups (nearly 90%).

TEG analysis

As shown in Fig. 1a and 1b, higher hypercoagulability (high MA value) and faster coagulation speed (low R and K value, high α angle) were found in canine whole blood than that of human (the dotting line was covered by the solid curve). Compared with the whole blood groups, the curve of the plasma groups (figure c and d) also showed faster coagulation speed, however, low which was reflected from the smaller K value and α angle.

The MA (Table1) in each groups were: aw: 65.1 ± 8.0, vw: 65.7 ± 6.6, ap: 54.1 ± 6.6 and vp: 58.1 ± 8.2, respectively. A significantly higher MA was found in the whole blood groups compared with those of the plasma groups ($P < 0.001$).

MRI and histological findings

Figure 2 shows the different group signals on T2WI. As is shown in Fig. 2a, the top two lines were AW and VW, respectively. High signals (water-like) shaped with circle; crescent or irregularity encompassed the low signals (clot body). The inhomogeneous signals of the whole blood groups mainly resulted from the shrinking of the clots during thrombogenesis. Compared with the whole blood groups, the AP and VP groups (lower two lines) elucidated homogeneous signals. T2_RGB map (Fig. 2b) showed the visualized color changes which were in accord with the T2WI map.

As shown in Table 1, the T2WI values of the clots were quantified as follows. The T2 values of whole blood groups were significantly lower than that of plasma groups ($p < 0.001$). No significant differences were found between AW and VW or AP and VP groups ($p > 0.05$).

Figure 3a demonstrated that in the whole blood groups, red blood cells enriched the view, while fibrin can hardly recognized. However, in the plasma groups, fibrin can be visualized due to the increased ratio (Fig. 3b). The results of the HE staining confirmed with the blood routine test.

Tension test analysis for clots

Our results (Table 1) showed the whole blood clots needed higher retrieve strength compared with that of plasma clots ($p < 0.001$). Pearson correlational analysis (Fig. 4) elucidated MA was significantly correlated with clot retrieving strength (AW: $p = 0.021$, $R = 0.512$, VW: $P = 0.030$, $R = 0.487$, AP: $P = 0.028$, $R = 0.491$; VP: $P = 0.026$, $R = 0.495$, respectively). Moreover, we found that whole blood clots were tended to be more fragile compared with plasma clots (Fig. 5).

Discussion

In this study, we analyzed and compared the whole blood clots and plasma clots from components, TEG analysis, MRI, histological examination and tension test. The result elucidated that whole blood contained rich RBCs has significantly higher MA and retrieval strength compared with plasma thrombus which is lack of RBCs, which was positively correlated with retrieval strength. Moreover, RBC-rich clots showed less homogenous compared with RBC-deficiency clots. The results were comparable whether the clots originated from arterial or venous blood.

Thrombi histopathologic characteristics in ischemic stroke could provide insights into stroke etiology and ideal treatment strategies [15–17]. The origin of thrombus from different subtypes of stoke, as defined by the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification, were still controversial. Previously, Kim et. al [18] analyzed retrieved clots from 22 acute ischemic stroke patients, and elucidated that cardioembolic clots had a significantly higher proportion of RBCs and a lower proportion of fibrin compared with those originated from large-artery atherosclerosis. However, recent studies [19, 20] showed that lower content of RBCs and higher content of fibrin/platelets was associated with cardioembolic origin. This discrimination may rise from the bias of inclusion criteria such as racial and individual properties, the use of alteplase or urokinase, mechanical treatment strategies as well as thrombus features including clot burden, location or generated time. Accordingly, autologous thrombus used in this study to semi-quantitively analyze their properties may be a good choice to find potential evidence.

Recent studies have thrown more light on the relationship between thrombus histopathology and mechanical thrombectomy. Among the properties of thrombus, the fibrin and red blood cell proportion in a thrombus determines its physical properties and how it responds to thrombectomy [17]. High RBC proportion of RBCs has been proved to be associated with successful reperfusion [21, 22]. Gunning et.al [23]also demonstrated that fibrin-rich thrombi comprising < 20% RBCs would decrease revascularization rates regardless of the technique employed. In our study, we found high proportion of RBCs in a thrombus increased the retrieval strength, however, tended to be more fragile during stent retrieval. One potential reason is the heterogeneity of the RBC-rich thrombus (as shown in Fig. 2) increased the surface adhesive friction, which has been considered as a predominant factor interacting with stent [8]. Another reason may underlie that mature clot rich in fibrin is firmer and less deformable when interacting with stent retriever [17]. However, the fibrin-rich clots used in our study were fresh made, which made them soft enough to be trapped and extracted. These potential reasons need further evidences proved by ultrastructural tests, such as electron microscopy.

Except for diverse clinical manifestations, image findings give us more direct diagnostic information of cerebral ischemic events. The presence of hyperdense artery sign (HAS) on CT and susceptibility vessel sign (SVS) on MRI are early signs of brain ischemic stroke, which have been recognized as higher RBC fraction within retrieved thrombus [10, 18, 21] and better clinical outcomes [21, 24]. Compare with HAS, SVS is reported to be higher sensitivity (about 70% VS. 30%) [10, 24]. In the present study, we used GRE T2*MR imaging sequence to identify SVS and found consistent results with previous studies. Moreover, we also found an interesting phenomenon that RBC-rich clots transformed to be more irregular during thrombogenesis under quiet circumstance in vitro. Under the ‘water-hammer’ effect in vivo, whether the

flexibility of RBC-rich thrombus affected the ultimate shape of a clot or the interaction with a stent still needs more investigation.

High MA were tended to be female gender, lower hemoglobin levels, high platelet counts and fibrinogen, inflammation, and were more likely to have intracranial artery stenosis and large-vessel subtype strokes [12]. We also found a moderate association of high TEG-MA with increased clot retrieving strength. High retrieval strength reflects the increased friction between the vessel wall and a clot trapped in a stent. When the fiction exceeds the ductility of a clot or the interaction strength between a thrombus and the stent, the clot would be dropped or fragmented. Therefore, a high radio force stent would be theoretically more suitable for stroke patients with high MA. Moreover, arterial and venous MA exhibited no significant difference, which was in accordance with previous in vitro study [25].

This study has some limitations. Firstly, this is an in vitro designed study, aiming to minimize the possible bias. However, the content of blood cells was semi-quantified, and the in vivo repeatability needs further proofs. Secondly, despite of the fact that mechanical thrombectomy with stent was till the mainstream method for ischemic stroke. Other effective strategies, such as thrombolysis or catheter aspiration, were not evaluated in the present study. Last but not least, although the results from this study demonstrated significant differences in thrombus properties, our study was not designed to deeply evaluate the possible mechanisms for these differences. None the less, based on the novel findings in the present study, we appreciate the fact that stroke patients with SVS on MRI (indicating a high RBC fraction clot) or with high MA tested by TEG, may need a higher radio force stent during mechanical thrombectomy. This suggestion might be difficult to accept by clinicians, a bold new approach is needed to address this novel calling.

Declarations

Author contributions

Xing-Long Liu, Qing-Quan Zu, Sheng Liu contributed to the conception of the study;

Xing-Long Liu, Bin Wang, Qing-Quan Zu performed the experiment;

Xing-Long Liu, Bin Wang, Hai-Bin Shi contributed significantly to analysis and manuscript preparation;

Xing-Long Liu, Bin Wang performed the data analyses and wrote the manuscript;

Sheng Liu, Hai-Bin Shi helped perform the analysis with constructive discussions.

Data availability statement

All data used in the study were available from the corresponding author by request.

Competing Interests Statement

The authors declare no competing interests.

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Figures

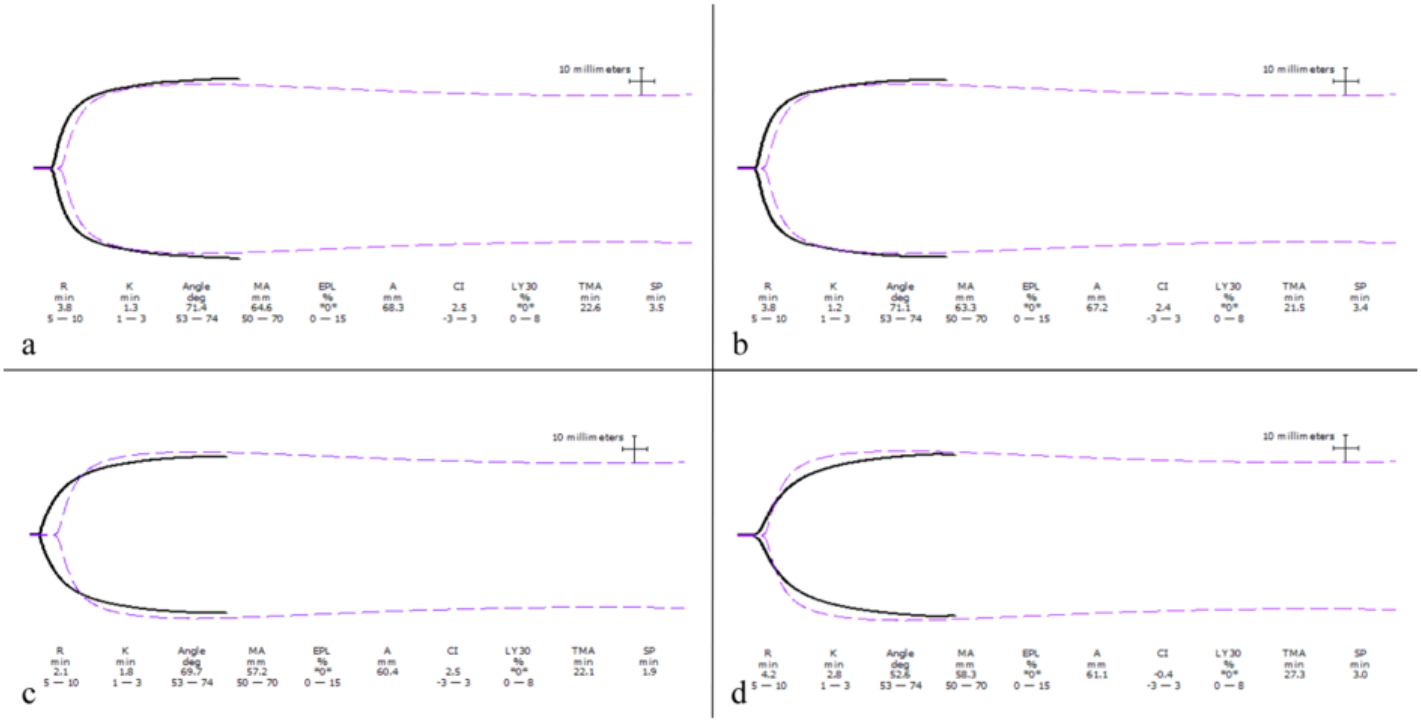

Figure 1

Figure shows the TEG (beagle 9) in different groups.

Figure 1a and 1b shows the typical TEG of different groups, the TEG curve of canine whole blood groups (solid line) covered the dotting line, which means higher hypercoagulability and faster coagulation speed compared with that of human (dotting line). Compared with whole blood groups, the plasma groups (figure c and figure d) showed lower MA.

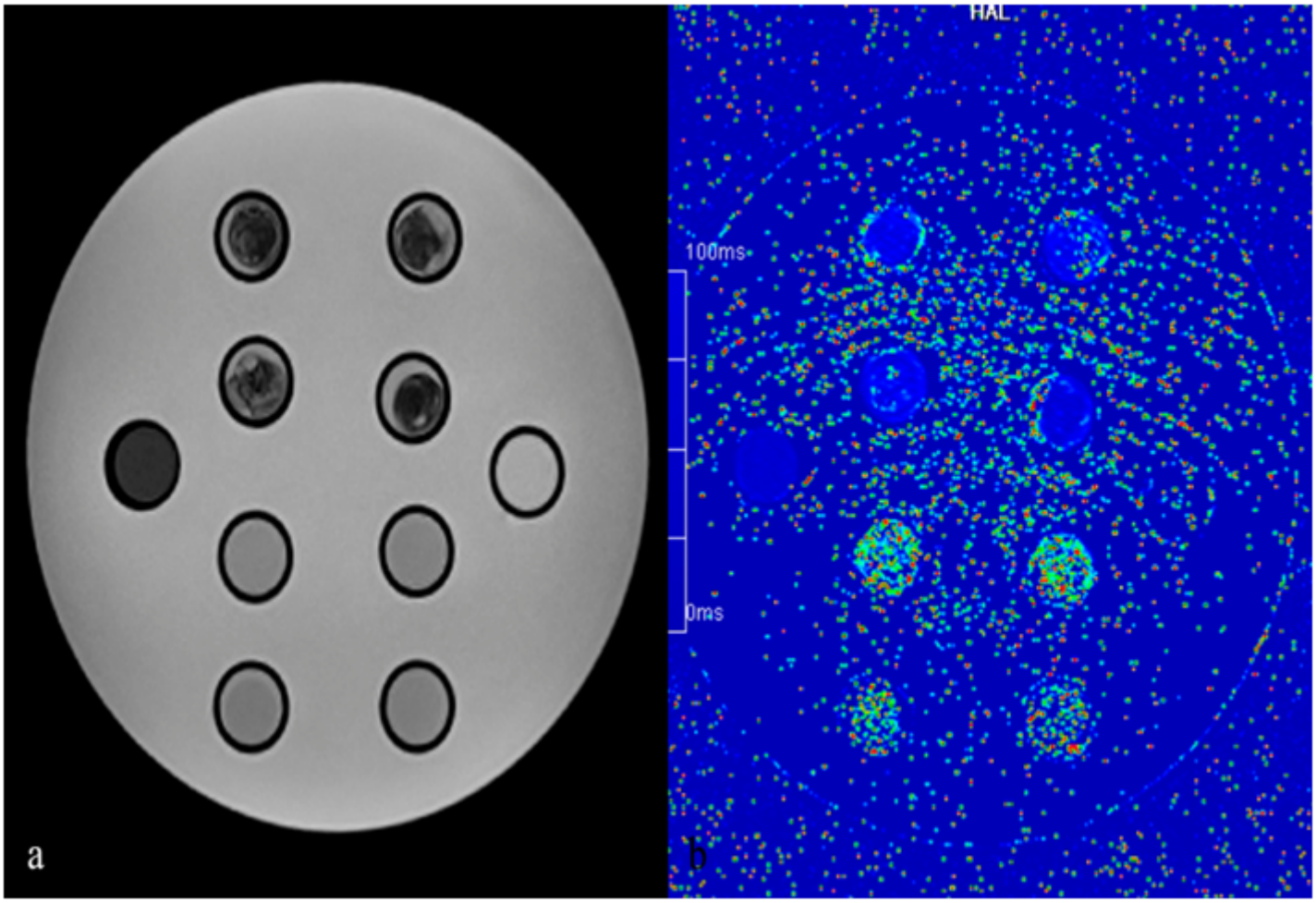

Figure 2

Figure shows the different group signals on T2WI.

As is shown in figure 2a, the top two lines were AW and VW, respectively. High signals (water-like) shaped with circle; crescent or irregularity encompassed the low signals (clot body). The inhomogeneous signals of the whole blood groups mainly resulted from the shrinking of the clots during thrombogenesis. Compared with the whole blood groups, the AP and VP groups (lower two lines) elucidated homogeneous signals. T2_RGB map (figure 2b) showed the visualized color changes which were in accord with the T2WI map.

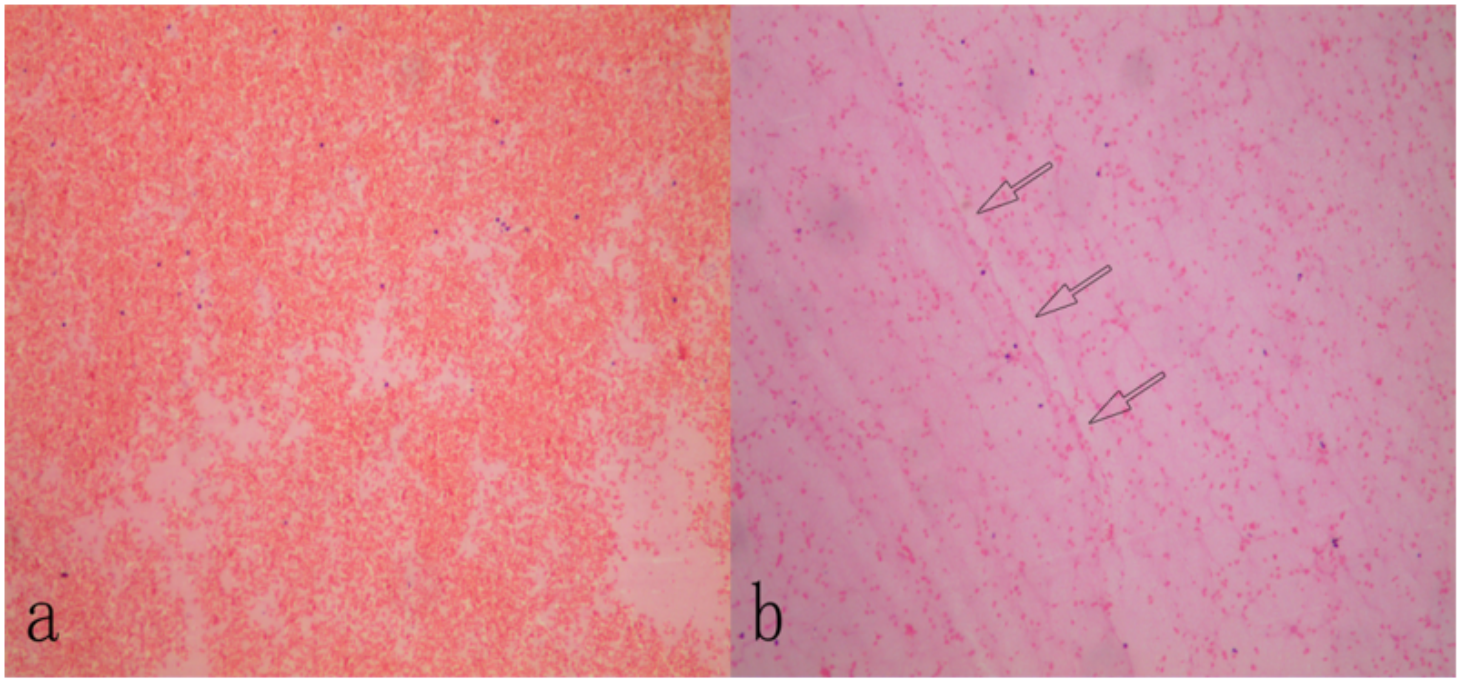

Figure 3

shows the HE staining of RBC-rich and RBC-deficient clots

Figure 3a demonstrates that in the whole blood groups, red blood cells enriched the view, while fibrin can hardly recognized. Figure 3b shows that in the plasma groups, fibrin can be visualized due to the increased ratio.

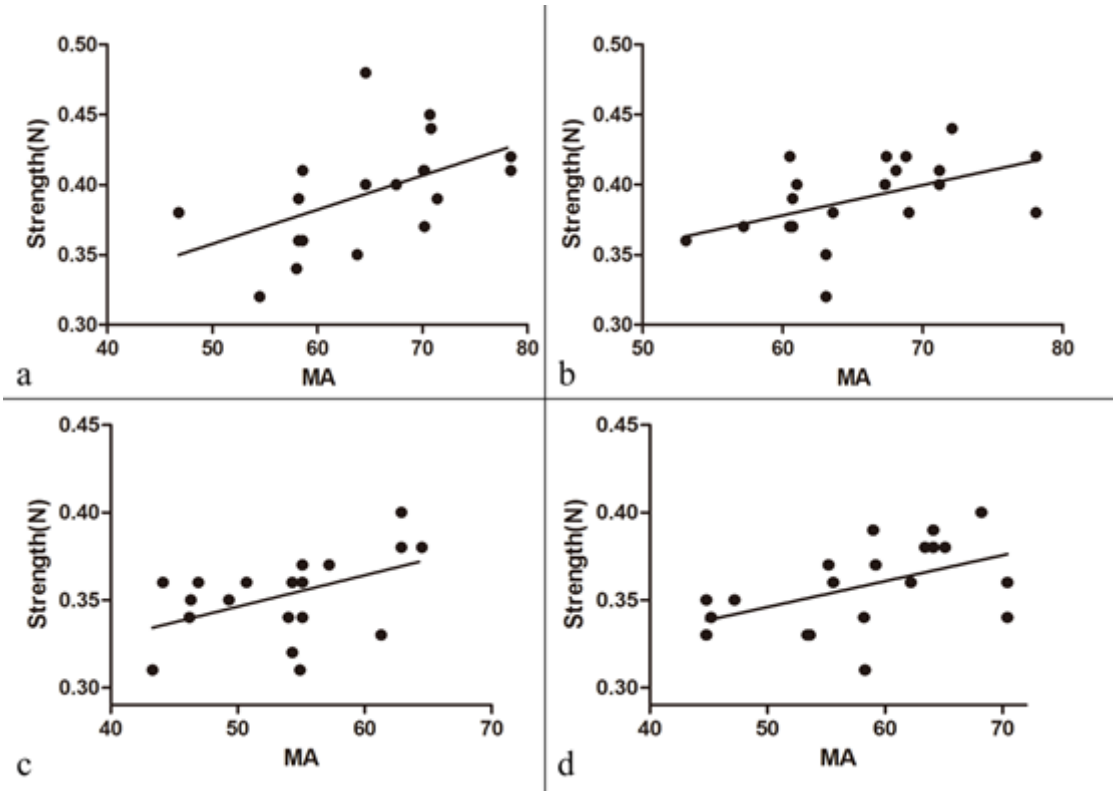

Figure 4

Figure shows the results of Pearson correlational analysis of MA and clot retrieving strength in different groups.

Figure 4a-d represents the group of AW, VW, AP and VP, respectively. Significant correlation was found in these groups.

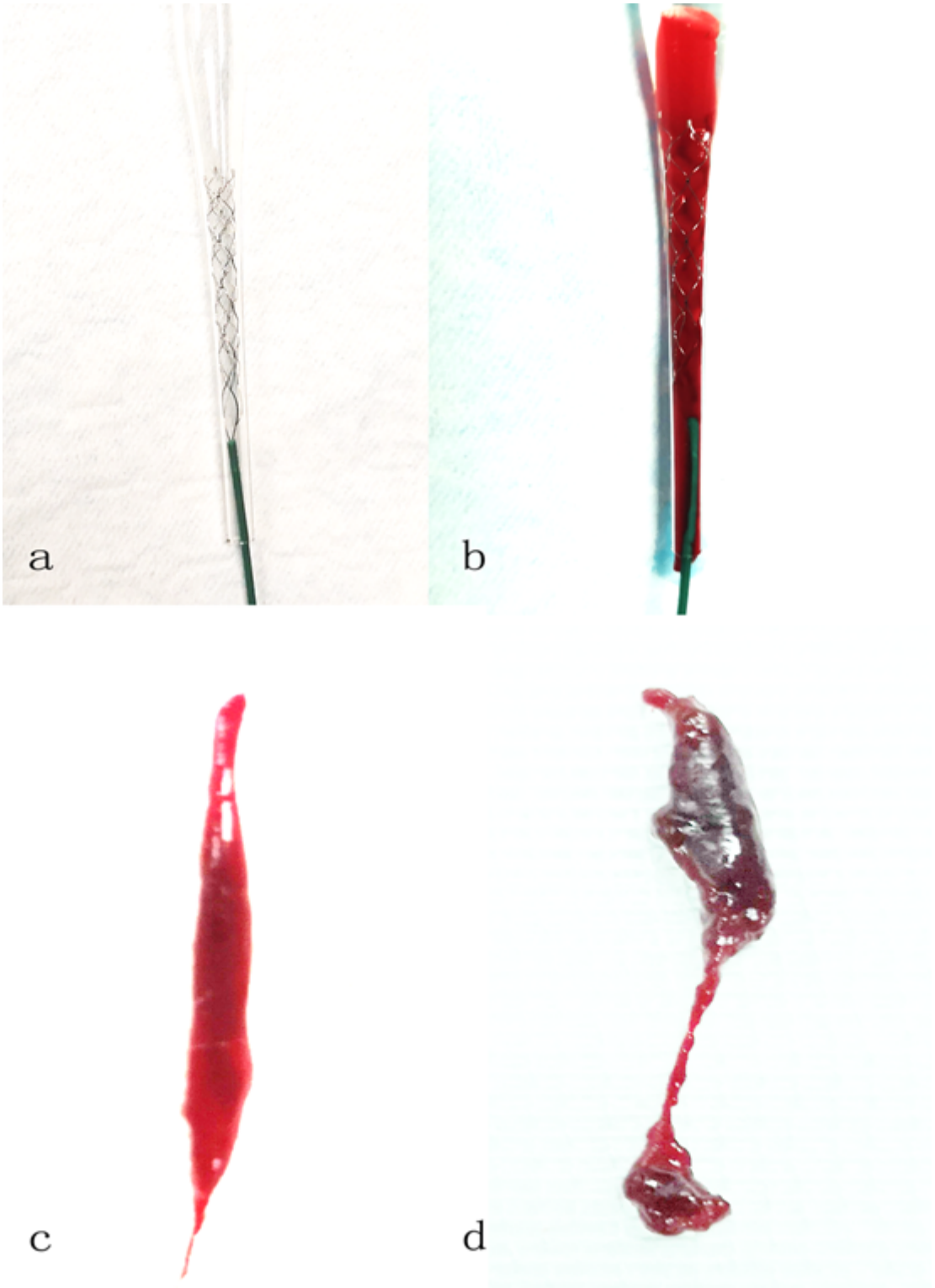

Figure 5

Figure shows the clot retrieving procedure in vitro.

Figure 5a and 5b shows the clot before and during retrieving in vitro, respectively. Figure 5c shows the RBC-rich clot was more regular and smooth compared with RBC-deficient clot (figure 5d) which tended to be more fragile.