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Convergent Evidence for a Distinct Intravascular Matrix: Integrated Morphological, Elemental, and Proteomic Analysis of Anomalous Intravascular Casts

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Abstract

Background: Anomalous intravascular casts (AICs) are elongated, lumen-conforming intravascular structures increasingly reported since early 2021. Their unusual elasticity, persistence, and resistance to fragmentation distinguish them from both ordinary postmortem clots and conventional antemortem thrombi, raising the possibility that they represent a distinct, previously unrecognised intravascular phenomenon.

Methods: We performed an integrated analysis of AICs across three independent analytical domains: morphology/histology, elemental composition, and proteomic architecture. Gross and histological examinations, elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS), and proteomic profiling by HPLC-MS/MS were conducted using blinded analytical workflows.

Results: Histological examination demonstrated a reproducible structural phenotype characterized by elongated branching casts, marked elastic resilience, dense fibrinous architecture, sparse cellular inclusion, and regionally restricted Lines of Zahn, indicating at least partial formation under active blood flow. ICP-MS revealed a reproducible elemental profile incompatible with a conventional fibrin-dominant clot matrix, including sulfur depletion relative to theoretical fibrinogen stoichiometry and relative phosphorus enrichment. These findings indicate that the matrix cannot be explained by fibrin(ogen) alone. Proteomic analysis identified 541 human proteins, with dominance of fibrin-family components in markedly abnormal proportions. Fibrinogen chains were present in a non-physiological mean ratio of approximately 1:7:3 (alpha:beta:gamma), while plasminogen abundance was extremely low, suggesting impaired intrinsic fibrinolytic potential.

Conclusions: Structural, elemental, and proteomic findings converge on a coherent interpretation that AICs are not adequately explained as variants of ordinary postmortem clots or conventional thrombi. Rather, they represent a distinct intravascular matrix characterised by non-conventional fibrin organisation and altered biochemical composition.

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Keywords: Anomalous intravascular casts, histology, morphology, fibrin, vascular pathology, postmortem pathology, thrombus structure, fibrinous casts

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Introduction

Emergence of anomalous intravascular casts

Since early 2021, licensed embalmers and clinicians in multiple countries have reported the recurrent presence of unusual intravascular structures during routine postmortem care Hirschman, 2022. (1) These structures, here termed anomalous intravascular casts (AICs), are characteristically elongated, lumen-conforming, and frequently extend through vascular bifurcations, forming coherent casts of the vascular tree. In contrast to the friable, gelatinous material typical of ordinary postmortem clots, AICs exhibit a firm, rubber-elastic consistency, with notable resistance to fragmentation and, in some cases, measurable tensile strength.

Reports describing these structures have been internally consistent across independent observers and geographic regions Haviland, 2023, (2) with many practitioners noting an apparent increase in frequency relative to prior experience Haviland, 2024. (3) Although such observations initially emerged outside formal pathology literature, their reproducibility and distinctiveness have prompted closer examination. Importantly, similar fibre-rich, white material has also been documented in clinical contexts involving living patients, including post-operative drainage systems, indicating that these structures are not confined to postmortem settings and may form, at least in part, during life.

Taken together, these observations raise a fundamental question: do AICs represent a previously unrecognised variant of known clotting phenomena, or do they reflect a distinct intravascular process not adequately captured by existing classifications?

Limitations of current clot classification frameworks

Classical descriptions of intravascular clotting distinguish between antemortem thrombi and postmortem coagula, each defined by characteristic structural, cellular, and mechanical properties. Antemortem thrombi typically form under conditions of active blood flow and are characterised by laminated fibrin architecture, prominent cellular incorporation, and the presence of Lines of Zahn, reflecting cyclical deposition under pulsatile haemodynamic conditions. In contrast, postmortem clots arise passively after cessation of circulation and are generally described as gelatinous, non-adherent masses lacking structural organisation, commonly referred to as “currant-jelly” or “chicken-fat” coagula.

The anomalous intravascular casts described here do not conform readily to either category. Their lumen-conforming geometry, branching morphology, and partial presence of flow-associated features such as Lines of Zahn are inconsistent with passive postmortem coagulation. At the same time, their sparse cellular content, atypical fibrin organisation, and pronounced mechanical resilience differ substantially from conventional antemortem thrombi. Notably, no recognised postmortem processes are known to produce cohesive, elastic, and laminated intravascular structures of this type.

These discrepancies highlight a limitation in current classification frameworks: they are well suited to describing established clot types but may not accommodate structurally and compositionally atypical intravascular materials. As a result, the observed features of AICs cannot be satisfactorily reconciled within existing pathological categories based on morphology alone.

Rationale for integrated multi-domain analysis

Determining the nature of anomalous intravascular casts requires an analytical approach that extends beyond any single domain of investigation. Morphological and histological analyses provide essential information regarding structural organisation, mechanical properties, and cellular incorporation, but cannot by themselves resolve the biochemical or molecular composition of the material. Conversely, compositional analyses can define elemental and molecular characteristics but do not directly capture the architectural context in which these features arise.

In this study, three independent analytical domains are applied to the same or overlapping specimen set: (i) morphological and histological characterisation, (ii) elemental analysis using inductively coupled plasma mass spectrometry (ICP-MS), and (iii) proteomic profiling by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Each domain provides an orthogonal line of evidence: structure, composition, and molecular architecture, respectively.

Importantly, these domains are not redundant. Distinct biological matrices may exhibit superficially similar fibrillar morphology while differing substantially in elemental composition or protein organisation. Likewise, the presence of fibrin-family proteins does not guarantee conventional fibrin architecture or function. Only through the integration of independent structural, elemental, and proteomic

data can constraints be placed on the identity and behaviour of the material.

Accordingly, the central premise of this work is that no single analytical approach is sufficient to classify AICs; rather, convergence across independent domains is required to determine whether these structures are consistent with known clot biology or represent a distinct intravascular matrix.

Aim of the study

The aim of this study is to determine whether anomalous intravascular casts constitute a discrete intravascular entity through integrated analysis across three independent analytical domains.

Specifically, this work seeks to:

1. Characterise the gross morphology and histological architecture of AICs and assess their relationship to recognised clot types;
2. Determine the elemental composition of AICs and evaluate their compatibility with conventional fibrin-based thrombus biochemistry;
3. Define the proteomic architecture of AICs, including fibrinogen chain composition and the presence of fibrinolytic components;
4. Integrate structural, elemental, and proteomic findings to assess whether these observations converge on a coherent and distinct biological phenotype.

Through this approach, the study aims to establish whether AICs can be understood within existing frameworks of thrombus biology or whether they represent a previously uncharacterised form of intravascular matrix requiring further investigation.

Materials and Methods

Sample acquisition, continuity, and blinding

Anomalous intravascular casts (AICs) analysed in this study were obtained from postmortem waste streams generated during routine embalming and mortuary procedures between 2022 and 2024. Specimens were collected by licensed embalmers and pathologists using sterile forceps and placed into clean, labelled containers containing 10% buffered formalin for preservation and transport.

All contributors provided written attestations confirming the circumstances of collection. A chain-of-custody log was maintained from the point of retrieval through to laboratory receipt. Upon arrival at analytical sites, each specimen was assigned a random alphanumeric identifier to ensure full anonymisation.

The specimens analysed across the three analytical domains—morphological/histological, elemental, and proteomic—were drawn from the same sample pool or directly overlapping subsets thereof, ensuring continuity of material and comparability of findings across all analyses.

Ten independent donors contributed specimens to the overall study, with overlapping subsets analysed within each analytical domain. Only representative subsamples <5g were required for laboratory analyses, leaving substantial portions of the original specimen collection archived for future analyses.

Morphological and histological assessments were performed independently by two laboratories under blinded conditions, with findings subsequently reviewed by a third independent laboratory.

Cross-referencing of specimen identities between analytical domains was performed only after completion of primary analyses.

All laboratory procedures were conducted under blinded conditions. Participating laboratories received no information regarding specimen provenance, morphological characteristics, geographic origin, embalmer observations, or study hypotheses. Analytical criteria and evaluation frameworks were predefined prior to processing to minimise bias and ensure methodological consistency.

Overview of analytical domains

Three independent analytical domains were applied to characterise AICs:

1. Morphological and histological analysis, to define gross structure, mechanical behaviour, and micro-architectural organisation;
2. Elemental analysis (ICP-MS), to determine bulk atomic composition and assess compatibility with conventional fibrin-based clot stoichiometry;
3. Proteomic analysis (LC–MS/MS), to identify protein composition, fibrinogen chain relationships, and the presence of fibrinolytic components.

Each analytical domain was conducted independently under blinded conditions using harmonised protocols across participating laboratories. Integration of findings was performed only after completion of domain-specific analyses, ensuring that structural, elemental, and proteomic results repre-

sent independent lines of evidence.

Morphological and histological analysis

Gross morphological assessment

Each coded specimen underwent standardised gross examination under controlled lighting conditions on a sterile dissection surface. High-resolution photographic documentation was performed using a calibrated imaging system with a fixed macro lens, colour reference card, and metric scale.

Measured parameters included:

- total length (cm)
- maximum and minimum diameter (mm)
- dry weight (g)
- degree of lumen conformity and branching geometry
- surface texture and visual appearance

Mechanical properties were assessed qualitatively but consistently across samples, including:

- elasticity (extent of stretch without fracture)
- resistance to cutting (including acoustic emission during transection)
- tensile behaviour under longitudinal traction
- fragmentation behaviour (cohesive vs friable)

These descriptors were selected to differentiate AICs from conventional postmortem clots, which are typically gelatinous, non-elastic, and readily fragment under minimal mechanical stress.

Histological processing and evaluation

Representative sections of each specimen were fixed in 10% neutral buffered formalin for 24–48 hours, processed through graded ethanol, cleared in xylene, and embedded in paraffin using standard histopathological protocols Bancroft, 2019. (4)

Paraffin blocks were sectioned at 4–5 µm thickness and mounted onto glass slides. Sections were stained with haematoxylin and eosin (H&E) to visualise nuclei, fibrin architecture, cellular inclusions, and structural organisation.

Slides were independently evaluated by blinded analysts using a predefined scoring framework. Evaluation criteria included:

- fibrinous lamination
- presence and distribution of Lines of Zahn
- fibre architecture (orientation, density, bundling)
- cellular inclusions (erythrocytes, leukocytes, platelets)
- matrix homogeneity versus heterogeneity
- presence of voids or vacuolation

Inter-observer agreement was high, with discrepancies resolved by consensus.

Elemental analysis

Sample preparation

Subsamples of AIC material were prepared for elemental analysis using standardised protocols for biological tissues. Specimens were air-dried or gently dried under controlled conditions to remove free moisture and enable consistent mass normalisation across samples.

Dried material was subjected to closed-vessel, microwave-assisted acid digestion using ultra-pure nitric acid with hydrogen peroxide as an oxidising agent, producing clear solutions suitable for elemental analysis Rodushkin & Axelsson, 2000. (5)

To minimise contamination, all procedures were conducted using low-contamination practices, including acid-washed vessels, ultra-pure reagents, and pre-rinsed tools. Procedural blanks, reagent blanks, and duplicate preparations were included as quality control measures.

ICP-MS analysis

Elemental determinations were performed using inductively coupled plasma mass spectrometry (ICP-MS) under harmonised protocols across participating laboratories Becker, 2007. (6)

Elemental analysis was performed on eight specimens derived from the overall donor pool, with blinded ICP-MS analyses conducted independently across three analytical laboratories.

The analytical panel included:

- phosphorus and sulfur as principal elements relevant to biological matrix characterisation;
- physiologically relevant electrolytes and metals (e.g., sodium, potassium, calcium, magnesium, iron, zinc);
- selected trace elements to assess potential contamination.

Calibration was performed using multi-element standards in matched acid matrices. Internal standards were used to correct for instrumental drift and matrix effects. Analytical results were accepted only when quality control criteria for calibration linearity, recovery, and precision were met.

Stoichiometric benchmarking

Elemental compositions were evaluated against:

- whole-blood reference ranges
- first-principles stoichiometric expectations derived from fibrinogen

This approach allowed assessment of whether AICs were consistent with a predominantly proteinaceous fibrin matrix or required substantial non-protein contributions.

Proteomic analysis

Protein extraction and digestion

Proteins were extracted from AIC specimens using standard lysis buffers and enzymatically digested with trypsin to generate peptide fragments suitable for mass spectrometric analysis.

LC–MS/MS analysis

Peptide mixtures were analysed by high-resolution liquid chromatography–tandem mass spectrometry (LC–MS/MS) using a hybrid mass spectrometer operated in data-dependent acquisition mode Aebersold & Mann, 2003. (7) Peptides were separated by reverse-phase chromatography prior to MS/MS fragmentation.

Proteomic analysis was performed on four representative specimens derived from the overall donor pool and analysed under blinded conditions by an independent specialist proteomics laboratory.

Instrument parameters were optimised to maximise peptide identification depth while maintaining mass accuracy and reproducibility across samples.

Bioinformatic processing and quantification

Raw MS/MS data were processed using established proteomic pipelines with defined false discovery rate thresholds. Peptides were assigned to corresponding human proteins, and relative protein abundance was estimated using normalised spectral intensity measures averaged across replicate samples Zhang et al., 2013. (8)

Fibrinogen chain ratios (α, β, γ) were calculated by comparing relative abundances within each sample and averaging across samples. These ratios were used descriptively to assess deviations from conventional fibrin stoichiometry and were not intended as absolute molar quantification.

No pathway enrichment or network-level analyses were performed, as the focus of this study was compositional and structural characterisation.

Ethical considerations

All materials analysed in this study were anonymised postmortem biological specimens obtained from waste streams generated during routine mortuary procedures. No living individuals were involved, no identifiable personal data were accessed, and no intervention or clinical procedure was performed for research purposes.

In accordance with prevailing ethical guidelines, research involving anonymised, discarded postmortem materials does not constitute human subject research and does not require formal institutional review National Ethics Advisory Committee, 2021. (9)

Results

Morphological and histological phenotype

Gross morphology

Anomalous intravascular casts (AICs) exhibited a reproducible gross phenotype distinct from both conventional postmortem clots and recognised antemortem thrombi.

Specimens ranged from a few millimetres to greater than 25 cm in length and frequently demonstrated branching patterns consistent with vascular bifurcation geometry. The casts were typically cream to opaque white in colour, with minimal red discolouration confined to focal regions. In contrast to the amorphous, non-adherent nature of postmortem coagula, AICs formed coherent, lumen-conforming structures that preserved the architecture of the vascular tree.

Mechanical assessment revealed marked elasticity and tensile resilience Cai et al., 2023. (10) When subjected to traction, casts demonstrated extension without immediate fracture, and during transection produced a characteristic acoustic emission ("squeak"), consistent with a tension-bearing, stick-slip phenomenon, indicating a cohesive internal structure not dissimilar to vulcanised rubber. Fragmentation was minimal, and the material resisted the friable disintegration typical of postmortem clots.

No specimen exhibited the gelatinous texture or passive sedimentation characteristics associated with "currant-jelly" or "chicken-fat" postmortem clots.

*Histological architecture (**Figure 1**)*

Histological examination demonstrated a heterogeneous but internally consistent fibrinous architecture across samples.

At low magnification, sections displayed irregular fibrillar matrices with variable cellular density and the presence of void regions consistent with internal channels or processing artefact. Intermediate magnification revealed a network of fine fibrillar strands supporting dispersed cellular elements, with regions of both cellular enrichment and relative acellularity (absence of intact cells within a tissue or material).

A defining feature was the presence of regionally restricted Lines of Zahn, consisting of alternating fibrin-rich and cellular layers. These laminations indicate formation under conditions of active blood flow and distinguish AICs from passive postmortem coagulation. However, the distribution of these features was non-uniform, with laminated regions coexisting alongside dense, homogeneous fibrillar zones.

At high magnification, the matrix was composed of densely interwoven fibrin-like fibres arranged in directionally aligned networks. Cellular inclusions were sparse, and intact erythrocytes were notably absent. Instead, evidence of erythrocyte degradation, including extracellular hemoglobin and cellular debris, was observed. Leukocytes were present in distorted forms, frequently exhibiting membrane disruption and nuclear extrusion.

Taken together, these features define a structural phenotype characterised by:

- dense fibrillar architecture
- heterogeneous lamination
- sparse cellular incorporation
- evidence of formation under variable haemodynamic conditions

Elemental composition

Global elemental profile

Elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS) revealed a reproducible and internally consistent elemental profile across AIC samples.

The elemental spectrum was consistent with a biological matrix, dominated by light elements and physiologically common electrolytes. Major detected elements included phosphorus, sulfur, sodium, potassium, calcium, magnesium, iron, and zinc. Trace elements such as copper, boron, tin, and aluminium were present at low concentrations consistent with physiological background or incidental exposure.

No elements were detected at concentrations suggestive of synthetic polymers, industrial fillers, or exogenous particulate materials. Inter-sample variability in absolute concentrations was observed but remained within ranges expected for heterogeneous biological tissues subject to postmortem handling and dehydration.

Relative abundance patterns were consistent across samples, with phosphorus and sodium among the most prominent elements and sulfur consistently present at lower relative levels than expected for a protein-dominant matrix (**Table 1**, **Figure 2**).

Deviations from conventional fibrin-based composition

Comparison of elemental composition with theoretical fibrinogen stoichiometry and whole-blood reference values revealed systematic and reproducible deviations.

The most prominent findings were:

- **Sulfur depletion:** Mean sulfur concentrations were substantially lower than those predicted from theoretical fibrinogen stoichiometry for a fibrin(ogen)-dominated matrix;
- **Phosphorus enrichment**: Phosphorus levels were elevated relative to expectations for proteinaceous clot material, both in absolute terms and relative to other bulk elements;
- **Elemental imbalance**: Relationships among bulk elements were inconsistent with those expected for conventional fibrin-based thrombi.

Together, these deviations constrain the bulk composition of AICs, indicating that the matrix is not dominated by protein content in the manner of conventional fibrin clots (**Table 2**).

Internal consistency and heterogeneity

Despite variability in absolute elemental concentrations across samples, the defining compositional features—particularly sulfur depletion and relative phosphorus enrichment—were consistently observed across all analysed specimens and laboratories.

No discrete subpopulations or bimodal distributions were identified. Subsampling within individual casts did not reveal systematic regional variation beyond expected biological and sampling variability.

Furthermore, no consistent correlation was observed between elemental composition and gross morphological or histological features, indicating that the compositional phenotype represents a global property of the AIC matrix rather than a feature of specific structural subtypes (**Figure 3**).

Proteomic composition

Protein identification and distribution

Proteomic analysis identified a total of 541 human proteins across analysed AIC samples. Refer to Fig. 4. Protein abundance distributions were highly skewed, with a small subset of proteins accounting for a substantial proportion of total signal intensity, while the majority were present at low relative abundance.

The dominant proteins included:

- hemoglobin subunit beta
- fibrinogen beta chain
- fibrinogen gamma chain
- hemoglobin subunit alpha
- myeloperoxidase
- fibrinogen alpha chain

All remaining proteins were present at less than 1% relative abundance and collectively accounted for approximately half of the total proteomic signal (**Figure 4**).

The distribution demonstrates a pronounced dominance of a small subset of proteins, with the majority present at low relative abundance (**Figure 5**).

Fibrinogen chain composition

Fibrinogen chains were detected in markedly non-physiological proportions. The observed mean ratio of α:β:γ chains was approximately 1:7:3, in contrast to the near-equimolar ratios expected in conventional fibrin clots.

This imbalance was consistent across samples and represents a reproducible feature of the AIC proteomic profile.

Fibrinolytic components

Plasminogen was detected at extremely low abundance, comprising approximately 0.13% of total protein signal. Tissue plasminogen activator (tPA) was detected only in a single sample and at very low levels.

These findings indicate a marked depletion of intrinsic fibrinolytic components within the AIC matrix.

Additional protein features

In addition to fibrin-family proteins, the proteomic profile included proteins associated with inflammatory and cellular processes, including myeloperoxidase, cathepsin G, immunoglobulin heavy chains, and cytoskeletal proteins such as actin. These proteins were present at moderate to low abundance relative to dominant structural components.

The majority of detected proteins were present at very low abundance and did not contribute substantially to the bulk proteomic signal. Nevertheless, their potential biological relevance cannot be excluded on the basis of abundance alone.

Summary proteomic phenotype

The proteomic profile of AICs is characterised by:

- dominance of fibrin-family proteins;
- marked imbalance in fibrinogen chain composition;
- severe depletion of fibrinolytic components;
- presence of a broad background of low-abundance proteins normally found in circulating blood.

Cross-Domain Convergence

Resolution of structural and compositional constraints

The morphological, elemental, and proteomic analyses each impose independent constraints on the nature of anomalous intravascular casts (AICs). When considered in isolation, these domains describe features that are not fully compatible with conventional clot biology. When considered together, however, they converge on a coherent and internally consistent description of the AIC matrix.

Morphological and histological analysis demonstrates a dense fibrillar architecture consistent with fibrin-like organisation, including the presence of laminated structures and regionally distributed Lines of Zahn indicative of formation under active blood flow. These observations establish that protein-based fibre assembly contributes to the structural framework of the casts.

In contrast, elemental analysis demonstrates that the bulk composition of AICs is not consistent with a matrix composed predominantly of fibrin(ogen).

The observed depletion of sulfur relative to theoretical fibrinogen stoichiometry constrains the contribution of fibrin(ogen) to the overall matrix, while relative phosphorus enrichment indicates the incorporation of additional phosphorus-containing proteinaceous and possibly non-proteinaceous constituents.

Proteomic analysis further refines this picture by demonstrating that fibrin-family proteins are present and structurally dominant within the detectable protein fraction, but exhibit markedly non-physiological organisation, including severe imbalance in fibrinogen chain ratios and depletion of fibrinolytic components.

Taken together, these findings resolve the apparent tension between fibrin-like morphology and non-protein elemental composition. AICs are best described as fibrin-bearing structures in which protein provides a structural scaffold but does not account for the majority of bulk mass. This distinction reconciles the presence of organised fibrillar architecture with compositional constraints derived from elemental analysis.

Relationship between molecular architecture and mechanical properties

The observed mechanical behaviour of AICs, including elasticity, tensile resilience, and resistance to fragmentation, can be interpreted in light of the proteomic findings.

The marked depletion of the fibrinogen α chain relative to β and γ chains represents a significant deviation from expected fibrin chain stoichiometry. The α chain contributes domains involved in lateral aggregation, fibre extensibility, and network compliance. Its relative deficiency is therefore expected to alter fibre assembly, favouring the formation of more densely packed, structurally constrained fibrillar networks.

Such an architecture is consistent with the gross mechanical properties observed in AICs, including their ability to sustain deformation without fragmentation and their resistance to shear. At the same time, the elemental findings suggest that compositional factors beyond fibrin-family proteins may contribute to matrix organisation and mechanical behaviour, potentially reinforcing or modifying the fibrin scaffold.

Accordingly, the mechanical phenotype of AICs can be understood as arising from the combined effects of altered fibrin architecture and incorporation of non-protein constituents, rather than from conventional fibrin assembly alone.

Integration of elemental and proteomic constraints

Elemental and proteomic analyses provide complementary but distinct insights into matrix composition, with each dataset constraining different aspects of the underlying molecular architecture.

Proteomic analysis identifies the proteins present within the analysed material and their relative abundance within the detectable protein fraction, whereas elemental analysis characterises the bulk chemical composition of the matrix.

The proteomic dataset demonstrated the presence of abundant fibrin-family proteins together with numerous additional protein species. However, the observed elemental composition was not consistent with a matrix composed predominantly of fibrin(ogen) alone. In particular, sulfur concentrations were substantially lower than those predicted from theoretical fibrinogen stoichiometry, while phosphorus concentrations were relatively enriched.

The marked depletion of the fibrinogen α chain relative to the β and γ chains provides additional context for interpretation of the sulfur data. Although this altered chain distribution may modestly influence the sulfur content of the fibrin component, the magnitude of the observed sulfur depletion cannot be explained solely by fibrin chain imbalance. Rather, the combined findings indicate that fibrin-family proteins, while clearly present and likely structurally important, represent only one component of a more compositionally complex matrix.

These observations are readily interpreted by recognising that proteomic measurements reflect relative abundance within the detectable protein pool rather than absolute contribution to total matrix composition. The combined elemental and proteomic data therefore support a model in which fibrin-family proteins form an organised structural framework embedded within, or associated with, a broader matrix containing additional proteinaceous constituents and possibly non-proteinaceous phosphorus-containing components.

The relative enrichment of phosphorus without corresponding calcium levels sufficient to indicate bulk mineralisation suggests incorporation of phosphorus-containing constituents in non-apatitic forms. These may include phosphorus-containing proteinaceous or non-proteinaceous constituents, although their specific chemical identities cannot be deter-

mined from elemental analysis alone.

Fibrinolytic capacity and structural persistence

A defining feature of AICs is their apparent persistence and resistance to degradation, which can be interpreted through the combined structural and proteomic findings.

Proteomic analysis demonstrates marked depletion of plasminogen and minimal detection of tissue plasminogen activator, indicating a profound reduction in intrinsic fibrinolytic capacity. In physiological clotting, incorporation of plasminogen into the fibrin network enables subsequent enzymatic degradation following vascular repair. The absence of this machinery in AICs implies that the matrix is not configured for normal resolution.

This biochemical constraint aligns with the observed mechanical properties of AICs, including their resistance to fragmentation and persistence as coherent structures. The combination of altered fibrin architecture and impaired fibrinolytic capacity provides a consistent explanation for the formation of elongated, lumen-conforming casts that are not readily degraded.

Thus, structural persistence in AICs is not solely a mechanical phenomenon but reflects a convergence of molecular architecture and enzymatic deficiency.

Convergent interpretation

The integration of structural, elemental, and proteomic data yields a coherent and internally consistent description of anomalous intravascular casts.

Across independent analytical domains, AICs are characterised by:

- organised fibrin-like architecture formed under conditions of active blood flow;
- bulk composition incompatible with a fibrin-dominant protein matrix;
- non-physiological fibrinogen chain stoichiometry;
- marked depletion of fibrinolytic components;
- mechanical properties consistent with a cohesive, persistent intravascular structure.

No single domain, considered in isolation, is sufficient to define this phenotype. However, the convergence of these independent lines of evidence supports the interpretation that AICs represent a distinct intravascular matrix that departs from conventional thrombus biology in both composition and function. Importantly, this interpretation arises from mutually constraining observations rather than from extrapolation beyond the available data. The agreement between independently derived structural, elemental, and proteomic findings provides internal validation of the observed phenotype and establishes a framework for further investigation.

Discussion

Relationship to established clot types

The integrated findings presented here indicate that anomalous intravascular casts (AICs) cannot be adequately classified within existing frameworks of either antemortem thrombi or postmortem coagula.

Classical antemortem thrombi are characterised by prominent lamination, substantial cellular incorporation, and balanced fibrin architecture consistent with near-equimolar fibrinogen chain composition. In contrast, AICs exhibit only regionally restricted lamination, markedly reduced cellular content, and pronounced deviation from conventional fibrin stoichiometry. These features collectively distinguish AICs from conventional thrombi formed under physiological haemostatic conditions.

Similarly, AICs differ fundamentally from postmortem clots, which arise passively following cessation of circulation and are typically gelatinous, friable, and non-adherent. The lumen-conforming geometry, branching structure, elastic resilience, and presence of flow-associated features such as Lines of Zahn observed in AICs are not compatible with passive postmortem coagulation.

Taken together, these distinctions indicate that AICs do not represent variants of recognised clot types but instead occupy a separate position within the spectrum of intravascular material.

Structural and biochemical constraints on matrix identity

The combined structural, elemental, and proteomic data impose important constraints on the identity of the AIC matrix.

Histological analysis demonstrates the presence of organised fibrin-like architecture, establishing that fibrin-family proteins contribute to the structural framework. However, elemental analysis indicates that the matrix cannot be explained by fibrin(ogen) alone, as evidenced by sulfur depletion relative to theoretical fibrinogen stoichiometry together with relative phosphorus enrichment.

Polyphosphate has recognised roles in coagulation and fibrin organisation and could contribute to phosphorus enrichment; however, the present data do not permit identification of specific phosphate-bearing constituents Morrissey, 2015. (11) Polyphosphate (polyP) is known to modulate multiple aspects of haemostasis, including thrombin generation, fibrin formation, clot architecture, and resistance to fibrinolysis, and can influence the retention and interaction of coagulation-associated proteins within developing thrombi. However, these effects are generally understood to alter clot structure and coagulation kinetics rather than generate a novel bulk matrix. Consequently, although polyP could potentially contribute to the elevated phosphorus content observed in AICs, it would not be expected to account for the marked sulfur depletion or the broader compositional divergence from conventional fibrin-rich thrombi. Proteomic findings further demonstrate that, while fibrin-family proteins are present and likely structurally significant, their internal organisation is markedly non-physiological.

These constraints collectively indicate that AICs are not composed of conventional fibrin in an altered physical state. Rather, they represent a compositionally complex matrix in which fibrin-family proteins form an organised structural framework associated with additional proteinaceous constituents and possibly non-proteinaceous components.

The identity of these additional constituents cannot be determined from the present data. However, the observed enrichment of phosphorus without corresponding evidence of bulk mineralisation suggests the presence of phosphorus-containing constituents in non-apatitic forms. This interpretation remains constrained by the limitations of elemental analysis, which cannot resolve chemical speciation.

Non-conventional fibrin architecture

Proteomic analysis demonstrates a marked deviation from conventional fibrinogen chain stoichiometry, with relative depletion of the α chain and corresponding enrichment of β and γ chains.

In physiological clot formation, the α chain contributes domains critical for lateral aggregation, fibre extensibility, and network compliance. Its relative depletion is therefore expected to alter fibrin assembly, favouring the formation of thinner, more densely packed fibres with reduced extensibility compared to a classical fibrin mesh clot built over a platelet plaque. Such an architecture is consistent with the observed mechanical properties of AICs, including their resistance to fragmentation and sustained structural integrity.

Importantly, the presence of fibrin-family proteins does not, in this context, imply normal fibrin function. Rather, the data indicate that fibrin is incorporated into a structurally atypical configuration that departs from the balanced, self-limiting architecture of physiological clotting. The absence of normal platelet involvement may be consistent with the observed lack of platelets in the AICs.

Impaired fibrinolysis and persistence

A defining feature of AICs is their apparent persistence as coherent intravascular structures. The proteomic data provide a mechanistic constraint on this behaviour through the marked depletion of plasminogen and minimal detection of fibrinolytic components.

In physiological systems, incorporation of plasminogen into the fibrin network enables subsequent clot resolution through enzymatic degradation once haemostatic function has been fulfilled. The absence or severe reduction of this machinery in AICs indicates that the matrix is not configured for normal fibrinolytic resolution.

When considered alongside altered fibrin architecture and the presence of non-protein components, this deficiency provides a consistent explanation for the persistence of these structures. Rather than representing an exaggerated form of normal clotting, AICs appear to reflect a process in which formation and resolution are decoupled, resulting in stable, tension-bearing intravascular material.

Evidence of inflammatory and cellular involvement

The proteomic profile of AICs includes proteins associated with inflammatory and cellular processes, including myeloperoxidase, cathepsin G, immunoglobulin heavy chains, and cytoskeletal components such as actin.

These findings indicate that AIC formation occurs in a biological environment involving cellular participation and inflammatory activity. The presence of neutrophil-associated enzymes, myeloperoxidase (MPO) and cathepsin G, suggests that oxidative and proteolytic processes may contribute to modification of the matrix during formation. Similarly, the detection of immunoglobulins and cytoskeletal proteins is consistent with incorporation of plasma components and cellular debris under dynamic con-

ditions.

At the same time, the low abundance of most non-structural proteins suggests that many of these components are incorporated incidentally rather than serving primary structural roles. The dominant structural features of the matrix remain associated with fibrin-family proteins and the phosphorus-enriched, sulfur-depleted composition identified through elemental analysis.

Implications for intravascular function and pathology

Any process resulting in the formation of persistent solid material within the vascular lumen has the potential to affect blood flow, tissue perfusion, and vascular integrity.

The structural properties of AICs—coherence, elasticity, and resistance to degradation—are consistent with the capacity to maintain intravascular presence over extended periods. In large vessels, such material could contribute to obstruction and acute vascular events. In smaller vessels and the microvasculature, persistent intraluminal structures may impair perfusion in a more diffuse manner, potentially contributing to chronic tissue hypoxia and organ dysfunction.

In addition to direct obstruction, the presence of structurally abnormal intravascular material may influence local haemodynamics, endothelial function, and inflammatory responses. These effects may, in turn, promote further clot formation or vascular remodelling.

These considerations are presented as general implications of persistent intravascular material and do not establish specific clinical outcomes associated with AICs. Determination of clinical relevance requires further investigation in appropriately designed studies.

Limitations

Several limitations of the present study should be acknowledged.

First, all specimens were obtained from postmortem sources, and while structural and histological features indicate at least partial formation under conditions of active blood flow, direct clinical correlation is not available. The extent to which AICs occur in living patients, and under what conditions, requires further study.

Second, elemental analysis provides information on bulk composition but cannot determine the specific chemical identity or bonding state of detected elements. In particular, the form and origin of phosphorus within the matrix remain unresolved.

Although the present study is based exclusively on postmortem specimens, the authors have received numerous informal reports from surgeons, interventional cardiology personnel, and other clinical practitioners describing morphologically similar intravascular structures observed during clinical procedures. These observations were not collected systematically and were therefore not suitable for inclusion in the present analysis. Prospective clinical investigation will be required to determine the prevalence, characteristics, and clinical significance of such structures in living patients.

Third, proteomic analysis was performed using relative abundance measures derived from spectral intensity, which provide comparative but not absolute quantification. Additionally, raw mass spectrometry datasets are not publicly available due to confidentiality constraints associated with participating laboratories.

Fourth, the sample size, while sufficient to demonstrate reproducible patterns across analytical domains, remains limited. Broader sampling across different populations and clinical contexts will be required to establish the generality of the observed phenotype.

Finally, mechanistic interpretation is constrained by the observational nature of the study. While the data define structural, compositional, and molecular characteristics, they do not establish the biochemical pathways responsible for AIC formation. The present study was not designed to evaluate temporal, demographic, or exposure-related associations. Consequently, no conclusions can be drawn regarding potential relationships between the appearance of AICs and contemporaneous public health, environmental, pharmaceutical, or other population-level factors. Investigation of such questions will require dedicated epidemiological studies incorporating appropriate clinical and exposure data.

Future directions

The findings presented here generate a number of testable hypotheses that can guide further investigation.

Future studies should aim to:

- determine the chemical speciation of phosphor-

us and other non-protein components within the matrix;
- perform expanded proteomic analyses with independent replication and utilise quantitative proteomic approaches to determine the reproducibility and relative abundance of key protein signatures across specimens;
- investigate the structural organisation of the matrix using complementary imaging and biochemical techniques;
- assess the presence and characteristics of similar structures in clinical settings involving living patients;
- evaluate the relationship between AIC formation and known pathways of coagulation, inflammation, and vascular injury.

Such studies will be essential to establish the biochemical mechanisms underlying AIC formation and to determine their potential clinical significance.

Conclusion

Anomalous intravascular casts (AICs) exhibit a reproducible and internally consistent phenotype that is not adequately explained by existing models of either antemortem thrombus formation or postmortem coagulation.

Across three independent analytical domains, AICs are characterised by:
- a coherent, lumen-conforming fibrillar structure formed under conditions consistent with active blood flow;
- a bulk elemental composition incompatible with a protein-dominant fibrin matrix;
- a proteomic architecture defined by non-physiological fibrinogen chain stoichiometry and marked depletion of fibrinolytic components.

These findings are not redundant but mutually constraining. Structural analysis demonstrates organised fibrin-like architecture, elemental data limit the contribution of protein to total matrix mass, and proteomic analysis defines the altered molecular composition and impaired fibrinolytic capacity of the protein fraction. The convergence of these independent lines of evidence supports a unified interpretation.

Accordingly, AICs are best understood as a distinct intravascular matrix characterised by non-conventional fibrin organisation, reduced protein dominance, and resistance to physiological degradation. This phenotype departs in both composition and function from recognised clot types and cannot be attributed to passive postmortem processes.

The present study is observational and does not establish the biochemical pathways responsible for AIC formation. However, it defines a coherent set of structural, compositional, and molecular constraints that any explanatory model must satisfy. The reproducibility of these findings across independent analytical domains underscores the need for further investigation and independent replication.

Recognition of AICs as a distinct, previously unrecognised intravascular phenomenon provides a framework for future studies aimed at determining their origin, biochemical basis, and potential clinical relevance.

Ethics Statement

All materials analysed in this study were anonymised postmortem biological specimens obtained from waste streams generated during routine mortuary and laboratory procedures. No living human subjects were involved, no identifiable personal data were accessed, and no intervention or procedure was performed for research purposes. Under applicable local ethics regulations, research involving anonymised discarded postmortem materials does not constitute human subjects research and does not require formal ethics committee approval.

Author Contributions

B.R. conceived the study, coordinated data acquisition, performed analysis, and drafted the manuscript. M.S. contributed to study design, interpretation of results, and manuscript revision. All authors reviewed and approved the final manuscript.

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Competing Interests

The authors declare no competing interests.

Data Availability

Data may be made available to qualified researchers upon reasonable request, subject to confidentiality constraints.

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Clinical, mortuary, or sample access support

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Table 1. Mean Elemental Concentrations (ppm) of Clot Samples Analyzed in the Present Study, with Comparison to Adam' Clot Data and Whole-Blood Values

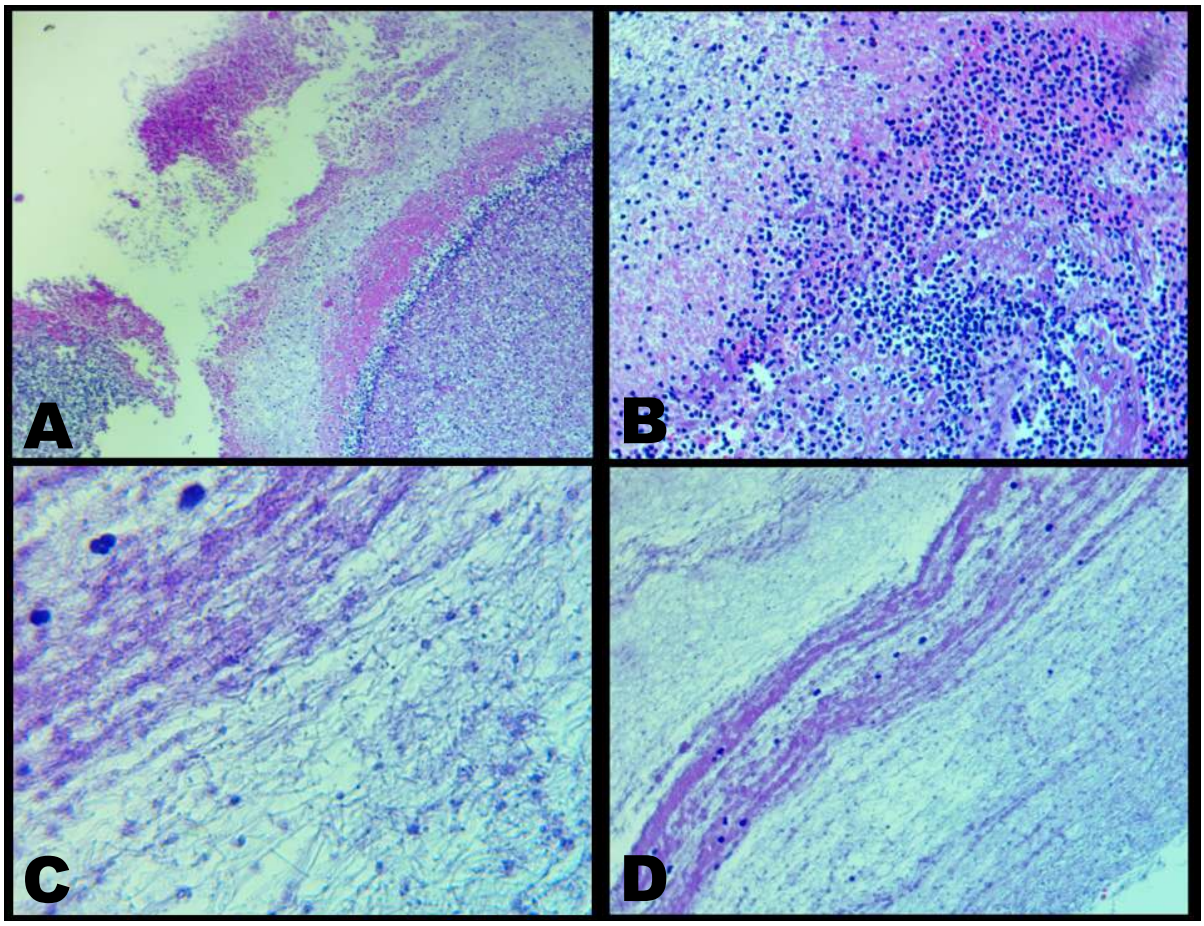
Element	94914	41490	71831	82359	85068	21682	55708	55709	Adams Clot	Adams Blood	Min	Max	Mean	Std. Dev.
Aluminium	1.1	1.1	1.0	1.0	1.0	1.1	1.0		1.3	1.3	1.0	1.3	1.0	0.1
Boron	0.6	0.6	0.5	0.5	0.5	0.6	0.5	1.2			0.5	1.2	0.6	0.2
Calcium	20.0	31.0	29.0	22.0	30.0	26.0	27.0	32.0			20.0	32.0	27.1	4.3
Copper	0.650	0.590	0.590	0.690	0.670	1.100	0.640	0.670			0.6	1.1	0.7	0.2
Iron	400	171	112	73	120	27	28	30	21	462	20.6	462.0	144.4	159.7
Magnesium	6.0	6.0	5.0	5.0	5.0	6.0	5.0	6.0	2	35	1.7	35.0	8.1	9.5
Phosphorus	1,130	1,390	1,290	1,380	1,560	1,430	1,600	1,510	4,900	1,130	1,130	4,900	1,732	1,124.7
Potassium	50	50	40	40	40	50	40	50	13		12.5	50.0	41.4	11.9
Sodium	2,600	2,800	2,700	2,500	2,800	2,600	2,600	2,600	1,050	1,050	1,050	2,800	2,330	681.2
Sulfur	1,540	1,480	1,470	1,180	1,380	1,330	1,130	12.0			12.0	1,540	1,190	497.4
Tin	0.110	0.110	0.100	0.100	0.100	0.110	0.100	0.120	0.163	0.163	0.1	0.2	0.1	0.025
Zinc	3.3	2.3	1.1	1.8	1.9	1.10	1.5	1.2	2.4	7.9	1.1	7.9	2.6	2.1

Table 2. Elemental Ratios Comparing AICs with Whole-Blood and Fibrinogen Reference Values

Element	Whole Blood Range (ppm)	Fibrinogen Stoichiometric Value (ppm)	Experimental Clot Mean ($\bar{x}$, ppm)	Ratio Expt. $\bar{x}$: Blood	Ratio Expt. $\bar{x}$: Fibrinogen
Aluminium (Al)	< 1.0	—	1.0	1.0	—
Boron (B)	0.02—0.10	—	0.6	1.0	—
Calcium (Ca)	90—105	—	27.1	0.3	—
Carbon (C)	100,000—120,000	516,400	—	—	—
Copper (Cu)	0.7—1.5	—	0.7	0.6	—
Hydrogen (H)	9,000—11,000	70,000	—	—	—
Iron (Fe)	400—500	—	144.4	0.20	—
Magnesium (Mg)	160—240	—	8.1	0.04	—
Nitrogen (N)	25,000—40,000	165,600	—	—	—
Oxygen (O)	50,000—60,000	239,900	—	—	—
Phosphorus (P)	900—1,500	—	1,732	1.44	—
Potassium (K)	1,500—2,000	—	41.1	0.02	0.02
Sodium (Na)	2,200—2,500	—	2,330.0	1.0	—
Sulfur (S)	700—900	8,100	1,190.3	1.5	0.15
Tin (Sn)	< 1.0	—	0.1	0.1	—
Zinc (Zn)	2.5—7.0	—	2.6	0.5	—
Total (normalized)		1,000,000			

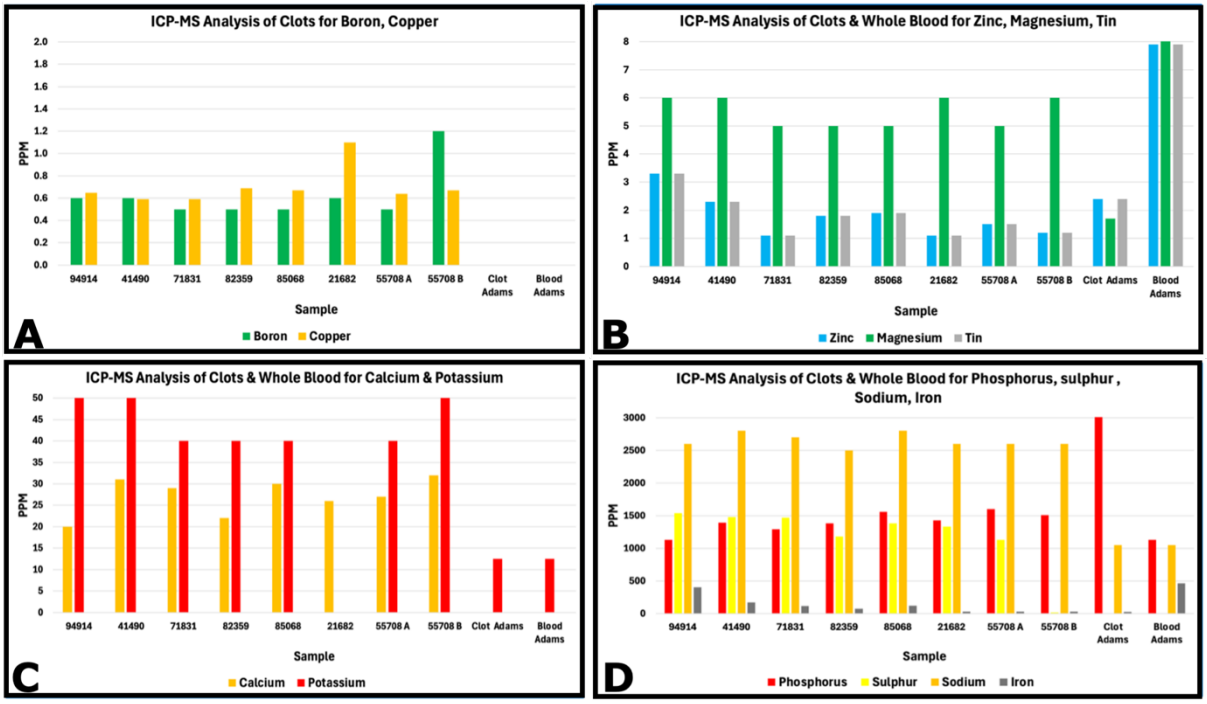
Key: red = excess, blue = deficit.

Figure 1. Microscopic morphology and structural heterogeneity of an anomalous intravascular cast (AIC)



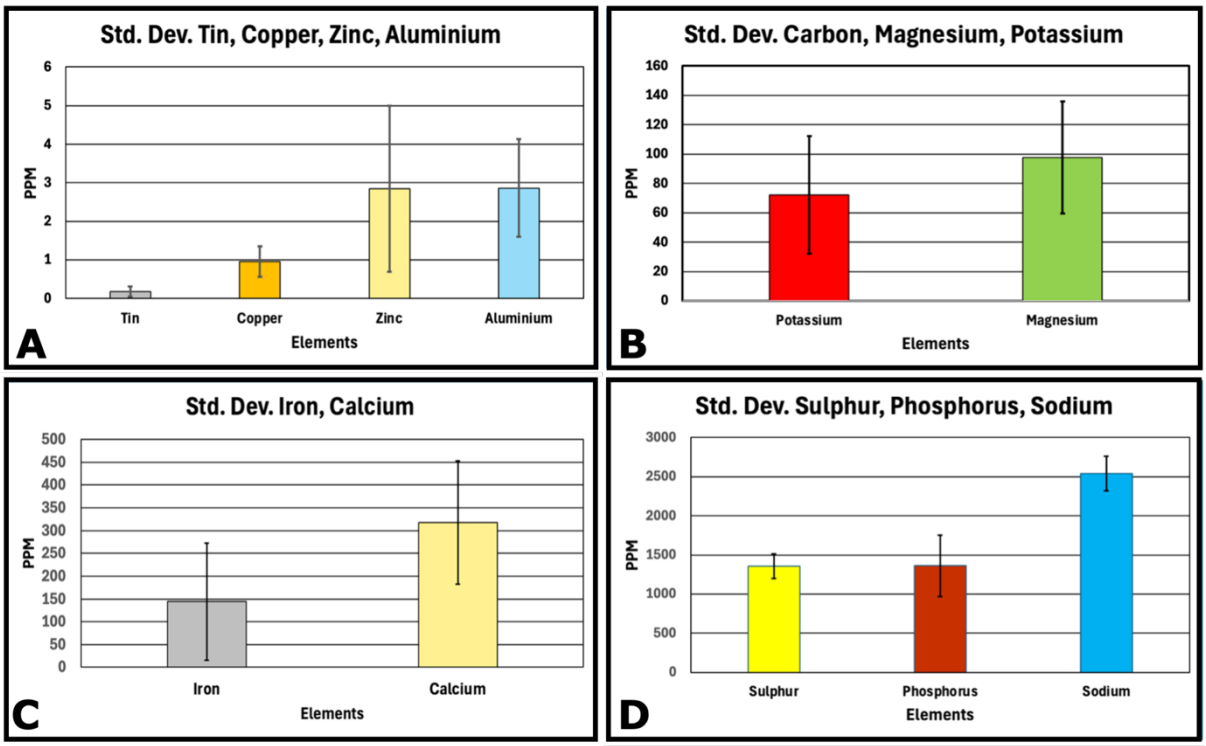
Panel A. Low-magnification (40×) H&E overview demonstrating the heterogeneous architecture of the AIC specimen. Large paraffin-filled voids reflect internal channels or loss of unsupported material during microtomy, while surrounding regions show an irregular fibrinous matrix with variable cellular density.
Panel B. Intermediate magnification (100×) highlighting heterogeneous distribution of cellular elements within a fibrin-dominant matrix. Hematoxylin-positive nuclei are scattered irregularly, and fine fibrillar strands support the sparse cellular content, indicating non-uniform microstructural organisation.
Panel C. High-magnification (400×) micrograph showing densely interwoven fibrin bundles interspersed with distorted leukocytes exhibiting ruptured membranes and nuclear extrusion. Hemoglobin streaking is visible in regions of erythrocyte lysis, consistent with the absence of intact red blood cells.
Panel D. High-magnification (400×) field illustrating non-homogeneous fibrin organisation, including banded zones reminiscent of partial lines of Zahn. Cellular inclusion is minimal, and fibrin fibres display directional alignment suggestive of formation under variable shear conditions.

Figure 2. ICP-MS analysis of clots & whole blood



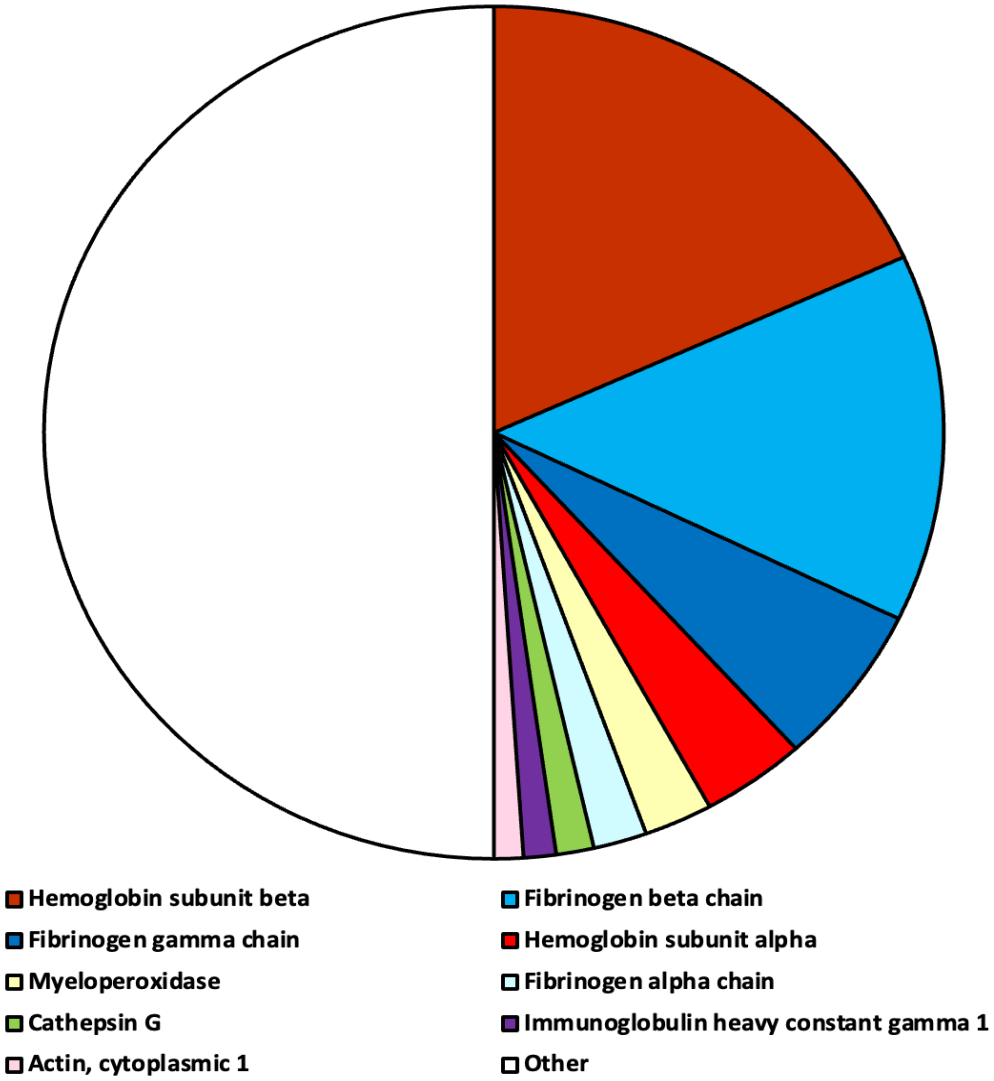
Panel A. Boron, copper.
Panel B. Zinc, magnesium, tin.
Panel C. Calcium, potassium.
Panel D. Phosphorus, sulfur, sodium, iron.

Figure 3. Standard deviation for elemental analysis



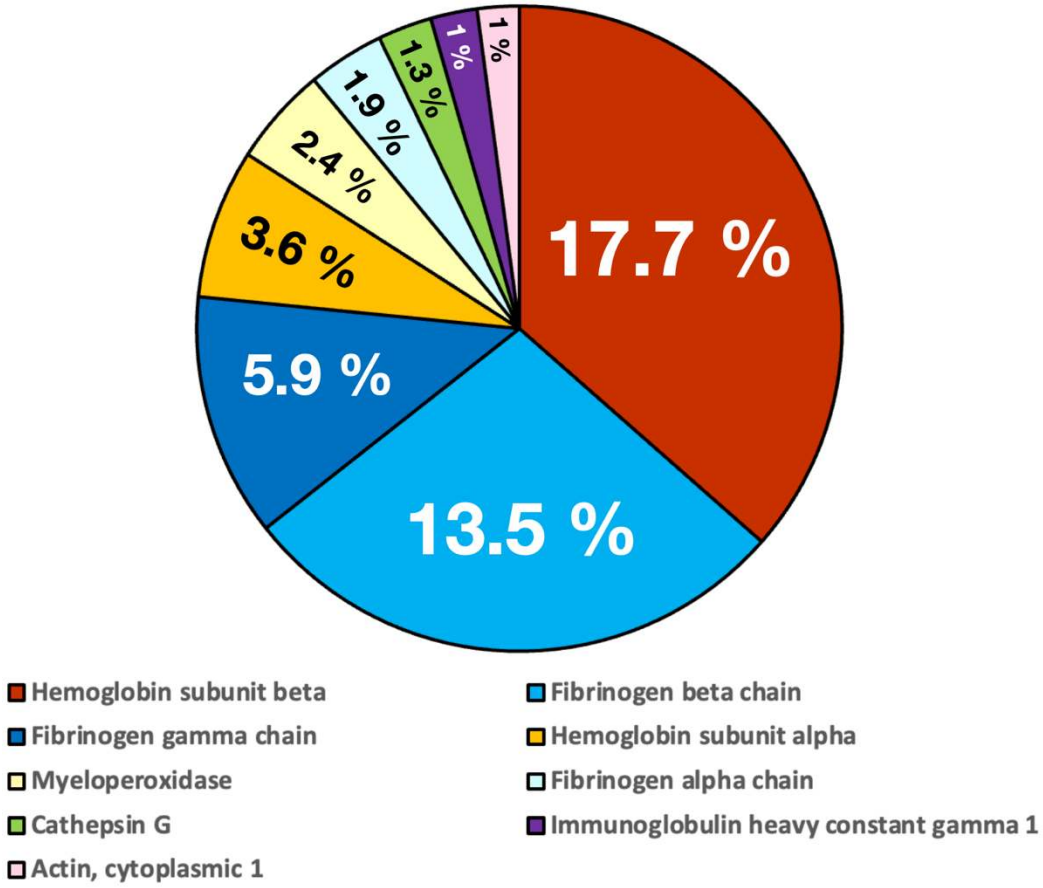
Panel A. Tin, copper, zinc, aluminium.
Panel B. Potassium, magnesium.
Panel C. Iron, calcium.
Panel D. Sulfur, phosphorus, sodium.

Figure 4. 541 human proteins by mean abundance (%)



If we ignore the 532 minor proteins that are all present at levels well below 1%, it is easier to visualize the main proteins of interest that dominate AIC proteome.

Figure 5. Top 9 human proteins by mean abundance (%)



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