

Title	Electrical impedance spectroscopy signatures of red blood cells and platelets in acute ischemic stroke clots in the Clotbase International Registry: Significance for etiology and first pass effect
Item Type	journal article
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Citation	Sahin, Cansu, Giraud, Alice, Sak, Nazan Güner, Liu, Wenyi, Messina, Pierluca, Bozsak, Franz, et al. (2025). Electrical impedance spectroscopy signatures of red blood cells and platelets in acute ischemic stroke clots in the Clotbase International Registry: Significance for etiology and first pass effect. Journal of NeuroInterventional Surgery, http://doi.org/10.1136/jnis-2025-023658
DOI	http://doi.org/10.1136/jnis-2025-023658
Publisher	BMJ Publishing Group
Rights	CC BY-NC
Download date	2026-10-09 04:52:16
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Link to Item	https://hdl.handle.net/10379/19239

ELECTRICAL IMPEDANCE SPECTROSCOPY SIGNATURES OF RED BLOOD CELLS AND PLATELETS IN ACUTE ISCHEMIC STROKE CLOTS IN THE CLOTBASE INTERNATIONAL REGISTRY: SIGNIFICANCE FOR ETIOLOGY AND FIRST PASS EFFECT

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Acknowledgements

The authors wish to express their profound gratitude to the individuals listed below for their substantial contributions to the development and execution of this research: technical support from Ronan Balland, Steven Chirre, and Lise Schaub at Sensome; Prof. Céline Verstuyft and Prof. Catherine Guettier, manager and co-manager of CRB PARIS SACLAY (BB-0033-00089) (AP-HP); and

the team at Assistance Publique Hôpitaux de Paris. Appreciation is also due to the clinical teams at the collaborating stroke centres, whose involvement was indispensable to the successful execution of this study.

Conflicting interests

The authors declare no conflict of interest.

Ethical approval

Appointed ethics committees, competent authorities and the University of Galway Research Ethics Committee (2019.10.008) approved the study according to local regulatory requirements.

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Abstract:

Background:

Clot composition plays a key role in the pathophysiology of Acute Ischemic Stroke (AIS) and impacts the effectiveness of treatments such as thrombolysis and mechanical thrombectomy (MT). Developing an electrochemical impedance spectroscopy (EIS)-based device to identify clot characteristics could improve stroke treatment outcomes. This study aims to estimate the red blood cell (RBC) and platelet content in extracted AIS clots and explore EIS's relevance to clinically important parameters, including stroke etiology and First Pass Effect (FPE).

Methods:

A total of 508 clots from 426 MT patients at five stroke centres in France, Japan, Serbia, and Spain (February 2021–2024) were analysed in the Clotbase International Registry. EIS was performed

on retrieved clots, followed by Martius Scarlet Blue staining and CD42b immunohistochemistry. EIS-based models to quantify RBCs and platelets were designed using a development dataset (n=309), validated on a blinded dataset (n=199) and combined in a full dataset (n=508). Components (median [IQR]) were quantified, correlating RBC and platelet percentages with impedance and clinical parameters.

Results:

Correlations between content as determined by EIS and histology were validated in a blind dataset for RBCs ($r=0.7$, $p<0.0001$) and platelets ($r=0.5$, $p<0.0001$). Similar correlations were observed in the full dataset. Large-artery atherosclerosis clots had significantly higher RBC (46.0% [25.7–67.7]) and lower platelet content (31.7% [22.4–42.7]) compared to cardioembolic (RBCs:34.9% [14.0–56.2]; platelets:36.5% [26.7–51.0]) and cryptogenic (RBCs:31.5% [17.4–63.6]; platelets:37.8% [28.4–50.8]). FPE was associated with significantly higher RBC (40.0% [25.6–55.4] vs. non-FPE:31.2% [6.6–50.2]) and lower platelet content (42.0% [32.0–54.0] vs. non-FPE:47.7% [30.6–65.0]), confirmed by histology.

Conclusions:

EIS of RBCs and platelets provides an accurate assessment of clot composition, correlating strongly with histology. EIS holds the potential to deliver clinically relevant information on clot characteristics at the point of care.

Key Messages

- What is already known on this topic

The composition of blood clots can significantly influence the success of interventions to treat Acute Ischemic Stroke (AIS). Developing a device based on electrochemical impedance spectroscopy (EIS) to analyse clot characteristics at point of care could enhance the effectiveness of stroke treatments and patient outcomes.

- What this study adds

For the first time, using a validated model of platelet signature in addition to RBC signature, we have shown that EIS provides an accurate assessment of AIS clot platelet and RBC composition, with robust correlation to histological results. This study is the first to demonstrate that combined EIS signature of RBCs and platelets has the potential to deliver clinically relevant information on clot characteristics at point of care.

- How this study might affect research, practice or policy

This study highlights EIS as a promising tool for accurate clot composition assessment at point of care, which could inform clinical decision-making and enhance AIS patient outcomes.

Introduction:

Acute ischemic stroke (AIS) clots are complex structures primarily composed of red blood cells (RBCs), white blood cells (WBCs), fibrin, and platelets ¹. RBCs, which are abundant in clots, contribute to their flexibility and are associated with higher rates of reperfusion. In contrast, the rigidity of the clot is largely determined by the platelet composition, especially under conditions of high mechanical strain ².

Cardioembolic (CE) and Large-Artery Atherosclerosis (LAA) clots exhibit distinct structural and compositional characteristics. There are conflicting reports in the literature on the relationship between clot composition and stroke etiology. Some studies have reported that LAA clots are higher in fibrin and lower in RBC content than CE clots ³⁻⁵. However, several studies, many more recent and with considerably larger sample sizes have shown the opposite finding that clots of LAA etiology are richer in RBCs than CE clots, and that CE clots are richer in fibrin and platelets ⁶⁻¹¹. On balance, it appears that the pendulum of evidence is swinging towards the latter view.

It has been shown that clots rich in fibrin and platelets are more challenging to retrieve and require more endovascular treatment passes to achieve recanalisation compared to those predominantly composed of RBCs ¹². This suggests that the composition of a clot may significantly influence the effectiveness of mechanical thrombectomy (MT). In the initial pass, clots removed exhibit the highest proportion of RBCs, which gradually decreases in subsequent procedural attempts ^{6,13}. Clots requiring four or more passes for removal show the highest proportion of fibrin, platelets, and other components ⁶. Studies have shown that higher platelet content in these clots is associated with a lower rate of successful First Pass Effect (FPE, modified Thrombolysis in Cerebral Infarction 2c/3), meaning that the chances of achieving successful reperfusion on the first attempt are reduced ¹⁴.

Electrochemical impedance spectroscopy (EIS) may provide a real-time and *in situ* solution for characterising clots in the emergency care setting, offering a rapid and high-resolution method ¹⁵. This capability is especially valuable in AIS, where the timely and precise removal of blood clots is critical for effective treatment ^{16,17}. Providing detailed insights into the properties of the clot could potentially guide clinical decisions regarding thrombolysis or MT, ultimately enhancing patient outcomes in AIS management.

We have previously published a mid-study analysis of the RBC EIS signature in 253 clot samples from 231 AIS patients ¹⁸. We demonstrated that EIS-based estimation of RBC content strongly correlated with histological findings, and a higher EIS-based RBC composition was associated with FPE ¹⁸. Building on this earlier work, we now present the results of the completed study. We have developed and validated models of platelets and RBC EIS signatures in a larger cohort of 508 clots from the Clotbase International Registry. Analysing two main components of AIS clots together enhances our understanding of their combined contribution to AIS clot characteristics and clinically relevant insights.

Methods

Study Cohort

A comprehensive analysis was performed on 508 clots obtained from 426 patients enrolled in the Clotbase International Registry. Containing clinical data, histological data and EIS measurements for AIS clots. Ethical approval was granted by the hospital research ethics committees of five stroke centres involved in the study according to local regulatory requirements: Purpan Hospital (Toulouse, France), Kobe City Medical Centre General Hospital (Kobe, Japan), Vall d'Hebron Hospital (Barcelona, Spain), University Clinical Centre of Serbia (Belgrade, Serbia), Bicêtre Hospital (Paris, France), and the University of Galway Research Ethics Committee (2019.10.008). The study's participants were adults aged 18 and above who had undergone MT treatment for AIS between February 2021 and February 2024. Data on the patients were collected for further examination.

Study Workflow

This study followed a systematic workflow encompassing multiple critical steps, as we previously published (**supplementary figure 1**)¹⁸. The process began with MT as a treatment for AIS, during which clots were retrieved from patients. EIS measurements were conducted *ex vivo* on the extracted clots to assess their electrical properties. Subsequently, the clots underwent histological assessment, where they were processed for structural and compositional analysis. Machine-learning techniques were applied to the EIS data to train the algorithm. Finally, correlation analysis was performed to link EIS and histological data with patient-specific clinical parameters, offering a deeper understanding of the relationship between clot properties and clinical outcomes.

Initially, a total of 569 clots were considered for analysis. However, some clots were excluded due to protocol deviations encountered during the study, to maintain database integrity. After these exclusions, a final cohort of 508 clots remained, ensuring a robust and comprehensive dataset for the analysis (**supplementary figure 2**).

Electrochemical Impedance Spectroscopy (EIS)

Immediately after MT, the clot was placed in a disposable cartridge and maintained moist with 750 µl of phosphate-buffered saline (PBS), and a gross photographic image was captured before EIS analysis. The custom-designed, single-use cartridge featured an array of 48 gold electrodes, spaced 450 µm apart, allowing for 45 distinct EIS measurements between neighbouring electrodes, as previously described¹⁷. Precautions were taken to ensure optimal contact between the clot and the electrode array. A high-precision electrical impedance analyser (ISX-3, ScioSpec GmbH, Germany) was used to perform the EIS measurements across a frequency range of 1 kHz to 30 MHz. The PBS signal from electrode pairs not in contact with the clot was isolated from the clot signal. Following this, the average number of valid EIS measurements per clot was calculated to be 20.9 ± 11.1 , referred to as usable EIS measurements. These measurements were then further processed to eliminate any electrical parasitic effects.

Evaluation of EIS-based models for RBC and platelet composition

EIS-based models were designed using a development dataset and validated on a blinded dataset. We determined that an EIS-based model can be effectively trained with clot samples (n=309), particularly for RBC content¹⁸. The remaining samples (n=199) were reserved for the blinded dataset for independent validation of the EIS-based models. This data split was not random but time-based, with earlier samples (collected before May 2023) allocated for model development and later ones reserved for validation. According to the Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) guidelines¹⁹, this non-random split provides a more robust evaluation framework than a purely random division. Two machine learning models were developed: one for determining RBC composition from RBC quantification using histological staining and another for determining platelet composition from platelet quantification using immunostaining. Both models followed the same methodological framework, described below.

A threshold (t) was defined to categorise the continuous variable of histology. Clot samples were assigned a class, "rich" or "poor," based on quantifications. Samples with composition values above t were labelled as "rich," while those below t were labelled as "poor." These class labels were then assigned to all usable EIS measurements within each clot sample. A machine learning algorithm was then trained at the EIS measurement level and once trained, the model produced the probability of an individual measurement belonging to the "rich" class. These probabilities were then averaged across all usable measurements within a clot sample to give an estimate of the fraction of the component (RBCs or platelets) in the clot.

The models were developed using the development dataset of 309 clot samples, employing a nested cross-validation strategy using group-stratified cross-validation ¹⁷. This approach optimised model selection by identifying the best combination of the threshold t , input features, and machine learning algorithm. Thus, for both RBCs and platelets, a single model was trained on the development dataset and applied to the blinded validation set. This methodology provided an objective evaluation of EIS-based model performance across the validation dataset.

Martius Scarlett Blue (MSB) Staining for Histological Assessment of Clot Composition

Clot specimens were rapidly preserved in 10% neutral buffered formalin and forwarded to the University of Galway for histological examination. The clots were processed using a fully enclosed tissue processor (ASP300s, Leica). Sections were prepared following a standard protocol, beginning with deparaffinisation and hydration through a series of graded alcohols. The sections were then stained using a sequence involving Bouin's Solution (HT10132, Sigma-Aldrich), Iron Ammonium-Celestine Blue (206342, Sigma-Aldrich), Mayer's Haematoxylin (MHS32, Sigma-Aldrich), Martius Yellow (2,4-Dinitro-1-naphthol) (D0839, TCI), Crystal Scarlet (Ponceau 6R) (C0644, Sigma-Aldrich), and Methyl Blue (M6900, Sigma-Aldrich). Finally, the sections were cleared in xylene and mounted with DPX Mountant. MSB staining enables the differentiation of clot components, specifically RBCs (yellow), white blood cells (WBCs) (purple), fibrin (red/dark

pink), platelets (grey-pinkish), and collagen (blue) as previously described^{6,18,20,21} (**supplementary figure 3A**).

Immunohistochemistry (IHC)

IHC was carried out using the Leica Bond-III Fully Automated IHC Staining System. Before staining, the slides were baked and Bond Dewax Solution (AR9222, Leica Biosystems) was applied to dissolve the paraffin at 72°C, followed by antigen retrieval for 20 minutes using the ethylenediaminetetraacetic acid (EDTA)-based BOND Epitope Retrieval Solution 2 (AR9640, Leica Biosystems). The slides were then incubated with rabbit monoclonal anti-CD42b antibody for 15 minutes (1:200, ab227669, Abcam), with the dilution optimised for different lots as recommended. This was followed by Post Primary alkaline phosphatase (AP) incubation for 20 minutes and Polymer AP for 30 minutes to amplify the signal. Next, the slides were stained with the BOND Polymer Refine Red Detection kit (DS9390, Leica Biosystems) for 15 minutes, and counterstained with Hematoxylin for 5 minutes. Between each step, the slides were washed with Leica Bond wash solution or deionised water.

Image Analysis and Quantification Techniques

Stained slides were scanned at 20x magnification with the Olympus VS120 Virtual Slide Scanner. Orbit Image Analysis (www.orbit.bio), a free, open-source software for whole slide scan analysis and component quantification^{22,23} was used to classify and quantify clot components.

Correlation of clot components with patient outcomes

Revascularisation outcome (final mTICI) and successful FPE were correlated with clot composition data analysed by histology and EIS. Clinical data was not integrated in EIS model development, which was based solely on clot histological quantification. The nested cross-validation strategy was employed to minimize overfitting in the development dataset. This enabled the inclusion of

the EIS-based composition of the cross-validated samples from the development dataset to correlate with clinical data.

Statistical Methods

Data normality was evaluated using the Kolmogorov-Smirnov test. As the data was not normally distributed, the Mann-Whitney U test was used for pairwise comparisons. For multiple group comparisons, the Kruskal-Wallis test followed by Dunn's post-hoc analysis was applied. Data were presented as median (interquartile range [IQR]; 25%–75%) values. All tests were two-sided, and results were considered statistically significant if the p-value was below 0.05. Spearman's correlation coefficient and 95% confidence interval (CI) were calculated for correlation analysis using a simple linear regression model. Statistical analysis was performed using GraphPad Prism version 10.4.1 (GraphPad Software, La Jolla, CA).

Results

Clot Composition Heterogeneity in AIS

As expected, and consistent with previous studies, there was substantial heterogeneity in the composition of the clots. The median (IQR) percentage composition of the main components in the 508 samples was as follows: For MSB staining, RBCs accounted for 36.4% (20.1–53.6), WBCs for 4.2% (2.5–6.8), and fibrin for 18.5% (10.3–31.7) of the overall composition. Using CD42b IHC, platelets constituted 43.6% (30.8–59.3) of the sample area. **Supplementary figure 3** shows a representative clot stained with CD42b and MSB.

Comprehensive Overview of Baseline Characteristics

Table 1 describes the baseline characteristics of the cohort. Of the 426 patients, 50% were female, and the median age of the patient population was 75 years. A third (34.5%) of the patient

population received recombinant tissue plasminogen activator (rtPA). The most common confirmed etiology was CE (63.6%), followed by cryptogenic (CRYPT) (12.7%) and LAA (10.8%). Occlusions were most frequently found in the M1 segment (49.0%) and intracranial ICA/terminus (21.1%). Single pass thrombectomy was performed in 59.2% of patients. A good reperfusion outcome (final mTICI score 2c-3) was achieved in 80.6% of patients. FPE was observed in 50.7% of patients. At admission, 59.4% of patients had severe strokes with the National Institutes of Health Stroke Scale (NIHSS) score greater than 15 (NIHSS >15). By discharge, 71.6% had mild to moderate deficits (NIHSS ≤15), while 18.5% remained severe. Almost 90% of patients exhibited an NIHSS delta score, defined as the difference between on admission and at discharge NIHSS scores, with a median of 8 (IQR 3–13). At 90 days, 36.2% had good functional outcomes with the modified Rankin Scale (mRS) score of 0 to 2 (mRS 0–2).

Table 1. Baseline characteristics

Variable	Cohort
Sex	n (%) of patients
Female	213 (50)
Male	205 (48.1)
Missing	8 (1.9)
Age	median (IQR) of patients
	75 y (IQR 64–83)
rtPA	n (%) of patients
Yes	147 (34.5)
No	279 (65.5)
Confirmed Etiology	n (%) of patients
LAA	46 (10.8)
CE	271 (63.6)
CRYPT	54 (12.7)
Other	38 (8.9)
Missing	17 (4.0)
Occlusion Locations	n (%) of clots
M1	249 (49.0)
Intracranial ICA/terminus	107 (21.1)
M2	52 (10.2)

Basilar	38 (7.5)
Cervical ICA	17 (3.3)
Vertebral	3 (0.6)
P1	2 (0.4)
P2	2 (0.4)
A1	1 (0.2)
Multiple	37 (7.3)
Number of passes	n (%) of clots
1 pass	304 (59.8)
>1 pass	204 (40.2)
Number of passes	n (%) of patients
1 pass	252 (59.2)
>1 pass	174 (40.8)
Final mTICI	n (%) of patients
0	5 (1.2)
1	3 (0.7)
2a	10 (2.3)
2b	65 (15.3)
2c	91 (21.4)
3	252 (59.2)
FPE	n (%) of patients
FPE	216 (50.7)
Non-FPE	210 (49.3)
NIHSS on admission	n (%) of patients
Mild + Moderate ≤15	167 (39.2)
Severe stroke >15	253 (59.4)
Missing	6 (1.4)
NIHSS at discharge	n (%) of patients
Mild + Moderate ≤15	305 (71.6)
Severe stroke >15	79 (18.5)
Missing	42 (9.9)
NIHSS delta	n (%), median (IQR) of patients
	383 (89.9), 8 (3-13)
mRS at 90 days	n (%) of patients
Score 0-2	154 (36.2)
Score 3-6	248 (58.2)
Missing	24 (5.6)

Correlation and Validation of EIS Signature-Based RBC and Platelet Composition with Histological Analysis

In the development set (n=309), the observed correlation between the EIS-based RBC values and histologically assessed RBC content had a Spearman's coefficient of $r=0.7$ ($p<0.0001$, 95% CI [0.6-0.8]). The corresponding linear regression equation was $Y=0.9X+7$, with a statistically significant slope ($p<0.0001$, 95% CI [0.8-1.0]). The model's performance was further evaluated on the validation dataset (n=199), where Spearman's coefficient was $r=0.7$ ($p<0.0001$, 95% CI [0.6-0.7]), and the regression equation was $Y=0.8X+7.8$, with a statistically significant slope ($p<0.0001$, 95% CI [0.7-1.0]). The combined full dataset (n=508) had a Spearman's coefficient of 0.7 ($p<0.0001$, 95% CI [0.6-0.7]) and the linear regression equation of $Y=0.8X+7.3$, with a statistically significant slope ($p<0.0001$, 95% CI [0.8-0.9]) (**supplementary figure 4A**).

Similarly, for platelets, using the development set (n=309), a correlation was observed between the histology and EIS platelet content with a Spearman's coefficient of $r=0.5$ ($p<0.0001$, 95% CI [0.5-0.6]) and the linear regression equation of $Y=0.4X+16.9$, with a statistically significant slope ($p<0.0001$, 95% CI [0.3-0.5]). In the validation dataset (n=199), the Spearman's coefficient remained comparable at $r=0.5$ ($p<0.0001$, 95% CI [0.4-0.6]) and the regression equation was $Y=0.5X+16.7$, with a statistically significant slope ($p<0.0001$, 95% CI [0.4-0.5]). The combined full dataset (n=508) had a Spearman's coefficient of 0.5 ($p<0.0001$, 95% CI [0.5-0.6]) and the linear regression equation of $Y=0.4X+16.9$, with a statistically significant slope ($p<0.0001$, 95% CI [0.4-0.5]) (**supplementary figure 4B**).

Comparative Analysis of RBC and Platelet Measurements Across Different Etiologies Using Histology and EIS-Based Methods

The comparison of RBC and platelet measurements across different etiologies (LAA, CE, and CRYPT) revealed significant differences in both histological and EIS-based assessments (**table 2**). For RBC (MSB), LAA demonstrated higher levels (48.2%) compared to CE (35.1%) and CRYPT

(37.3%), with a statistically significant difference ($H(2)=7.5$, $p=0.02$). Platelet measurements (CD42b, IHC) were highest in CRYPT (48.2%) and CE (46.5%), with significant differences observed between LAA and both CE and CRYPT ($H(2)=13.5$, $p=0.001$). EIS-based RBC measurements mirrored this trend, with LAA (46.0%) showing higher levels than both CE (34.9%) and CRYPT (31.5%) ($H(2)=6.9$, $p=0.03$). Similarly, EIS-based platelet measurements were higher in CRYPT (37.8%) and CE (36.5%) compared to LAA (31.7%), with a significant difference ($H(2)=7.4$, $p=0.03$). These findings highlight significant variations in RBC and platelet composition across etiologies, with LAA exhibiting higher RBC levels and lower platelet levels compared to both CE and CRYPT (**table 2**), which is in line with previously published literature ^{6,24}.

Table 2. Correlation of histological RBC and platelet composition with EIS signatures by stroke etiology

Variable	MSB	IHC	EIS Measurements	
Etiology	RBC%	CD42b%	RBC%	Platelets%
LAA	48.2 (32.7-57.2)	34.0 (24.7-47.0)	46.0 (25.7-67.7)	31.7 (22.4-42.7)
CE	35.1 (18.5-51.7)*	46.5 (32.5-61.8)*	34.9 (14.0-56.2)*	36.5 (26.7-51.0)*
CRYPT	37.3 (22.5-51.0)	48.2 (35.0-57.1)*	31.5 (17.4-63.6)	37.8 (28.4-50.8)
Test, P-value	H(2)=7.5, p=0.02	H(2)=13.5, p=0.001	H(2)=6.9, p=0.03	H(2)=7.4, p=0.03
Median and interquartile range (IQR; 25%–75%) were reported.				
* $p \leq 0.05$ vs LAA, Dunn's post-hoc analysis				

Impact of RBC and Platelet Components on Revascularisation Outcomes: A Comparative Analysis of Procedural Variables

Revascularisation outcomes were significantly associated with RBC and platelet markers, as assessed histologically, across various procedural factors. Clots requiring more than one pass to remove had lower RBC (33.1% vs. 39.4%, $p=0.04$) and higher platelet levels (48.0% vs. 42.9%, $p=0.03$) compared to those requiring one pass, as measured by both histology (**table 3**) and EIS (**supplementary table 1**). Poor recanalisation outcome (mTICI 0-2b) was identified using EIS

measurements in samples with low levels of RBCs and high levels of platelets (**table 3 and supplementary table 1**). Regarding FPE (mTICI 2c/3), patients with FPE had higher median RBC (40.0% vs. 31.2%, $p=0.008$) and lower median platelet (42.0% vs. 47.7%, $p=0.03$) (**table 3**). These findings underscore the important role of RBC and platelet markers in revascularisation success and their association with procedural factors.

Table 3. Histologic assessment of main clot components analysed the number of passes, final mTICI score, and successful recanalisation outcome (FPE).

Variable	MSB	IHC
Revascularisation outcome	RBC%	CD42b%
Number of passes		
1 pass	39.4 (23.1-55.6)	42.9 (30.2-55.4)
>1 pass	33.1 (18.4-50.6)	48.0 (31.2-65.0)
Test, P-value	U=27745, $p=0.04$	U=27571, $p=0.03$
Final mTICI		
0-2b	33.9 (14.4-48.3)	50.6 (32.5-62.7)*
2c	38.0 (22.8-57.8)	39.9 (27.6-55.4)
3	37.2 (20.0-52.1)	44.4 (32.2-59.3)
Test, P-value	H(2)=4.0, $p=0.1$	H(2)=6.4, $p=0.04$
FPE		
FPE	40.0 (25.6-55.4)	42.0 (32.0-54.0)
Non-FPE	31.2 (16.6-50.2)	47.7 (30.6-65.0)
Test, P-value	U=19290, $p=0.008$	U=19969, $p=0.03$
Median and interquartile range (IQR; 25%–75%) were reported.		
* $p \leq 0.05$ vs final mTICI score 2c, Dunn's post-hoc analysis		

Association of RBC and Platelet EIS-based Quantification with Revascularisation Outcomes

The results for RBC and platelet EIS-based quantification showed the same pattern of effects as observed in histology. Patients who experienced FPE showed higher median RBC EIS-based values (40.6%) compared to those non-FPE (27.3%), with a statistically significant difference ($U=19134$,

p=0.005). In contrast, platelet EIS-based content was lower in FPE patients (34.3%) compared to non-FPE patients (37.5%), and this difference was also significant (U=20061, p=0.04). **Figure 1**, FPE analysis illustrates the differences in RBC and platelet percentages, both through histological assessments and EIS-based measurements.

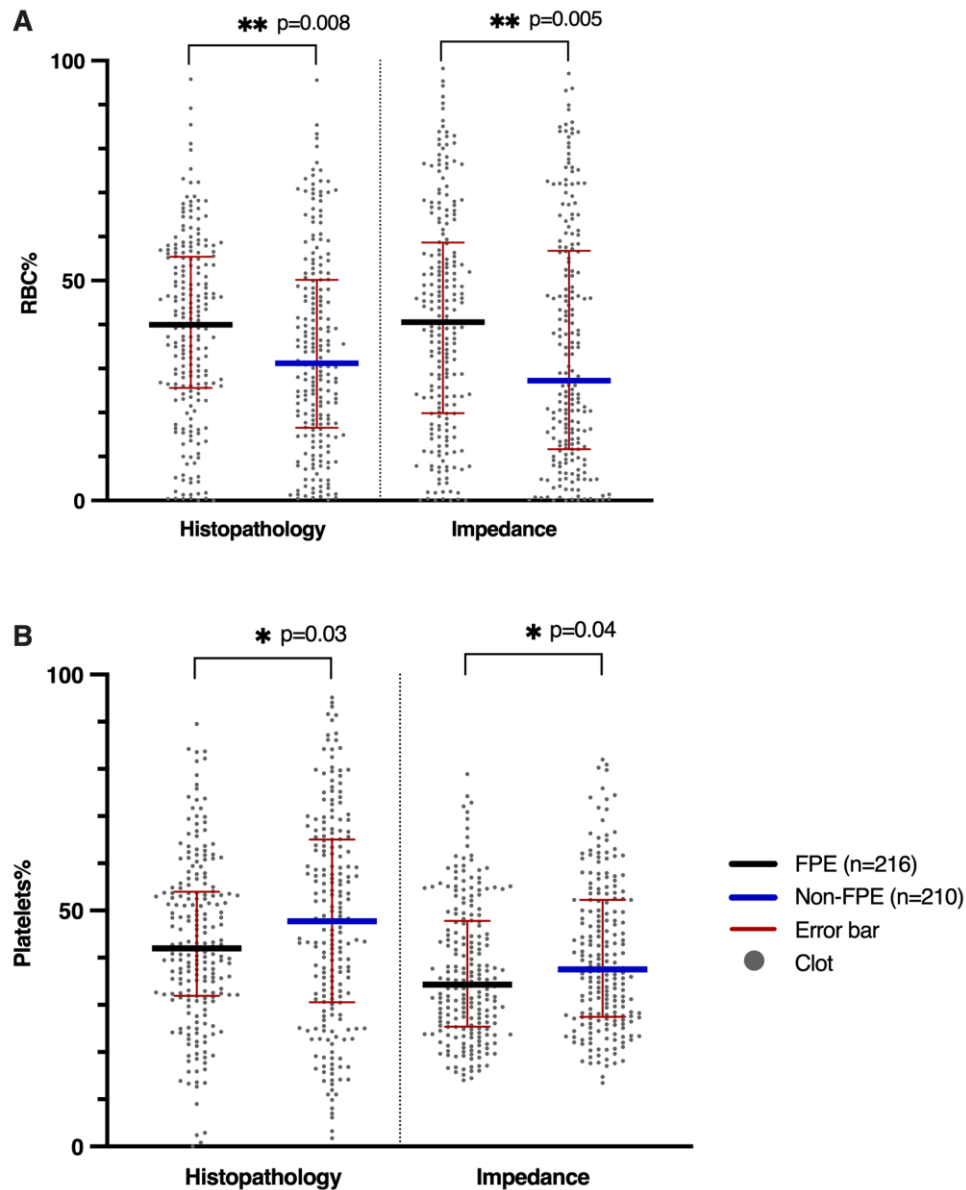


Figure 1. FPE analysis of (A) RBC and (B) platelet percentages by histology and EIS-based measurements.

Association of Clot Components with Other Patient Data

Correlation of histological data with baseline characteristics is shown in **supplementary table 1**. Correlation of EIS measurements across baseline characteristics is shown in **supplementary table 2**.

The administration of rtPA did not have a significant effect on any clot component. However, RBC content varied significantly across occlusion locations, with the highest in basilar occlusions and the lowest in M2 occlusions. On admission, WBC content was significantly elevated in patients with severe strokes (NIHSS >15). Patients with NIHSS ≤15 at discharge had higher RBC content than those with severe strokes. When we compared NIHSS delta in patients who had severe strokes on admission with those admitted with mild to moderate strokes, we observed a greater improvement in severe cases (n=226, median 9.5 [IQR 3-16] vs n=157, median 7 [IQR 3-10]) respectively (U=13959, p=0.0004). Additionally, patients with good functional outcomes (mRS 0–2) had significantly higher RBC content as analysed by histology and EIS (**supplementary tables 1 and 2**).

Discussion

This study confirms the promising potential of the EIS method for analysing clot composition by demonstrating its correlation with histological analysis of *ex vivo* clots with a specific focus on two main components: RBCs and platelets. This study reinforces the previously published findings^{16,18} that demonstrated the capability of EIS to identify RBC content in AIS clots *ex vivo*. By testing our models with a blinded validation dataset, we strengthened the reliability of our mid-study findings regarding the strong correlation between RBC content from EIS-based models and histology quantification¹⁸ and, additionally, we also demonstrated a robust correlation for platelet content. For both the RBC and platelet EIS-based models, the 95% CI for the slope and Spearman's coefficient overlapped between the development and the validation datasets, minimising concerns of model overfitting. The observed correlations indicate a high level of agreement of the

histology and EIS-qualification methods despite their intrinsic differences. While both methodologies measure the same object they do so with different techniques. Histology provides a two-dimensional representation from a clot section, whereas impedance measurement captures the three-dimensional composition of the clot portion in close contact with the electrodes. This considerable methodological difference may partially account for the observed variations between the EIS-based and histology quantification.

The correlation with platelet content was less strong than the correlation observed for RBCs which is likely due to multiple factors, including the small size of platelets (almost eight times smaller than RBCs) ²⁵, their shape-changing behaviour upon activation ²⁶, and the potential masking by fibrin ²⁷. Nonetheless, the results still indicate that EIS can provide reliable insights into platelet content in clots. The combined RBC and platelet quantification capability of EIS offers potential insights into clot composition in the acute care setting that could help to inform and optimise treatment strategies.

By examining the histological composition of AIS clots and correlating clot characteristics with clinically important parameters, we can gain valuable insights into the underlying mechanisms of AIS ^{6,24}. This knowledge may inform the development of more targeted and effective treatment strategies, such as novel thrombolytic agents or MT devices. Analysing AIS clot composition offers potential for improved recanalisation outcomes and optimised secondary stroke prevention by revealing characteristics hinting to stroke etiology ^{28,29}.

The comparison of RBC and platelet measurements across LAA, CE, and CRYPT revealed distinct differences. LAA exhibited higher RBC levels and lower platelet levels compared to CE and CRYPT. In contrast, CE and CRYPT showed higher platelet levels, consistent with previous studies ^{6,24}. The EIS-based measurements of RBCs and platelets aligned with the histological findings, validating the potential of EIS in providing clinically relevant information, such as insight into the underlying etiology of AIS.

Patients undergoing MT have a higher chance of good outcomes when the entire clot is removed in a single pass. Multiple passes, while sometimes necessary, can increase the risk of secondary embolism and poorer outcomes ¹⁵. Revascularisation success was positively linked to RBC levels and negatively linked to platelet markers. Clots requiring more than one pass tended to have lower RBC levels and higher platelet levels compared to single-pass procedures ^{6,13,30}. FPE was associated with higher RBC and lower platelet content, as previously shown ¹⁸. This consistency further strengthened the reliability of EIS as an effective tool for evaluating critical factors that impact MT outcomes.

Studies have consistently found that RBC levels were significantly higher in basilar clots compared to those in the anterior circulation ^{31,32}. In the present study, the variation in RBC content across different occlusion sites followed a similar pattern, with higher RBC levels observed in basilar artery occlusions and lower levels in M2 occlusions. This adds weight to the suggestion that the location of occlusion may be impacted by the thrombus composition. Moreover, previous research has established that an increased serum WBC count is independently correlated with higher stroke severity, as measured by the NIHSS on admission, and worse functional recovery at discharge ^{33,34}. Our current findings also indicate that patients with severe strokes exhibit higher WBC levels in their clots. In our mid-study analysis, we did not observe any significant differences in clot components ¹⁸ related to stroke severity. However, after increasing our sample size, we now observe that patients with milder deficits at discharge (NIHSS) had higher RBC content, suggesting a better response to treatment. Consistent with a recent study ³⁵, our data also showed a significant association of higher RBC content and reduced platelet content in clots with lower mRS at 90 days.

A limitation of this study was the potential influence of electrode contact quality with the tissue on EIS measurements, which could have led to some inaccuracy in composition readings. To mitigate poor clot–electrode contact, a cap was used to secure the clot in place. Additionally, efforts were made to prevent clot dehydration during EIS measurements using a PBS physiological solution.

In this investigation, we focused on the two major cellular clot components, RBCs and platelets. Measuring both RBCs and platelets together in clots using the EIS-based device provided a more comprehensive evaluation of clot composition. Both RBC and platelet EIS signatures reliably identified complementary key clot component characteristics, indicating red or white components within the clot.

Histology of the extracted clot can provide information on clot composition, but during the acute treatment phase the clinician is blind to this information. Using EIS signature to provide an indication of RBC and platelet components in the clot *in vivo* in real time could inform clinical decision making, offering insight into how likely it is a clot might be easily removed in a first pass, enabling more precise selection of thrombectomy device choice, which could improve procedural efficiency and success rates. Furthermore, insight into clot composition could inform both immediate and longer-term clinical decisions for secondary stroke prevention, ultimately leading to safer interventions and better patient long term outcomes.

Integration of EIS into an *in situ* device capable of real-time measurements during MT could thus support more informed clinical decisions in AIS treatment. Notably, Sensus has developed the Clotild® Smart Guidewire System (CSGS) to perform *in vivo* EIS-based characterisation of occlusions during endovascular stroke treatment and has recently completed a first interventional clinical trial (NCT04993079).

Conclusion

EIS was shown to be an effective tool for characterising AIS clot composition. EIS-based models for RBCs and platelets demonstrated robust correlations with the histological analysis. This study is the first to show that the combined EIS signatures of both RBCs and platelets have the potential

to estimate stroke etiology and key clinical outcomes, including the likelihood of first-pass clot retrieval and overall recanalisation success. These findings underscore the potential of EIS as a minimally invasive tool to refine current procedural practise and optimise patient management in AIS.

Funding

Research Ireland and the European Regional Development Fund (Grant Number 13/RC/2073 & 13/RC/2073_P2) and Sensome

Contributorship

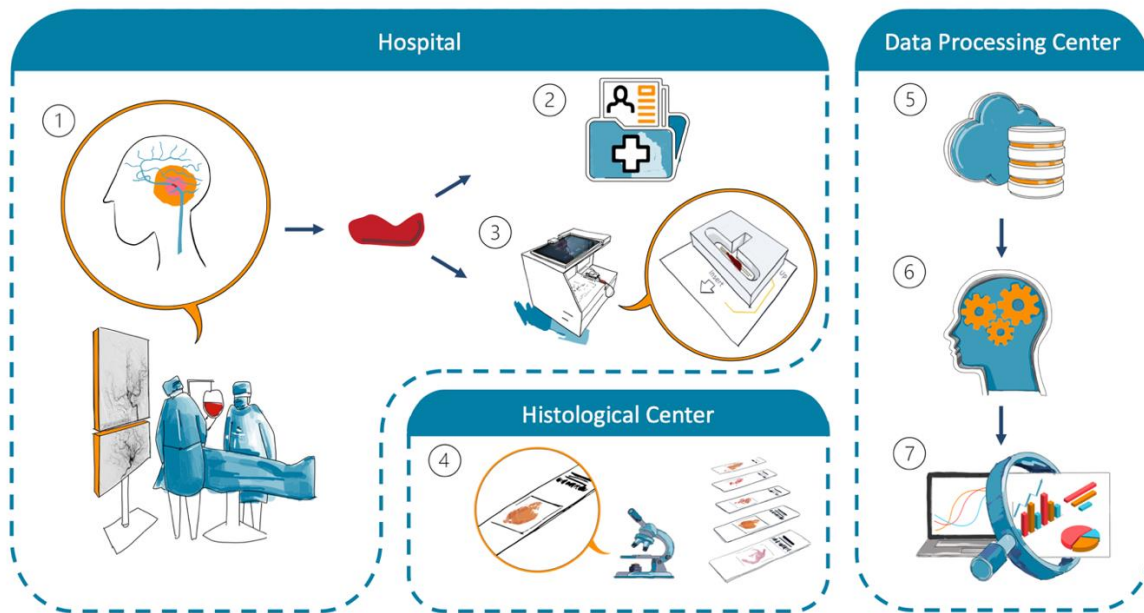
All authors contributed to data collection. CS, AG, NGS and WL performed the analysis. KD, PM, FB and CC conceived and designed the analysis. JD, FS, ACJ, GB, JP, JJ, HI, TO, LS, VC, UM, PS, IV, MR, NS, and CC performed the thrombectomy at several hospitals and collected samples and procedural data. CS and KD prepared the manuscript. All authors reviewed it. The funder did not influence the results/outcomes of the study despite author affiliations with the funder.

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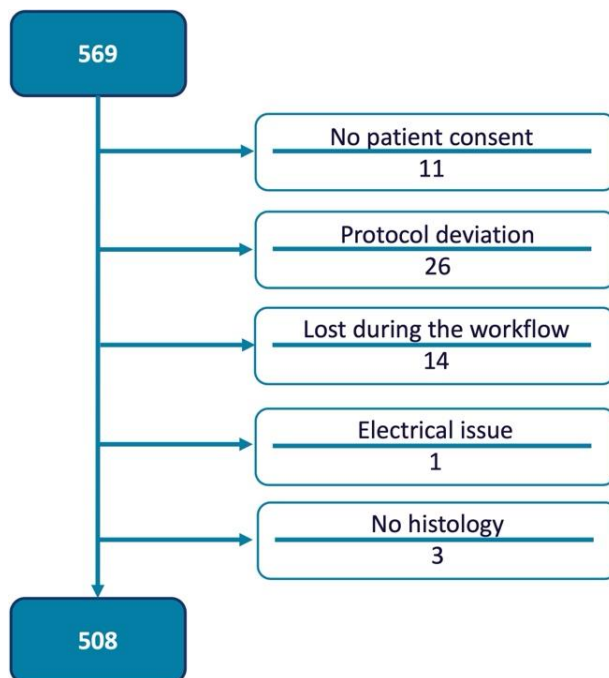
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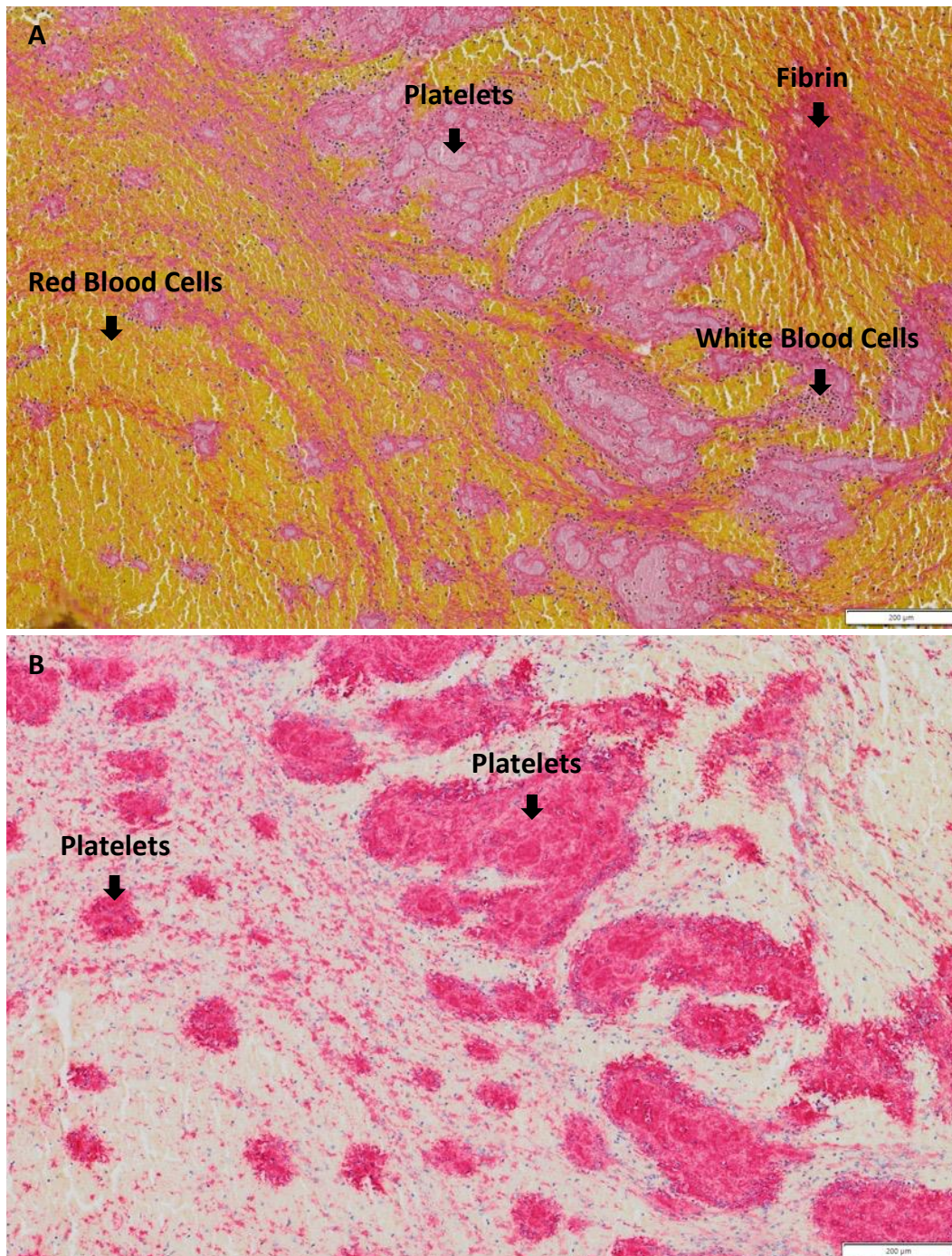
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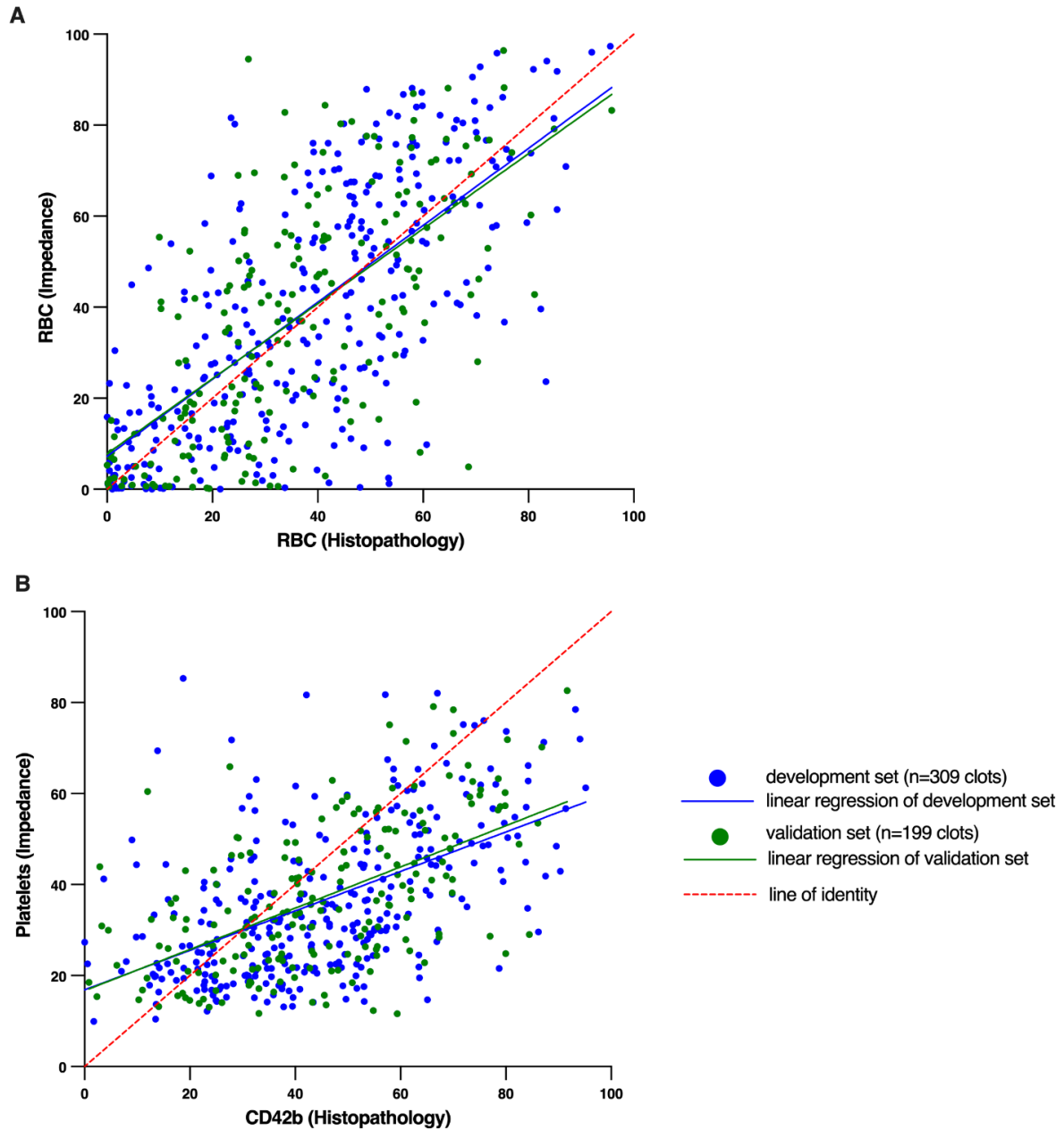
Supplementary Figure 1: Workflow: mechanical thrombectomy (1), patient information (2), EIS measurements (3), histology (4), cloud database (5), machine learning (6), and correlation analysis (7) (Sahin et al., 2024; Reprinted (CC BY)).



Supplementary Figure 2: Clot samples selection flowchart



Supplementary Figure 3. The clot is visualised with MSB staining, which highlights RBCs (yellow), white blood cells (WBCs) (purple), fibrin (red/dark pink), and platelets (grey-pinkish) (A). Platelets were also identified by immunohistochemistry (IHC) using CD42b (red) (B). Scale bar: 200 µm



Supplementary Figure 4. Correlation of histological and EIS-based RBC and platelet composition: RBC (A) and platelet (B) correlations with development (n=309, blue) and validation (n=199, green) sets.

Supplementary Table 1. Histological clot composition data correlate with baseline characteristics.

Variable	<u>MSB</u>			<u>IHC</u>
	RBC%	WBC%	Fibrin%	CD42b%
rtPA				
Yes	36.1 (23.4-51.7)	4.4 (2.7-7.7)	23.0 (12.8-34.2)	45.3 (32.3-61.8)
No	36.3 (18.5-53.9)	4.1 (2.5-6.6)	18.2 (9.6-31.8)	43.4 (31.4-57.8)
Test, P-value	U=20432, p=1.0	U=18724, p=0.1	U=18217, p=0.06	U=19344, p=0.3
Confirmed Etiology				
LAA	48.2 (32.7-57.2)	3.7 (2.6-8.1)	17.8 (10.6-28.8)	34.0 (24.7-47.0)
CE	35.1 (18.5-51.7)	4.4 (2.7-6.8)	21.4 (11.4-33.9)	46.5 (32.5-61.8)
CRYPT	37.3 (22.5-51.0)	4.2 (2.9-7.0)	16.1 (7.7-29.6)	48.2 (35.0-57.1)
Test, P-value	H(2)=7.5, p=0.02* Dunn's test: LAA vs. CE, p=0.02*	H(2)=0.01, p=1.0	H(2)=3.6, p=0.2	H(2)=13.5, p=0.001** Dunn's test: LAA vs. CE, p=0.0008***, LAA vs. CRYPT, p=0.02*
Occlusion Locations				
M1	34.1 (19.3-52.0)	4.4 (2.6-7.2)	18.7 (10.5-32.2)	43.7 (31.3-59.9)
Intracranial ICA/terminus	38.5 (18.5-54.9)	3.9 (2.5-6.2)	18.9 (9.6-31.5)	43.3 (30.5-57.9)
M2	28.6 (15.1-47.8)	4.0 (2.3-6.7)	21.1 (9.6-33.5)	48.2 (29.4-62.5)
Basilar	45.7 (33.3-58.4)	3.8 (2.9-5.9)	14.2 (9.2-17.5)	43.5 (32.0-56.9)
Cervical ICA	44.0 (18.5-57.6)	3.1 (1.3-8.3)	26.9 (13.4-32.2)	43.0 (25.0-59.3)
Test, P-value	H(4)=10.2, p=0.04* Dunn's test M2 vs. Basilar, p=0.03*	H(4)=2.6, p=0.6	H(4)=8.1, p=0.09	H(4)=0.6, p=1.0
Number of passes				
1 pass	39.4 (23.1-55.6)	4.1 (2.5-6.4)	17.0 (10.1-30.5)	42.9 (30.2-55.4)
>1 pass	33.1 (18.4-50.6)	4.2 (2.6-7.6)	22.3 (11.5-33.2)	48.0 (31.2-65.0)
Test, P-value	U=27745, p=0.04*	U=29284, p=0.3	U=27338, p=0.02	U=27571, p=0.03*
Final mTICI				
0-2b	33.9 (14.4-48.3)	3.6 (2.3-6.5)	22.5 (9.2-35.0)	50.6 (32.5-62.7)
2c	38.0 (22.8-57.8)	4.1 (2.5-6.3)	17.5 (11.1-28.8)	39.9 (27.6-55.4)
3	37.2 (20.0-52.1)	4.4 (2.9-7.0)	20.0 (10.4-32.4)	44.4 (32.2-59.3)
Test, P-value	H(2)=4.0, p=0.1	H(2)=4.0, p=0.1	H(2)=1.8, p=0.4	H(2)=6.4, p=0.04* Dunn's test: 0-2b vs. 2c, p=0.04*

FPE				
FPE	40.0 (25.6-55.4)	4.3 (2.6-6.3)	18.2 (10.3-31.8)	42.0 (32.0-54.0)
Non-FPE	31.2 (16.6-50.2)	4.1 (2.6-7.7)	21.0 (10.9-33.6)	47.7 (30.6-65.0)
Test, P-value	U=19290, p=0.008**	U=22364, p=0.8	U=21129, p=0.2	U=19969, p=0.03*
NIHSS on admission				
Mild + Moderate ≤15	36.9 (19.9-53.6)	3.7 (2.5-6.1)	17.6 (9.6-33.7)	43.7 (32.1-59.3)
Severe stroke >15	35.7 (19.3-51.8)	4.4 (2.7-7.7)	20.6 (11.8-31.4)	43.7 (31.4-59.9)
Test, P-value	U=20474, p=0.6	U=18519, p=0.03*	U=20136, p=0.4	U=21010, p=0.9
NIHSS at discharge				
Mild + Moderate ≤15	38.0 (22.8-54.1)	4.2 (2.6-6.5)	18.5 (10.6-32.8)	43.4 (31.4-60.6)
Severe stroke >15	31.5 (14.9-51.7)	4.3 (2.4-8.5)	23.8 (14.5-31.5)	43.4 (33.0-56.4)
Test, P-value	U=10279, p=0.04*	U=11318, p=0.4	U=11021, p=0.2	U=11835, p=0.8
mRS at 90 days				
Score 0-2	39.2 (25.2-53.5)	4.3 (2.7-6.6)	18.4 (10.3-31.8)	43.2 (31.8-59.2)
Score 3-6	33.8 (17.6-51.9)	4.2 (2.6-7.6)	20.0 (11.2-33.4)	43.9 (31.8-59.4)
Test, P-value	U=16873, p=0.05*	U=18669, p=0.7	U=18338, p=0.5	U=18571, p=0.6
Median and interquartile range (IQR; 25%–75%) were reported. *p≤0.05, **p≤0.01				

Supplementary Table 2. Correlation of clot composition data obtained by EIS measurements with baseline characteristics.

Variable	EIS Measurements	
	RBC%	Platelets%
rtPA		
Yes	37.9 (18.6-55.5)	37.4 (28.1-48.2)
No	34.9 (12.7-58.8)	35.4 (25.7-50.3)
Test, P-value	U=19477, p=0.4	U=19006, p=0.2
Confirmed Etiology		
LAA	46.0 (25.7-67.7)	31.7 (22.4-42.7)
CE	34.9 (14.0-56.2)	36.5 (26.7-51.0)
CRYPT	31.5 (17.4-63.6)	37.8 (28.4-50.8)
Test, P-value	H(2)=6.9, p=0.03* Dunn's test: LAA vs. CE, p=0.03*	H(2)=7.4, p=0.03* Dunn's test: LAA vs. CE, p=0.03*
Occlusion Locations		
M1	34.2 (13.1-57.2)	35.1 (24.9-48.3)

Intracranial ICA/terminus	33.6 (13.2-66.7)	33.7 (21.8-48.5)
M2	24.9 (7.2-52.9)	37.7 (24.7-50.2)
Basilar	53.3 (22.6-67.5)	28.6 (21.1-44.0)
Cervical ICA	41.0 (22.9-77.8)	30.7 (19.9-47.0)
Test, P-value	H(4)=10.8, p=0.03*	H(4)=6.0, p=0.2
Number of passes		
1 pass	42.7 (19.0-62.7)	31.8 (22.2-45.6)
>1 pass	28.4 (9.2-56.4)	34.7 (24.3-50.6)
Test, P-value	U=25678, p=0.001**	U=27827, p=0.05*
Final mTICI		
0-2b	26.2 (9.2-55.1)	39.0 (29.4-52.3)
2c	42.2 (20.0-59.0)	31.2 (24.5-46.6)
3	36.8 (16.2-56.4)	36.7 (27.1-50.3)
Test, P-value	H(2)=5.9, p=0.05 Dunn's test: 0-2b vs. 2c, p=0.05*	H(2)=7.9, p=0.02* Dunn's test: 0-2b vs. 2c, p=0.02*)
FPE		
FPE	40.6 (19.9-58.7)	34.3 (25.4-47.8)
Non-FPE	27.3 (11.7-56.8)	37.5 (27.5-52.3)
Test, P-value	U=19134, p=0.005**	U=20061, p=0.04*
NIHSS on admission		
Mild + Moderate ≤15	36.8 (15.2-63.5)	34.1 (25.3-48.8)
Severe stroke >15	34.8 (14.5-53.7)	37.3 (28.0-50.3)
Test, P-value	U=19750, p=0.3	U=19019, p=0.08
NIHSS at discharge		
Mild + Moderate ≤15	36.8 (16.0-60.5)	35.7 (26.2-49.4)
Severe stroke >15	28.7 (15.4-52.5)	36.1 (29.6-50.1)
Test, P-value	U=11009, p=0.2	U=11212, p=0.3
mRS at 90 days		
Score 0-2	42.1 (19.5-64.1)	34.1 (25.3-46.2)
Score 3-6	30.4 (13.4-54.1)	37.2 (27.7-52.5)
Test, P-value	U=15986, p=0.006**	U=16624, p=0.03*
Median and interquartile range (IQR; 25%–75%) were reported. *p≤0.05, **p≤0.01		