Journal of Biomechanics 129 (2021) 110731

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech

Measuring the effect of thrombosis, thrombus maturation and thrombolysis on clot mechanical properties in an in-vitro model

Anushree Dwivedi [a], Aoife Glynn [a], Sarah Johnson [a,b], Sharon Duffy [a,c], Behrooz Fereidoonnezhad [b], Patrick McGarry [b], Michael Gilvarry [a], Ray McCarthy [a,*], On behalf of the INSIST investigators[1]

[a] Cerenovus (Johnson & Johnson), Galway Neuro Technology Centre, Galway, Ireland
[b] Biomedical Engineering Department, National University of Ireland Galway, Galway, Ireland
[c] Galway Medical Technologies Centre, Galway-Mayo Institute of Technology, Galway, Ireland

ARTICLE INFO

Keywords:
Acute ischemic stroke
Thrombolysis
Clot mechanical properties
Clot microstructure

ABSTRACT

Changes in acute ischemic stroke thrombi structure and composition may result in significant differences in treatment responsiveness. Ischemic stroke patients are often treated with a thrombolytic agent to dissolve thrombi, however these patients may subsequently undergo mechanical thrombectomy to remove the occlusive clot. We set out to determine if rt-PA thrombolysis treatment of blood clots changes their mechanical properties, which in turn may impact mechanical thrombectomy. Using a design-of-experiment approach, ovine clot analogues were prepared with varying composition and further exposed to different levels of compaction force to simulate the effect of arterial blood pressure. Finally, clots were treated with three r-tPA doses for different durations. Clot mass and mechanical behaviour was analysed to assess changes due to (i) Platelet driven contraction (ii) Compaction force and (iii) Thrombolysis. Clots that were exposed to r-tPA for longer duration showed significant reduction in clot mass ($p < 0.001$). Exposure time to r-tPA ($p < 0.001$) was shown to be an independent predictor of lower clot stiffness. A decrease in energy dissipation ratio during mechanical compression was associated with longer exposure time in r-tPA ($p = 0.001$) and a higher platelet concentration ratio ($p = 0.018$). The dose of r-tPA was not a significant factor in reducing clot mass or changing mechanical properties of the clots. Fibrinolysis reduces clot stiffness which may explain increased distal clot migration observed in patients treated with r-tPA and should be considered as a potential clot modification factor before mechanical thrombectomy.

1. Introduction

Treatment of acute ischemic stroke (AIS) involves restoring blood supply in the vessel occluded by a thrombus. The recanalization approach can either involve administration of intravenous recombinant-tissue plasminogen activator (r-tPA) within 4.5-hours of stroke onset and/or mechanical thrombectomy to recover the viable tissue in the penumbra region (Alonso de Leciñana et al., 2014; del Zoppo et al., 1992). Fibrinolysis is mediated by specific molecular interactions between r-tPA, fibrin and plasminogen whereby r-tPA becomes activated in presence of fibrin. It converts inactive plasminogen into active plasmin to degrade fibrin fibers (Collen, 1987; Murray et al., 2010). AIS patients presenting within a 24-hour time window may qualify for mechanical thrombectomy (MT) regardless of the r-tPA treatment for the same ischemic stroke event. Difference in clot mechanical behaviour, due to extensive compositional variation, has been associated with different responses to AIS endovascular therapies (Abbasi et al., 2021; Simons et al., 2015). Therefore, it is important to understand the effect of thrombolysis on clot mechanical properties, which may in turn impact MT interventions for AIS patients.

Recanalization rate in AIS patients using r-tPA is approximately 33% (Seners et al., 2016), while in some patients thrombolysis may lead to incomplete recanalization, re-occlusion or bleeding-related side effects. AIS thrombi composition, physical properties, thrombus length and location of occlusion have shown to impact fibrinolysis (De Meyer et al., 2017; Linfante et al., 2002; Mehta and Nogueira, 2012; Riedel et al.,

* Corresponding author at: CERENOVUS, Block 3, Ballybrit Business Park, Galway H91 K5YD, Ireland.
E-mail address: rmccart9@its.jnj.com (R. McCarthy).
[1] The INSIST investigators are given in the Appendix.

https://doi.org/10.1016/j.jbiomech.2021.110731
Accepted 25 July 2021
Available online 6 September 2021

2011; Rohan et al., 2014). In an animal stroke model, red blood cell (RBC) rich thrombi were found to be more susceptible to thrombolysis (Jang et al., 1989), while white thrombi, composed mainly of fibrin and platelets, displayed more resistance to thrombolysis (Sambola et al., 2016). Thrombolysis may also enhance clot fragility and fragmentation leading to distal embolization by softening the clot and facilitating reperfusion (Alves et al., 2019; Mistry et al., 2017; Tsivgoulis et al., 2015).

Many studies have focussed on understanding the efficacy of r-tPA on clots with different compositions, stiffness or viscoelasticity (Fang and Tsui, 2015; Jeong et al., 2021; Li et al., 2020; Longstaff et al., 2013; Mercado-Shekhar et al., 2018; Niessen et al., 2003; Weisel, 2007) while other studies have focussed on assessing clinical outcome post thrombolysis (Campbell et al., 2021; Hasnain et al., 2020; Poggio et al., 2018; Willeit et al., 2015). Some studies have focussed on examining the effect of fibrinolysis on fibrin fibres of different diameters and arrangements (Collet et al., 2000; Kolev et al., 2005); however, we didn't find studies that have directly investigated the effect of r-tPA on whole-clot mechanical properties. The novelty of this study is that the effect of r-tPA on clots is examined for a broad range of known thrombus variables such as RBC, platelet and fibrin composition as well as compaction of the clots prior to thrombolysis. Considering that AIS clots present with a broad range of composition and material properties (Abbasi et al., 2021; Chueh et al., 2011; Fitzgerald et al., 2021), we feel that this study amounts to a comprehensive evaluation of the fibrinolysis effect on AIS thrombi.

2. Materials and methods

Ovine blood clots were used in this study; however, confirmatory tests were first performed to establish if r-tPA would have a similar effect on ovine blood clots as human blood clots. Retracted ovine and human clot analogues were prepared using whole blood for this assessment; their mass was recorded before and after r-tPA treatment. Scanning electron microscope (SEM) analysis of both types of clots was also carried out to examine their microstructure as per the protocol described by Johnson et al. (2020). Following this comparative study, the main experiment analyzed clot mechanical properties using ovine clot analogues. Clot samples were created using a design of experiment (DoE) approach from blood mixtures with different levels of RBCs, plasma and platelets. Following coagulation, the clots were exposed to different levels of compaction force to simulate the compressive effect of arterial blood pressure. Finally, the clots were treated with a range of r-tPA doses for different durations. Clot mass and mechanical properties were then analyzed to assess changes due to (i) Platelet driven contraction (ii) Compaction force and (iii) Thrombolysis as shown in Fig. 1. The main objective of the experiment was to examine the mechanical behaviour of the clots, therefore, to ensure acceptable test samples for compression testing, just partial reduction in mass by thrombolysis was targeted.

2.1. Experiment design

Having identified the experimental factors for inclusion as listed in Table 1, Design-Expert® software was used to design the experiment (Chang et al., 2013) using an optimal response surface model with a coordinate exchange type of design. A high and low setting for each of the factors was identified based on technical and clinical knowledge as shown in Table 1. The final design resulted in multiple levels for each factor, arranged in such a way to ensure that individual contributions from each factor, as well as combined effects could be identified. A random run order including 39 different experimental runs was arrived at (See Supplementary Table 1). Each test sample consisted of a corresponding control sample that underwent the same treatment as the test sample except for exposure to r-tPA. The control samples were also prepared to account for changes in mass, which may occur due to handling of the sample during the experiment.

Table 1
Factors and their minimum and maximum ranges selected for experimental design; RBC: Red Blood Cells; Factor A, B and D refer to concentration in blood mixtures.

Factor	Name	Minimum setting	Maximum setting
A	% RBC	0 %	50%
B	Time in rt-PA	0	120 min
C	Compaction force	0	0.5 kg (equivalent to >70 mm of Hg[1])
D	% PRP in plasma	0 %	100 %
E	Dose of r-tPA	7 (μg/ml)	21 (μg/ml)
F	Blood mixture volume	10 ml	20 ml

[1] Compaction force on the clot resulted in generating pressure greater than or equivalent to 70 mm of Hg.

2.2. Blood clot preparation

2.2.1. Preliminary tests on ovine and human clots

To compare efficacy of r-tPA on ovine blood clots and human blood clots, whole blood was drawn into a vacuette container. The whole blood spontaneously coagulated and it was then allowed to contract overnight to form a platelet retracted whole blood clot. Clot samples were stored at 4 °C for < 24 h before testing, as described by Duffy et al. (2016). The human and ovine blood clot analogues were cut into dimensions of 4 mm × 4 mm × 2 mm. Human whole blood was obtained by sterile venepuncture from healthy donors from the National Blood Centre, Dublin, Ireland. Ovine blood was obtained from Ash Stream Ltd, Co. Mayo, Ireland. All clot analogues were formed in a static environment.

2.2.2. Ovine clot preparation for mechanical testing

Fresh ovine blood was collected for clot preparation in a sterile container pre-loaded with anti-coagulant solution ACPD (adenine citrate phosphate dextrose). The blood was maintained at room temperature prior to use and clots were prepared within 5 h of blood collection. Platelet rich plasma (PRP), platelet poor plasma (PPP) and red blood cells (RBC) were collected as described previously by Johnson et al. (2021, 2020). Clots with varying compositions were prepared according to the experimental plan (See Supplementary Figure 1). Blood mixtures were prepared by varying the RBC and plasma fractions to create blood mixtures in the range of 0–50% haematocrit. Platelet concentration was controlled by varying the quantity of PRP in the plasma fraction from 0 to 100%. Additional plasma from the same sheep was prepared and stored at −20 °C for use during fibrinolysis. Two volumes of blood mixture were prepared (10 ml and 20 ml) according to a randomized order, to ascertain any clot-size bias in the experiment. Blood mixtures were coagulated by adding calcium chloride. The samples were formed

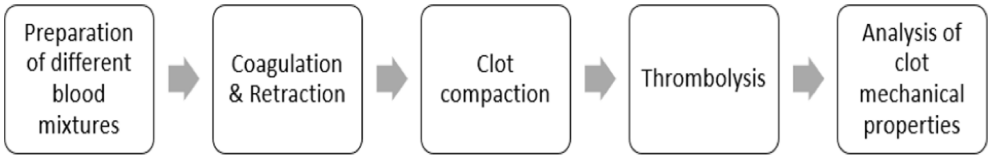

Fig. 1. Flow chart of the experimental plan showing step by step experimental set-up.

in the same size cylindrical containers (internal diameter of 28 mm). They were placed in an oven at 37 °C for 2 h and then maintained at room temperature at atmospheric pressure conditions overnight. The following day all the clots were weighed along with the serum expelled from the clot to measure mass loss due to platelet retraction.

2.3. Mechanical compaction

After the clots matured, clots were subjected to compressive loading to simulate the action of arterial blood pressure on the clot in an occluded artery. In order to compact the clots mechanically, samples were carefully placed in a cylindrical chamber between 2 flat plates as shown in Fig. 2. A fixed weight was applied to the top plate to compress the clots for 5 min, any fluid expelled from the clots could drain away through a circumferential gap between both platens and the wall of the chamber. The fixed loads of 0.25 kg and 0.5 kg exerted a force to the clot equivalent to a pressure of at least 35 or 70 mmHg, respectively. These settings were selected to approximate physiological pressure gradients measured (Sorimachi et al., 2011) in AIS patients (mean pressure = 60 mm Hg, with proximal, distal pressures (mean ± SD) of 95.26 ± 13.2 mm Hg and 35.96 ± 13.5 mm Hg respectively)). The mass of the clot sample was recorded immediately after the load was removed. Details of the calculation to measure the response have been displayed in supplementary Figure 2.

2.4. Fibrinolysis

Following mechanical compaction, clots were treated with r-tPA (Actilyse®, Boehringer Ingelheim), at 37 °C. Rt-PA (1 mg/ml) was prepared by mixing 50 mg of Actylise lypholized powder with 50 ml of sterile water. Aliquots of r-tPA were stored at −20 °C. Clots were subjected to 7 μg/ml, 14 μg/ml or 21 μg/ml r-tPA concentration in plasma. The dose range was selected to span the target systemic r-tPA concentration of 14ug/ml used for in clinical thrombolysis. Each clot was submerged in a fixed volume of plasma, which equated to twice the volume of plasma in the blood mixture from which the clot was prepared. Trials were carried out on a selection of different size clots exposed to r-tPA for 2 h to choose a blood volume for the main experiment. This was to prevent excessive reduction in mass of the clots, so that sufficient sample for mechanical testing remained and the clots did not become fragmented.

Clots were exposed for 0, 1 or 2 h to r-tPA. The r-tPA dose was replenished after every 30 min. After the clots were exposed to r-tPA for the required duration, the clots were subsequently placed in serum from the same sheep from which the clot was derived. The clots were maintained in serum for 1 h following fibrinolysis until r-tPA activity had reduced to a negligible level. The control clots underwent the same process in parallel to the test samples with exception of r-tPA treatment. To assess relative mass loss post thrombolysis, the mass loss experienced by corresponding control samples was applied to the mass of the test samples.

2.5. Mechanical compression testing

Clots were tested under unconfined compression in saline at 37 °C using a Zwick Rowell instrument with a 10 N load cell, as outlined in Johnson et al. 2020. The samples were placed between two platens and the crosshead position of the machine was adjusted so that the top platen was slightly touching the top of the sample at the beginning of the test. The clot specimens were loaded to a compressive nominal strain of 50% at a constant axial strain-rate magnitude of 10% per second and then unloaded to their initial configuration at the same axial strain-rate magnitude. This strain value was chosen so that the clots did not fragment during mechanical testing. Nominal stress-nominal strain plots were constructed and clot tangent modulus was calculated from the slopes of straight lines fitted to the initial and final linear portions of the non-linear nominal stress–strain curves: a low strain tangent modulus (initial 20% strain approximately), and a large strain tangent modulus (final 2% strain approximately). Energy dissipation ratio was calculated by normalizing area under the stress strain curve with respect to the applied loading energy (Bennett and Ker, 1990).

2.6. Statistical analysis

Design Expert® 12 software was used to design a reduced response surface model with a total of 39 runs for 6 factors and was also used to perform statistical analysis. ANOVA was used to evaluate statistical difference between model terms for each response after satisfying the requirement for normality (data transformation was used if required). Statistical significance was taken as $p < 0.05$ and model terms with a p-value < 0.05 were included in the model. A backwards elimination method was used to determine the model terms to remove or retain in the model until the best model was found.

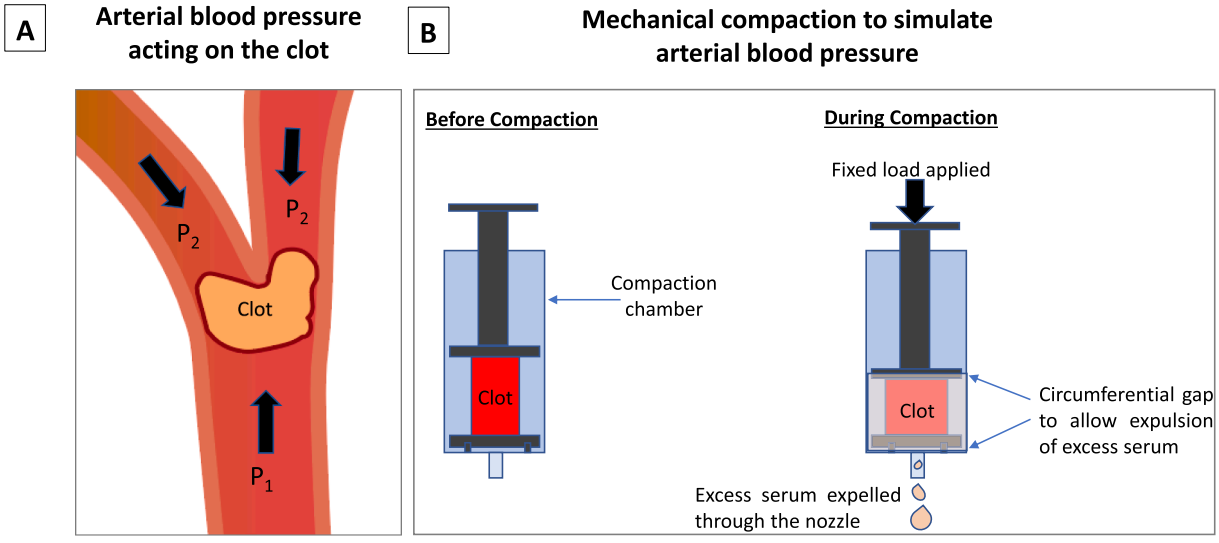

Fig. 2. Experimental set up to simulate compaction force on the clot acting due to arterial blood pressure (A) P1 and P2 represent antegrade and retrograde pressure respectively generating a pressure gradient across the clot. (B) Experimental set-up showing a clot being compacted inside a compaction chamber; 1 mm of circumferential gap was maintained between the top and bottom plate and the compaction chamber to allow expulsion of excess serum.

3. Results

3.1. Comparison of human and ovine clot analogues following thrombolysis

Human and ovine whole blood clot analogues had similar mass loss after r-tPA treatment for 2 h, with final mass of 18.75 ± 4.3 % and 24 ± 3.8 % remaining at 2 h as highlighted in Fig. 3A. Human and ovine clot microstructure was compared using SEM imaging. SEM images of human analogues had similar fibrin and RBC microstructural arrangement, with evidence of both long, thick fibrin fibres and short, thin hyperbranched fibrin, as well as RBC polyhedrocytes (Fig. 3 B); as previously reported in ovine clot analogues (Johnson 2019).

3.2. Effect of thrombolysis on clot mass and clot mechanical properties

The mass of the clots was analysed after the clots matured. Following thrombosis, the clots were subjected to mechanical compaction and thereafter the clots were treated with r-tPA. The mechanical properties of the clots were measured after thrombolysis using an unconfined compression test. A summary of the statistical analysis of all results are provided in Table 2. Interactions between different factors are also noted where dependencies between two factors were uncovered.

3.3. Changes in clot mass following platelet contraction, compaction and thrombolysis

Change in clot mass was assessed following the cumulative effect of platelet contraction, mechanical compaction and thrombolysis (Table 2). Unsurprisingly, blood volume was an independent predictor of clot size ($p < 0.001$), (Supplementary Figure 1). Importantly for our analysis, clots with different size due to the blood volume behaved similarly which is indicated by the absence of significant interactions between blood volume and with RBC content, platelet contraction ($p = 0.879$), compaction force ($p = 0.266$) and thrombolysis parameters ($p = 0.727$). Considering that the eventual size of all clots varied significantly from one another, it was important to establish the impact of clot size on the experimental model. Thus, having established the independence of blood volume on the experimental conclusions, the percent fraction of clot mass after coagulation (*% clot mass-thrombosis and mechanical compaction*) and after thrombolysis (*% clot mass-thrombolysis*) were used for all subsequent data analysis.

% Clot mass loss-thrombosis: We confirmed previous findings (Johnson et al., 2020) that clots with increasing platelet concentration (%PRP) in the blood mixture caused increased mass loss after coagulation ($p < 0.001$, graph not shown here), and that a decreasing % RBC leads to smaller clots , $p = 0.029$ (Supplementary Figure 1).

% Clot mass loss-post mechanical compaction: Mechanical loading applied to clot samples to simulate compression due to arterial blood pressure conditions also changed the clot mass. Unsurprisingly, clots that experienced greater mechanical force underwent significant mass loss. Clots created from blood mixtures with lower % RBC displayed greater mass loss following mechanical loading, $p < 0.001$ (Fig. 4A). Clots that had previously undergone significant platelet contraction changed less due to mechanical compression than clots that hadn't undergone platelet contraction, $p = 0.041$ (Fig. 4A).

% Clot mass-thrombolysis. Clots that were treated with r-tPA for a longer duration exhibited greater mass loss ($p < 0.01$). Mean clot degradation of 10.7 % (confidence interval range from 8.1 % −13.5 %) and 24.6% (confidence interval range from 19.3 % to 30.5 %) was observed after 1 h and 2 h of exposure to r-tPA, respectively (Fig. 4B). The other factors varied in the experiment, (RBC concentration, platelet retraction, mechanical compaction, and drug dose) didn't influence the rate of clot degradation.

3.4. Effect of r-tPA on clot mechanical properties

As shown for a typical sample in Fig. 5, mechanical compression testing of clots reveals a non-linear stress–strain relationship with significant strain stiffening and hysteresis between loading and loading. Treatment of the clots with r-tPA resulted in reduced stiffness and reduced hysteresis between loading and loading. No variable was found to have a significant impact at low strain tangent modulus. However at large strain tangent modulus, increasing fibrin content (decreasing % RBC) in blood mixtures resulted in increased clot stiffness, while increasing %PRP, which determines the extent of contraction, was also found to increase clot stiffness (Fig. 6 A). Increased duration of exposure to r-tPA resulted in a significant decrease in clot stiffness (Fig. 6A).

Additionally, clot energy dissipation ratio was calculated to examine clot viscoelasticity. Energy dissipation ratio relates to relative energy dissipation due to internal friction within the clots. This also affects clot viscoelasticity and its stiffness. Clots that underwent platelet driven contraction (i.e. high %PRP) showed a significant decrease in energy dissipation ratio ($p = 0.018$). Clots that were exposed to r-tPA for longer durations had a significant reduction in energy dissipation ratio ($p < 0.001$) (Fig. 6 B).

4. Discussion

Clinical studies for large vessel occlusions in AIS have proven that a

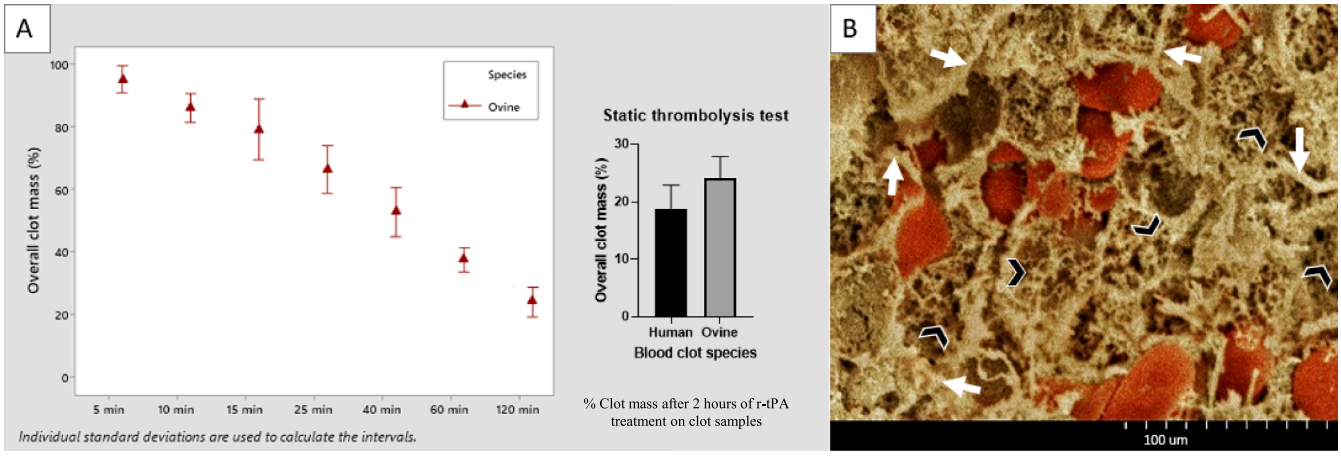

Fig. 3. (A) Graph highlighting overall percentage clot mass remaining following fibrinolysis. Whole blood clot analogues were prepared from human and ovine blood respectively (B) SEM imaging of the interior of a human whole blood clot showing thin hyper-branched fibrin fibres along with compressed RBCs. Black arrow heads showing thin hyperbranched fibrin while black arrows are showing thick fibrin fibres.

Table 2
Statistical summary of all the variables that were analysed in the study.

		Final clot mass (g)	Mass loss after thrombosis (%)	Mass loss after compaction (%)	Mass loss post thrombolysis (%)	Tangent stiffness at high strain	Energy dissipation ratio
A	% RBC	$P < 0.001$ (AC; AD) (-)	$P = 0.029$ (-)	$P < 0.001$ (AC) (-)	$P = 0.116$	$P < 0.001$ (-)	$P = 0.0811$
B	r-tPA exposure time	$P = 0.0435$ (+)	NA	NA	$P < 0.001$ (+)	$P < 0.001$ (-)	$P < 0.001$ (-)
C	Mechanical compaction	$P = 0.0447$ (AC) (-)	NA	$P < 0.001$ (AC) (+)	$P = 0.41$	$P = 0.188$	$P = 0.195$
D	%PRP	$P = 0.002$ (AD) (-)	$P < 0.001$ (+)	$P = 0.041$ (-)	$P = 0.683$	$P = 0.017$ (+)	$P = 0.018$ (-)
E	r-tPA dose	$P = 0.102$	NA	NA	$P = 0.445$	$P = 0.425$	$P = 0.0545$
F	Blood Vol	$P < 0.001$ (+)	$P = 0.879$	$P = 0.266$	$P = 0.727$	$P = 0.734$	$P = 0.349$

'(+)' indicates positive correlation; '(-)' indicates negative correlation; The two variables in bracket indicate interaction and 'NA' indicates 'not applicable'.

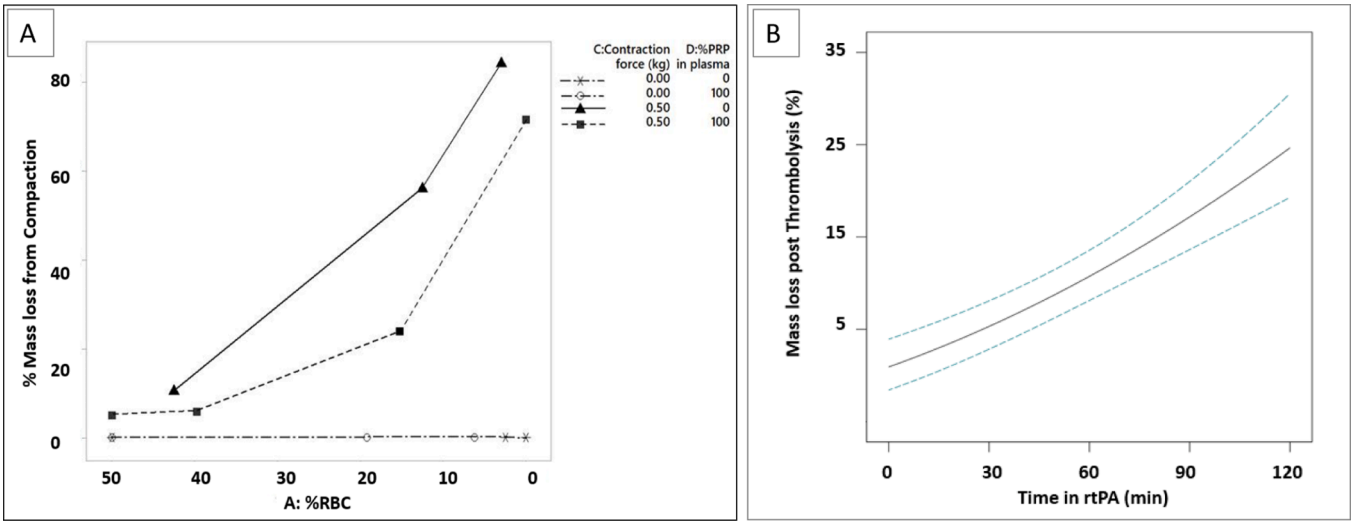

Fig. 4. (A) % Mass loss from compaction as a function of contraction force and % PRP in blood mixture; (B) Relative mass loss post r-tPA treatment; Graph B represents main effects plot from the Design Expert model. The dotted lines in graph B represents 95% confidence interval bands.

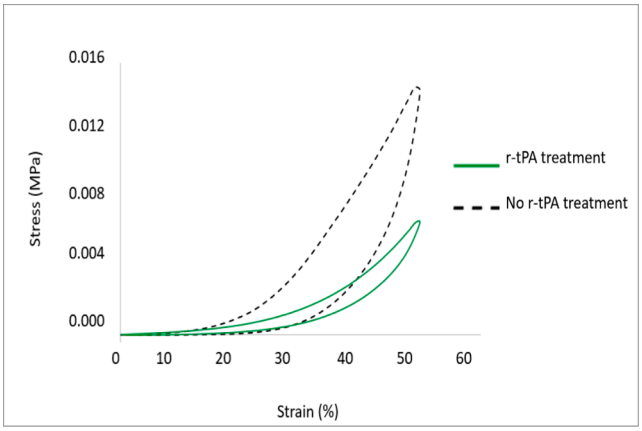

Fig. 5. Stress strain representative curve of a 50% RBC (blood mixture) platelet rich clot with (shown in green) and without (shown in black) r-tPA treatment.

combined approach involving thrombolysis and MT has shown improved recanalization rate, better functional outcomes, and reduced mortality than thrombolysis alone (Gariel et al., 2018; Goyal et al., 2016). In comprehensive stroke centres, where patients present directly to a hospital capable of performing MT, there is an argument for bypassing clinical thrombolysis treatment altogether (Kaesmacher et al., 2019; Schlemm et al., 2020; Suzuki et al., 2021; Yang et al., 2020). However, in the so-called hub-and-spokes treatment model, where patients are transferred from a primary stroke hospital to a comprehensive stroke hospital for MT, patients will continue to receive thrombolytics prior to MT. As thrombus mechanical properties may play a significant part in determining success of MT outcomes, it is important to understand the effect of r-tPA on clot mechanical behaviour (Hernández-Fernández et al., 2021; Johnson et al., 2020; Mercado-Shekhar et al., 2018; Niessen et al., 2003)

Clot lysis has been shown to be affected by a number of factors influencing clot microstructure, such as clot composition (Collet et al., 2000; Hudson, 2017), fibrin fibre diameter, orientation of fibrin fibres, porosity of fibrin network (Hudson, 2017) and platelet content in the clots (Tomkins et al., 2011). The current study investigates the influence of RBC content, platelet retraction, mechanical compaction, r-tPA dose and duration of r-tPA exposure on clot mass and mechanical behaviour. The interaction between these variables was also examined. A statistically significant reduction in clot mass occurred over time after the clots were exposed to r-tPA verifying that the experimental model using ovine blood is effective.

RBC-rich clots have shown more favourable outcomes with thrombolysis while clots with high fibrin and platelet content have shown greater resistance to thrombolysis (Bagoly et al., 2019; Denorme et al., 2016; Jolugbo and Ariëns, 2021; Shin et al., 2018; Tomkins et al., 2011; Weisel and Litvinov, 2008); highlighting that thrombus composition is an important factor that might influence clot degradation with r-tPA treatment. In contrast to these studies, we observed that factors such as clot RBC content and platelet concentration which greatly change the retracted size of clots, and change their microstructure, did not influence

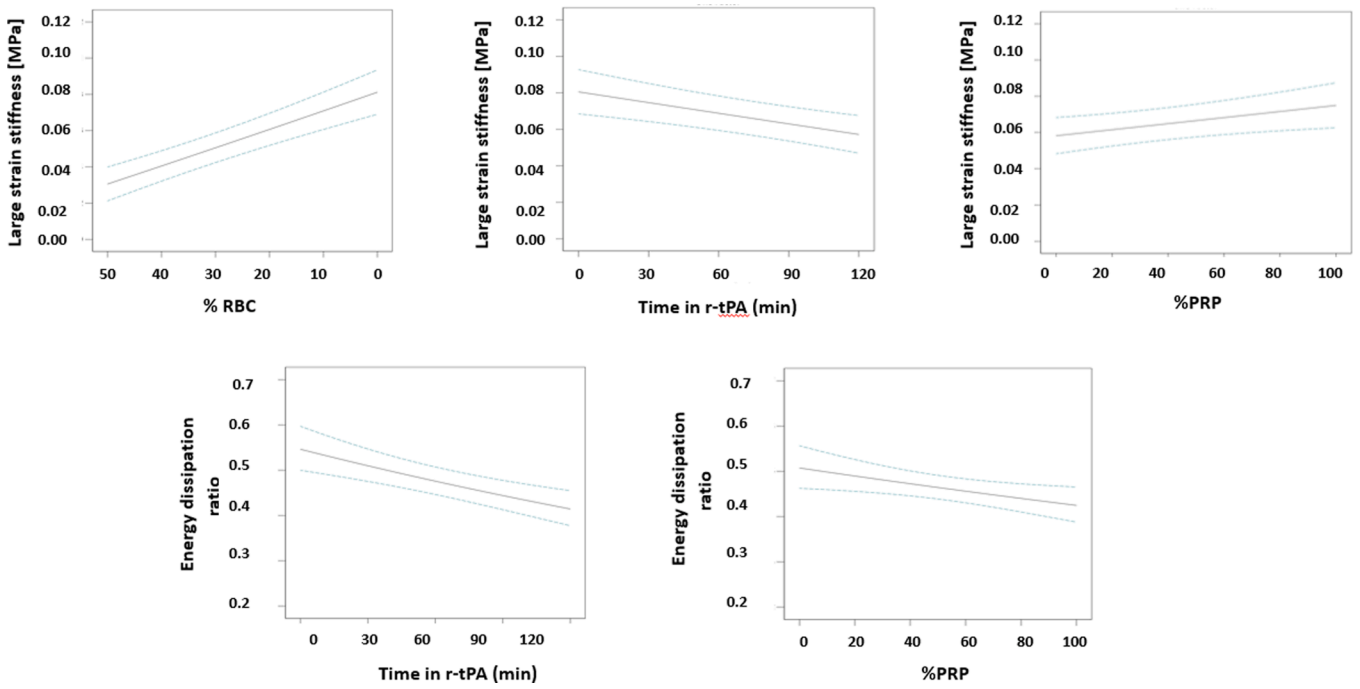

Fig. 6. Main effect plots obtained from DoE model. Dotted lines represent 95 % confidence interval bands (A) Large strain stiffness was calculated by analysing slope at last 2% of the maximum applied strain (B) Energy dissipation ratio from the clot sample analysed by calculating area under the hysteresis curve.

the effectiveness of r-tPA in reducing clot mass. It should be stated however, that our experiment did not proceed to full or even significant clot lysis; furthermore, it was carried out using unconstrained clots under static flow conditions. As such, RBC composition and platelet retraction may have been more apparent in clots that experience greater mass reduction and/or are subjected to dynamic flow conditions. In our study, mechanical properties of the clots were affected by RBC content, platelet retraction and exposure time to r-tPA. Each of these variables were found to be independent predictors of clot mechanical properties. Interestingly, %RBC and platelet retraction of blood clot did not influence the r-tPA effect on clot mass reduction and mechanical properties.

A significant observation in our investigation highlights that clot stiffness is impacted by fibrinolysis. Others have shown that clot fibrin conformation and network density significantly resisted clot lysis (Miniati et al., 2010; Niessen et al., 2003; Wohner et al., 2011). It signifies that changes in the stiffness of the material can occur without large changes in the mass of the clots, presumably brought about by cleavage of individual fibrin fibres within the clot's fibrin mesh. Taken together with the observations that platelet retraction and RBC content are key determinants of clot stiffness even prior to the thrombolysis effect, it may explain, in-part, why some clots are more responsive to r-tPA than others. For example, the effect of reducing the stiffness of an already highly deformable RBC-rich clot may have a larger effect than a similar reduction in stiffness of a tough, fibrin rich clot. Indeed, studies have shown that r-tPA thrombolysis is less effective on fibrin rich clots than RBC rich clots (Jang et al., 1989; Kirchhof et al., 2004).

We observed that clot samples that underwent r-tPA treatment for longer duration also showed significantly reduced viscoelastic behaviour. It can be proposed that reduction in modulus brought on by fibrinolysis may lead to continued distal clot migration which would reduce the size of the infarcted brain territory. However, it may also move occlusive clots into distal vessels that are inaccessible for mechanical thrombectomy. It is also notable that using a 50% or 150% dose of r-tPA did not impact the rate of clot lysis. A plausible reason could be that the available plasminogen attached to the fibrin fibres is the limiting factor rather than the amount of r-tPA available in the circulating blood. Other studies have also reported similar results, highlighting that other blood components may play a superior role in determining clot lysis (Staykov et al., 2011; Ziai et al., 2012).

The following limitations should be acknowledged in this study. This study was performed in an *in vitro* static condition which limits the direct applicability to the dynamic *in vivo* animal and human studies. The homogenous nature of the clots may are not representative of thrombi retrieved from AIS patients, which tend to be heterogenous in nature. However, for the purpose of this study the use of homogenous clot analogues enables repeatable and comparable testing based on clot composition. The influence of r-tPA on the fracture properties of blood clots was not assessed in this study which may have implications on procedural embolization in MT.

Our investigation established that thrombolysis has a significant effect on reducing clot stiffness. This may lead to increased clot migration prior to MT, thereby resulting in increased risk of incomplete reperfusion for distal thrombus with challenging access; alternately it may lead to softening of the clot, which could be beneficial in retrieving clots during MT. Future work focused on examining the benefits and risks of changing the mechanical properties of clots prior to MT may help treating physicians decide the best treatment combination for AIS patients. Moreover, this experimental investigation may also prove beneficial in supporting numerical models of thrombolysis for AIS (Konduri et al., 2020; Luraghi et al., 2021b; Luraghi et al., 2021a).

5. Conclusion

Our study examined the effect of thrombolysis on clot mechanical properties using ovine blood clot analogues that were reduced in mass by up to 25%. Thrombolysis was shown to significantly affect clot mechanical properties by reducing their stiffness, which may explain thrombus migration observed clinically (Alves et al., 2019). Such alterations of clot mechanical behaviour may also impact the outcome of MT and should be subject of future investigations.

Funding

This project has received funding from the European Union's

Horizon 2020 research and innovation program under grant agreement No 777072.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [AD, AG, SD, SJ and MG and RMcC report a financial relationship with Cerenovus outside the submitted work].

Appendix A

**INSIST Investigators*

Charles Majoie[1], Henk Marquering[1,6], Ed van Bavel[14], Alfons Hoekstra[15], Diederik Dippe[l2], Hester Lingsma[4], Aad van der Lugt[3], Noor Samuels[2,3,4], Nikki Boodt[2,3,4], Yvo Roos[1], Simon de Meyer[5], Senna Staessens[5], Sarah Vandelanotte[5], Henk Marquering[1,6], Praneeta Konduri[1,6], Nerea Arrarte Terreros[1,6], Bastien Chopard[7], Franck Raynaud[7], Remy Petkantchin[7], Mikhail Panteleev[11], Alexey Shibeko[11], Karim Zouaoui Boudjeltia[16], Vanessa Blanc-Guillemaud[8], Francesco Migliavacca[9], Gabriele Dubini[9], Giulia Luraghi[9], Jose Felix Rodriguez Matas[9], Sara Bridio[9], Patrick McGarry[10], Michael Gilvarry[12], Ray McCarthy[12], Kevin Moerman[10], Behrooz Fereidoonnezhad[10], Anushree Dwivedi[12], Sharon Duffy[12], Stephen Payne[13], Tamas Jozsa[13], Wahbi El-Bouri[13], Ed van Bavel[14], Sissy Georgakopoulou[14], Alfons Hoekstra[15], Raymond Padmos[15], Alfons Hoekstra[15], Victor Azizi[15], Claire Miller[15], Max van der Kolk[15], Raymond Padmos[15],

[1] Department of Radiology and Nuclear Medicine, Amsterdam University Medical Centers, location AMC, Amsterdam

[2] Department of Neurology, Erasmus MC University Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands

[3] Department of Radiology, Erasmus MC University Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands

[4] Department of Public Health, Erasmus MC University Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands

[5] Laboratory for Thrombosis Research, KU Leuven Campus Kulak Kortrijk, Kortrijk, Belgium

[6] Department of Biomedical Engineering & Physics, Amsterdam University Medical Centers, location AMC, Amsterdam

[7] Computer Science Department, University of Geneva, CUI, 7 route de Drize, 1227 Carouge, Switzerland

[8] Institut de Recherches Internationales Servier, Coubevoie Cedex, France

[9] Department of Chemistry, Materials and Chemical Engineering 'Giulio Natta', Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy.

[10] National Centre for Biomedical Engineering Science, School of Engineering, National University of Ireland Galway, Ireland.

[11] Faculty of computational mathematics and cybernetics, Moscow

[12] Cerenovus, Galway Neuro Technology Centre, Galway, Ireland.

[13] Department of Engineering Science, University of Oxford, Parks Road, Oxford OX1 3PJ, UK.

[14] Biomedical Engineering, Amsterdam University Medical Centers, location AMC, Amsterdam

[15] Computational Science Lab, Faculty of Science, Institute for Informatics, University of Amsterdam, Amsterdam, Netherlands

[16] Laboratory of Experimental Medicine (ULB222), Faculty of Medicine, Université libre de Bruxelles, CHU de Charleroi, Belgium

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiomech.2021.110731.

References

Abbasi, M., Kvamme, P., Layton, K.F., Hanel, R.A., Almekhlafi, M.A., Delgado, J.E., Pereira, V.M., Patel, B.M., Jahromi, B.S., Yoo, A.J., Nogueira, R.G., Gounis, M.J., Fitzgerald, S., Mereuta, O.M., Dai, D., Kadirvel, R., Kallmes, D.F., Doyle, K.M., Savastano, L.E., Cloft, H.J., Liu, Y., Thacker, I.C., Aghaebrahim, A., Sauvageau, E., Demchuk, A.M., Kayan, Y., Copelan, A.Z., Entwistle, J., Nazari, P., Cantrell, D.R., Bhuva, P., Soomro, J., Haussen, D.C., Al-Bayati, A., Mohammaden, M., Pisani, L., Rodrigues, G., Puri, A.S., Brinjikji, W., 2021. Per pass analysis of thrombus composition retrieved by mechanical thrombectomy. Interv. Neuroradiol. 7: 15910199211009119.

Alonso de Leciñana, M., Egido, J.A., Casado, I., Ribó, M., Dávalos, A., Masjuan, J., Caniego, J.L., Martínez Vila, E., Díez Tejedor, E., Fuentes Secretaría, B., Álvarez-Sabin, J., Arenillas, J., Calleja, S., Castellanos, M., Castillo, J., Díaz-Otero, F., López-Fernández, J.C., Freijo, M., Gállego, J., García-Pastor, A., Gil-Núñez, A., Gilo, F., Irimia, P., Lago, A., Maestre, J., Martí-Fábregas, J., Martínez-Sánchez, P., Molina, C., Morales, A., Nombela, F., Purroy, F., Rodríguez-Yañez, M., Roquer, J., Rubio, F., Segura, T., Serena, J., Simal, P., Tejada, J., Vivancos, J., 2014. Guía para el tratamiento del infarto cerebral agudo. Neurologia. 29, 102–122.

Alves, H.C., Treurniet, K.M., Jansen, I.G.H., Yoo, A.J., Dutra, B.G., Zhang, G., Yo, L., van Es, A.C.G.M., Emmer, B.J., van den Berg, R., van den Wijngaard, I.R., Lycklama À Nijeholt, G.J., Vos, J.A., Roos, Y.B.W.E.M., Schonewille, W., Marquering, H.A., Majoie, C.B.L.M., 2019. Thrombus Migration Paradox in Patients With Acute Ischemic Stroke. Stroke 50, 3156–3163.

Bagoly, Z., Szegedi, I., Kálmándi, R., Tóth, N.K., Csiba, L., 2019. Markers of Coagulation and Fibrinolysis Predicting the Outcome of Acute Ischemic Stroke Thrombolysis Treatment: A Review of the Literature. Front. Neurol. 10, 513.

Bennett, M.B., Ker, R.F., 1990. The mechanical properties of the human subcalcaneal fat pad in compression. J. Anat. 171, 131–138.

Campbell, B.C.V., Ma, H., Parsons, M.W., Churilov, L., Yassi, N., Kleinig, T.J., Hsu, C.Y., Dewey, H.M., Butcher, K.S., Yan, B., Desmond, P.M., Wijeratne, T., Curtze, S., Barber, P.A., De Silva, D.A., Thijs, V., Levi, C.R., Bladin, C.F., Sharma, G., Bivard, A., Donnan, G.A., Davis, S.M., 2021. Association of Reperfusion after Thrombolysis with Clinical Outcome across the 4.5- To 9-Hours and Wake-up Stroke Time Window: A Meta-Analysis of the EXTEND and EPITHET Randomized Clinical Trials. JAMA Neurol. 78, 236–240.

Chang, R.K., Raw, A., Lionberger, R., Yu, L., 2013. Generic development of topical dermatologic products, part II: Quality by design for topical semisolid products. AAPS J. 15, 1–8.

Chueh, J.Y., Wakhloo, A.K., Hendricks, G.H., Silva, C.F., Weaver, J.P., Gounis, M.J., 2011. Mechanical characterization of thromboemboli in acute ischemic stroke and laboratory embolus analogs. Am. J. Neuroradiol. 32, 1237–1244.

Collen, D., 1987. Molecular mechanisms of fibrinolysis and their application to fibrin-specific thrombolytic therapy. J. Cell. Biochem. 33, 77–86.

Collet, J.P., Park, D., Lesty, C., Soria, J., Soria, C., Montalescot, G., Weisel, J.W., 2000. Influence of Fibrin Network Conformation and Fibrin Fiber Diameter on Fibrinolysis Speed. Arterioscler. Thromb. Vasc. Biol. 20, 1354–1361.

De Meyer, S.F., Andersson, T., Baxter, B., Bendszus, M., Brouwer, P., Brinjikji, W., Campbell, B.C.V., Costalat, V., Dávalos, A., Demchuk, A., Dippel, D., Fiehler, J., Fischer, U., Gilvarry, M., Gounis, M.J., Gralla, J., Jansen, O., Jovin, T., Kallmes, D., Khatri, P., Lees, K.R., López-Cancio, E., Majoie, C., Marquering, H., Narata, A.P., Nogueira, R., Ringleb, P., Siddiqui, A., Szikora, I., Vale, D., von Kummer, R., Yoo, A. J., Hacke, W., Liebeskind, D.S., 2017. Analyses of thrombi in acute ischemic stroke: A consensus statement on current knowledge and future directions. Int. J. Stroke. 12, 606–614.

del Zoppo, G.J., Poeck, K., Pessin, M.S., Wolpert, S.M., Furlan, A.J., Ferbert, A., Alberts, M.J., Zivin, J.A., Wechsler, L., Busse, O., Greenlee, R., Brass, L., Mohr, J.P., Feldmann, E., Hacke, W., Kase, C.S., Biller, J., Gress, D., Otis, S.M., 1992. Recombinant tissue plasminogen activator in acute thrombotic and embolic stroke. Ann. Neurol. 32, 78–86.

Denorme, F., Langhauser, F., Desender, L., Vandenbulcke, A., Rottensteiner, H., Plaimauer, B., François, O., T, A., H, D., F, S., C, K., K, V., SF, D.M., 2016. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. Blood 127, 2337–2345.

Duffy, S., Farrell, M., McArdle, K., Thornton, J., Vale, D., Rainsford, E., Morris, L., Liebeskind, D.S., MacCarthy, E., Gilvarry, M., 2016. Novel methodology to replicate clot analogs withdiverse composition in acute ischemic stroke. J. NeuroIntervent. Surg. 1–7. https://doi.org/10.1136/neurintsurg-2016-012308.

Fang, J., Tsui, P.-H., 2015. Evaluation of thrombolysis by using ultrasonic imaging: an in vitro study. Sci. Reports 2015 51 5, 1–12.

Fitzgerald, S.T., Liu, Y., Dai, D., Mereuta, O.M., Abbasi, M., Larco, J.L.A., Douglas, A.S., Kallmes, D.F., Savastano, L., Doyle, K.M., Brinjikji, W., 2021. Novel Human Acute Ischemic Stroke Blood Clot Analogs for In Vitro Thrombectomy Testing. Am. J. Neuroradiol. https://doi.org/10.3174/ajnr.A7102.

Gariel, F., Lapergue, B., Bourcier, R., Berge, J., Barreau, X., Mazighi, M., Kyheng, M., Labreuche, J., Fahed, R., Blanc, R., Gory, B., Duhamel, A., Saleme, Suzana, Costalat, V., Bracard, S., Desal, H., Detraz, L., Consoli, A., Piotin, M., Marnat, G., Piotin, M., Blanc, R., Redjem, H., Ciccio, G., Smajda, S., Mazighi, M., Fahed, R., Desilles, J.P., Lapergue, B., Rodesch, G., Consoli, A., Coskun, O., Di Maria, F., Bourdain, F., Pierre Decroix, J., Wang, A., Tchikviladze, M., Evrard, S., Turjman, F., Gory, B., Emile Labeyrie, P., Riva, R., Mounayer, C., Saleme, Suzanna, Costalat, V., Bonafé, A., Eker, O., Gascou, G., Dargazanli, C., Bracard, S., Tonnelet, R., Laure Derelle, A., Anxionnat, R., Desal, H., Bourcier, R., Daumas-Duport, B., Berge, J., Barreau, X., Marnat, G., Djemmane, L., Ben Maacha, M., Labreuche, J., Duhamel, A., 2018. Mechanical thrombectomy outcomes with or without intravenous thrombolysis insight from the ASTER randomized trial. Stroke 49, 2383–2390.

Goyal, M., Menon, B.K., Van Zwam, W.H., Dippel, D.W.J., Mitchell, P.J., Demchuk, A.M., Dávalos, A., Majoie, C.B.L.M., Van Der Lugt, A., De Miquel, M.A., Donnan, G.A., Roos, Y.B.W.E.M., Bonafe, A., Jahan, R., Diener, H.C., Van Den Berg, L.A., Levy, E.I., Berkhemer, O.A., Pereira, V.M., Rempel, J., Millán, M., Davis, S.M., Roy, D., Thornton, J., Román, L.S., Ribó, M., Beumer, D., Stouch, B., Brown, S., Campbell, B. C.V., Van Oostenbrugge, R.J., Saver, J.L., Hill, M.D., Jovin, T.G., 2016. Endovascular thrombectomy after large-vessel ischaemic stroke: A meta-analysis of individual patient data from five randomised trials. Lancet 387, 1723–1731.

Hasnain, M.G., Paul, C.L., Attia, J.R., Ryan, A., Kerr, E., Oldmeadow, C., D'Este, C.A., Bivard, A., Hubbard, I.J., Milton, A.H., Levi, C.R., 2020. Thrombolysis implementation intervention and clinical outcome: a secondary analysis of a cluster randomized trial. BMC Cardiovasc. Disord. 2020 201 20, 1–8.

Hernández-Fernández, F., Ramos-Araque, M.E., Barbella-Aponte, R., Molina-Nuevo, J.D., García-García, J., Ayo-Martin, O., Pedrosa-Jiménez, M.J., López-Martinez, L., Serrano-Heras, G., Julia-Molla, E., Segura, T., 2021. Fibrin-Platelet Clots in Acute Ischemic Stroke. Predictors and Clinical Significance in a Mechanical Thrombectomy Series. Front. Neurol. 12, 443.

Hudson, N.E., 2017. Biophysical Mechanisms Mediating Fibrin Fiber Lysis. Biomed Res. Int. 2017.

Jang, I.K., Gold, H.K., Ziskind, A.A., Fallon, J.T., Holt, R.E., Leinbach, R.C., May, J.W., Collen, D., 1989. Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator. A possible explanation for resistance to coronary thrombolysis. Circulation 79, 920–928.

Jeong, Y.H., Kevin, B., Ahn, J.H., Chaudhary, R., Kang, M.G., Park, H.W., Koh, J.S., Park, Y., Tantry, U.S., Gurbel, P.A., 2021. Viscoelastic properties of clot formation and their clinical impact in East Asian versus Caucasian patients with stable coronary artery disease: a COMPARE-RACE analysis. J. Thromb. Thrombolysis 51, 454–465.

Johnson, S., Chueh, J., Gounis, M.J., McCarthy, R., Patrick McGarry, J., McHugh, P.E., Gilvarry, M., 2020. Mechanical behavior of in vitro blood clots and the implications for acute ischemic stroke treatment. J. Neurointerv. Surg. 12, 853–857.

Johnson, S., McCarthy, R., Gilvarry, M., McHugh, P.E., McGarry, J.P., 2021. Investigating the Mechanical Behavior of Clot Analogues Through Experimental and Computational Analysis. Ann. Biomed. Eng. 49, 420–431.

Jolugbo, P., Ariëns, R.A.S., 2021. Thrombus Composition and Efficacy of Thrombolysis and Thrombectomy in Acute Ischemic Stroke. Stroke. 53, 1131–1142.

Kaesmacher, J., Mordasini, P., Arnold, M., López-Cancio, E., Cerdá, N., Boeckh-Behrens, T., Kleine, J.F., Goyal, M., Hill, M.D., Pereira, V.M., Saver, J.L., Gralla, J., Fischer, U., 2019. Direct mechanical thrombectomy in tPA-ineligible and-eligible patients versus the bridging approach: A meta-analysis. J. Neurointerv. Surg. 11, 20–27.

Kirchhof, K., Sikinger, M., Welzel, T., Zoubaa, S., Sartor, K., 2004. Hängt das Ergebnis einer Thrombolyse mit rekombinantem Gewebe-Plasminogenaktivator (rt-PA) beim Kaninchen vom Erythrozyten- und Fibringehalt eines Thrombus ab? RoFo Fortschritte auf dem Gebiet der Rontgenstrahlen und der Bildgeb. Verfahren 176, 98–105.

Kolev, K., Longstaff, C., Machovich, R., 2005. Fibrinolysis at the fluid-solid interface of thrombi. Curr. Med. Chem. Cardiovasc. Hematol. Agents 3, 341–355.

Konduri, P.R., Marquering, H.A., van Bavel, E.E., Hoekstra, A., Majoie, C.B.L.M., 2020. In-Silico Trials for Treatment of Acute Ischemic Stroke. Front. Neurol. 11, 558125.

Li, Y., Wang, Heying, Zhao, L., Jian, Y., Dang, M., Jiang, Y., Zhang, Y., Zhang, L., Wang, Huqing, Zhang, R., Chen, M., Zhang, G., 2020. A case report of thrombolysis resistance: thrombus ultrastructure in an ischemic stroke patient. BMC Neurol. 2020 201 20, 1–6.

Linfante, I., Llinas, R.H., Selim, M., Chaves, C., Kumar, S., Parker, R.A., Caplan, L.R., Schlaug, G., 2002. Clinical and vascular outcome in internal carotid artery versus middle cerebral artery occlusions after intravenous tissue plasminogen activator. Stroke 33, 2066–2071.

Longstaff, C., Varjú, I., Sótonyi, P., Szabó, L., Krumrey, M., Hoell, A., Bóta, A., Varga, Z., Komorowicz, E., Kolev, K., 2013. Mechanical Stability and Fibrinolytic Resistance of Clots Containing Fibrin, DNA, and Histones *.288, 6946-6956.

Luraghi, G., Bridio, S., Rodriguez Matas, J.F., Dubini, G., Boodt, N., Gijsen, F.J.H., van der Lugt, A., Fereidoonnezhad, B., Moerman, K.M., McGarry, P., Konduri, P.R., Arrarte Terreros, N., Marquering, H.A., Majoie, C.B.L.M., Migliavacca, F., 2021a. The first virtual patient-specific thrombectomy procedure. J. Biomech. 126, 110622.

Luraghi, G., Matas, J.F.R., Dubini, G., Berti, F., Bridio, S., Duffy, S., Dwivedi, A., McCarthy, R., Fereidoonnezhad, B., McGarry, P., Majoie, C.B.L.M., Migliavacca, F., investigators, on behalf of the I., 2021b. Applicability assessment of a stent-retriever thrombectomy finite-element model. Interface Focus 11, 20190123.

Mehta, B.P., Nogueira, R.G., 2012. Should clot composition affect choice of Endovascular therapy? Neurology 79, s63–s67.

Mercado-Shekhar, K.P., Kleven, R., Rivera, H.A., Lewis, R., Karani, K.B., Vos, H.J., Abruzzo, T.A., Haworth, K.J., Holland, C.K., 2018. Effect of Clot Stiffness on Recombinant Tissue Plasminogen Activator Lytic Susceptibility In Vitro HHS Public Access. Ultrasound Med Biol 44, 2710–2727.

Miniati, M., Fiorillo, C., Becatti, M., Monti, S., Bottai, M., Marini, C., Grifoni, E., Formichi, B., Bauleo, C., Arcangeli, C., Poli, D., Nassi, P.A., Abbate, R., Prisco, D., 2010. Fibrin resistance to lysis in patients with pulmonary hypertension other than thromboembolic. Am. J. Respir. Crit. Care Med. 181, 992–996.

Mistry, E.A., Mistry, A.M., Nakawah, M.O., Chitale, R.V., James, R.F., Volpi, J.J., Fusco, M.R., 2017. Mechanical Thrombectomy Outcomes With and Without Intravenous Thrombolysis in Stroke Patients. Stroke 48, 2450–2456.

Murray, V., Norrving, B., Sandercock, P.A.G., Terént, A., Wardlaw, J.M., Wester, P., 2010. The molecular basis of thrombolysis and its clinical application in stroke. J. Intern. Med. 267, 191–208.

Niessen, F., Hilger, T., Hoehn, M., Hossmann, K.A., 2003. Differences in clot preparation determine outcome of recombinant tissue plasminogen activator treatment in experimental thromboembolic stroke. Stroke 34, 2019–2024.

Poggio, M.B. di, Finocchi, C., Brizzo, F., Altomonte, F., Bovis, F., Mavilio, N., Serrati, C., Malfatto, L., Mancardi, G., Balestrino, M., 2018. Management of acute ischemic stroke, thrombolysis rate, and predictors of clinical outcome. Neurol. Sci. 402 40, 319–326.

Riedel, C.H., Zimmermann, P., Jensen-Kondering, U., Stingele, R., Deuschl, G., Jansen, O., 2011. The importance of size: Successful recanalization by intravenous thrombolysis in acute anterior stroke depends on thrombus length. Stroke 42, 1775–1777.

Rohan, V., Baxa, J., Tupy, R., Cerna, L., Sevcik, P., Friesl, M., Polivka, J., Polivka, J., Ferda, J., 2014. Length of occlusion predicts recanalization and outcome after intravenous thrombolysis in middle cerebral artery stroke. Stroke 45, 2010–2017.

Sambola, A., Del Blanco, B.G., Ruiz-Meana, M., Francisco, J., Barrabés, J.A., Figueras, J., Bañeras, J., Otaegui, I., Rojas, A., Vilardosa, Ú., Montaner, J., García-Dorado, D., 2016. Increased von Willebrand factor, P-selectin and fibrin content in occlusive thrombus resistant to lytic therapy. Thromb. Haemost. 115, 1129–1137.

Schlemm, L., Endres, M., Nolte, C.H., 2020. Bypassing the closest stroke center for thrombectomy candidates what additional delay to thrombolysis is acceptable? Stroke 867–875.

Seners, P., Turc, G., Maïer, B., Mas, J.L., Oppenheim, C., Baron, J.C., 2016. Incidence and Predictors of Early Recanalization after Intravenous Thrombolysis: A Systematic Review and Meta-Analysis. Stroke 47, 2409–2412.

Shin, J.W., Jeong, H.S., Kwon, H.-J., Song, K.S., Kim, J., 2018. High red blood cell composition in clots is associated with successful recanalization during intra-arterial thrombectomy. PLoS One 13, e0197492.

Simons, N., Mitchell, P., Dowling, R., Gonzales, M., Yan, B., 2015. Thrombus composition in acute ischemic stroke: A histopathological study of thrombus extracted by endovascular retrieval. J. Neuroradiol. 42, 86–92.

Sorimachi, T., Morita, K., Ito, Y., Fujii, Y., 2011. Blood pressure measurement in the artery proximal and distal to an intra-arterial embolus during thrombolytic therapy. J. Neurointerv. Surg. 3, 43–46.

Staykov, D., Wagner, I., Volbers, B., Huttner, H.B., Doerfler, A., Schwab, S., Bardutzky, J., 2011. Dose effect of intraventricular fibrinolysis in ventricular hemorrhage. Stroke 42, 2061–2064.

Suzuki, K., Matsumaru, Y., Takeuchi, M., Morimoto, M., Kanazawa, R., Takayama, Y., Kamiya, Y., Shigeta, K., Okubo, S., Hayakawa, M., Ishii, N., Koguchi, Y., Takigawa, T., Inoue, M., Naito, H., Ota, T., Hirano, T., Kato, N., Ueda, T., Iguchi, Y., Akaji, K., Tsuruta, W., Miki, K., Fujimoto, S., Higashida, T., Iwasaki, M., Aoki, J., Nishiyama, Y., Otsuka, T., Kimura, K., 2021. Effect of Mechanical Thrombectomy without vs with Intravenous Thrombolysis on Functional Outcome among Patients with Acute Ischemic Stroke: The SKIP Randomized Clinical Trial. JAMA - J. Am. Med. Assoc. 325, 244–253.

Tomkins, A.J., Schleicher, N., Murtha, L., Kaps, M., Levi, C.R., Nedelmann, M., Spratt, N. J., 2011. Platelet rich clots are resistant to lysis by thrombolytic therapy in a rat model of embolic stroke. 7:2.

Tsivgoulis, G., Zand, R., Katsanos, A.H., Goyal, N., Uchino, K., Chang, J., Dardiotis, E., Putaala, J., Alexandrov, A.W., Malkoff, M.D., Alexandrov, A.V., 2015. Safety of Intravenous Thrombolysis in Stroke Mimics. Stroke 46, 1281–1287.

Weisel, J., Litvinov, R., 2008. The Biochemical and Physical Process of Fibrinolysis and Effects of Clot Structure and Stability on the Lysis Rate. Cardiovasc. Hematol. Agents Med. Chem. 6, 161–180.

Weisel, J.W., 2007. Structure of fibrin: Impact on clot stability. J. Thromb. Haemost. 5, 116–124.

Willeit, J., Geley, T., Schöch, J., Rinner, H., Tür, A., Kreuzer, H., Thiemann, N., Knoflach, M., Toell, T., Pechlaner, R., Willeit, K., Klingler, N., Praxmarer, S., Baubin, M., Beck, G., Berek, K., Dengg, C., Engelhardt, K., Erlacher, T., Fluckinger, T., Grander, W., Grossmann, J., Kathrein, H., Kaiser, N., Matosevic, B., Matzak, H., Mayr, M., Perfler, R., Poewe, W., Rauter, A., Schoenherr, G., Schoenherr, H.R., Schinnerl, A., Spiss, H., Thurner, T., Vergeiner, G., Werner, P., Wöll, E., Willeit, P., Kiechl, S., 2015. Thrombolysis and clinical outcome in patients with stroke after implementation of the Tyrol Stroke Pathway: A retrospective observational study. Lancet Neurol. 14, 48–56.

Wohner, N., Sótonyi, P., MacHovich, R., Szabó, L., Tenekedjiev, K., Silva, M.M.C.G., Longstaff, C., Kolev, K., 2011. Lytic resistance of fibrin containing red blood cells. Arterioscler. Thromb. Vasc. Biol. 31, 2306–2313.

Yang, P., Zhang, Yongwei, Zhang, L., Zhang, Yongxin, Treurniet, K.M., Chen, W., Peng, Y., Han, H., Wang, J., Wang, S., Yin, C., Liu, S., Wang, P., Fang, Q., Shi, Hongchao, Yang, J., Wen, C., Li, C., Jiang, C., Sun, J., Yue, X., Lou, M., Zhang, M., Shu, H., Sun, D., Liang, H., Li, Tong, Guo, F., Ke, K., Yuan, H., Wang, G., Yang, W., Shi, Huaizhang, Li, Tianxiao, Li, Z., Xing, P., Zhang, P., Zhou, Y., Wang, H., Xu, Y., Huang, Q., Wu, T., Zhao, R., Li, Q., Fang, Y., Wang, Laixing, Lu, J., Li, Y., Fu, J., Zhong, X., Wang, Y., Wang, Longde, Goyal, M., Dippel, D.W.J., Hong, B., Deng, B., Roos, Y.B.W.E.M., Majoie, C.B.L.M., Liu, J., 2020. Endovascular Thrombectomy with or without Intravenous Alteplase in Acute Stroke. N. Engl. J. Med. 382, 1981–1993.

Ziai, W.C., Muschelli, J., Thompson, C.B., Keyl, P.M., Lane, K., Shao, S., Hanley, D.F., 2012. Factors affecting clot lysis rates in patients with spontaneous intraventricular hemorrhage. Stroke 43, 1234–1239.