Toxic metal Burden in Intracranial Thrombi Retrieved During Mechanical Thrombectomy: An Observational Study

Manuel Scimeca[1], Alessandro Mauriello[1], Thanh N. Nguyen[2], Marco Nezzo[3], Federico Sabuzi[3], Renato Argirò[3], Daniele Morosetti[3], Mariafrancesca Trulli[3], Lorenzo Rocchi[4], Ilaria Maestrini[5], Valeria Palumbo[1], Giovanni Maria D'Amico[5], Michele Treglia[3], Francesca Di Giuliano[3], Jacopo Troisi[6-8], Francesco Garaci[3], Valerio Da Ros[3]

■ BACKGROUND: **Acute ischemic stroke caused by intracranial large vessel occlusion is associated with significant morbidity and mortality. Emerging evidence suggests that environmental exposures, including toxic metals, may be associated with cerebrovascular disease. However, the presence of toxic metals within intracranial thrombi and their relationship with clinical features of acute ischemic stroke remain largely unexplored.**

■ MATERIALS AND METHODS: **We conducted an observational cohort study including 59 patients with acute ischemic stroke due to large vessel occlusion treated with mechanical thrombectomy between November 2023 and April 2024. Retrieved thrombi were analyzed for aluminum, cadmium, nickel, and lead concentrations using Inductively Coupled Plasma—Mass Spectrometry. Histological and immunohistochemical analyses were performed to characterize thrombus composition. Associations between thrombus metal concentrations and clinical, radiological, and procedural variables, including NIHSS scores and first-pass recanalization, were assessed.**

■ RESULTS: **Significant correlations were observed between metals: aluminum with cadmium (r = 0.733,**

Key words
- Acute ischemic stroke
- Clinical outcomes
- Inductively coupled plasma mass spectrometry
- Intracranial thrombus
- Large vessel occlusion
- Mechanical thrombectomy
- Toxic metals

Abbreviations and Acronyms
ACA: Anterior Cerebral Artery
AIS: Acute Ischemic Stroke
Al: Aluminum
ASPECTS: Alberta Stroke Program Early CT Score
BA: Basilar Artery
CAD: Coronary Artery Disease
Cd: Cadmium
CE: Cardioembolism
CVD: Cerebrovascular Disease
CT: Computed Tomography
CTA: Computed Tomography Angiography
DAB: 3,3′-Diaminobenzidine
EDTA: Ethylenediaminetetraacetic Acid
ESUS: Embolic Stroke of Undetermined Source
FFPE: Formalin-Fixed, Paraffin-Embedded
FPR: First-Pass Recanalization
Hg: Mercury
HRP: Horseradish Peroxidase
ICA: Internal Carotid Artery
ICP-MS: Inductively Coupled Plasma—Mass Spectrometry
LAA: Large-Artery Atherosclerosis
LVO: Large Vessel Occlusion
MCA: Middle Cerebral Artery
MT: Mechanical Thrombectomy
NCCT: Non-Contrast Computed Tomography
Ni: Nickel
NIHSS: National Institutes of Health Stroke Scale
Pb: Lead
PBS: Phosphate-Buffered Saline
PCA: Posterior Cerebral Artery
ROS: Reactive Oxygen Species
TIA: Transient Ischemic Attack
TOAST: Trial of Org 10 172 in Acute Stroke Treatment
UD: Undefined
μg/g: Micrograms per Gram
μg/L: Micrograms per Liter

From the [1]Department of Experimental Medicine, TOR, University of Rome "Tor Vergata", Rome, Italy; [2]Department of Neurology, Boston Medical Center, Boston University Chobanian and Avedisian School of Medicine, Boston, Massachusetts, USA; [3]Department of Biomedicine and Prevention, University Hospital of Rome "Tor Vergata", Rome, Italy; [4]Department of Medical Sciences and Public Health, University of Cagliari, Cittadella Universitaria di Monserrato, Monserrato, Cagliari, Italy; [5]Stroke Center, Department of Systems Medicine, University Hospital of Rome "Tor Vergata", Rome, Italy; [6]Theoreo srl, Via degli Ulivi 3, Montecorvino Pugliano, Salerno, Italy; [7]European Institute of Metabolomics - Fondazione EIM, Baronissi, Salerno, Italy; and [8]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno, Baronissi, Salerno, Italy

To whom correspondence should be addressed: Valerio Da Ros, M.D., Ph.D. [E-mail: darosvalerio@gmail.com]

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$P < 0.001$) and nickel ($r = 0.558$, $P = 0.001$). Cadmium was positively associated with first-pass recanalization (FPR) success ($r = 0.648$, $P < 0.001$), while lead correlated with National Institutes of Health Stroke Scale (NIHSS) at onset ($r = 0.502$, $P < 0.001$). In 29% of thrombi containing all 4 metals, aluminum was inversely related to NIHSS at 12 hours ($r = -0.775$, $P < 0.001$).

CONCLUSION: **This study identifies significant associations between thrombus toxic metal concentrations and clinical outcomes, suggesting that thrombus metal composition may be relevant to acute ischemic stroke characteristics.**

INTRODUCTION

Acute ischemic stroke (AIS) due to intracranial large vessel occlusion (LVO) is a devastating neurological event associated with high morbidity and mortality. While the primary focus of research has traditionally been on vascular pathophysiology and neuroprotective strategies, emerging evidence suggests that environmental and occupational exposures, particularly to toxic metals, can play a role in the pathogenesis of cerebrovascular diseases (CVD), including AIS.[1,2] In this context, recent research indicates a significant link between exposure to toxic metals and CVD. Metals such as arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg), prevalent in contaminated water, soil, food, and industrial environments, can induce oxidative stress, inflammation, and endothelial dysfunction, thereby increasing the risk of hypertension, arhythmia, and atherosclerosis.[3-7] These effects are mediated through mechanisms including free radical accumulation, lipid peroxidation, and endocrine disruption.[4,8] Traditional cardiovascular risk models fail to account for the full burden of CVDs and stroke, underscoring the importance of considering environmental and dietary exposures.[8] Clinical trials, including those on chelation therapy, further support the pathogenic role of metals in CVD and its potential reversibility.[9]

Despite the strong association between toxic metal exposure and CVDs, their specific role in AIS remains poorly understood. In particular, it is unclear whether these metals contribute directly to thrombus formation or influence thrombus composition.

Recent advances in mechanical thrombectomy (MT) have enabled the retrieval of intracranial thrombi, offering a unique opportunity to study their biochemical composition.[10] While serum biomarkers reflect recent systemic exposure, thrombi may provide insight into local and potentially chronic deposition of toxic substances within the vasculature. Toxic metals, known to induce oxidative stress, endothelial dysfunction, and platelet aggregation, could plausibly accumulate in thrombi and influence their formation and mechanical behavior.

To date, no studies have directly evaluated the presence of toxic metals within intracranial thrombi. Existing research has focused primarily on serum biomarkers,[11] leaving a gap in understanding local accumulation at the site of vascular occlusion. This exploratory study aims to determine whether aluminum (Al), Cd, nickel (Ni), and Pb can be detected in thrombi from AIS patients undergoing MT, serving as our primary endpoint. To our knowledge, this represents the first analysis of toxic metal content within cerebral thrombotic material.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the ethics committee of our institution (Prot n°219.24). The human research conformed to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all patients or their next of kin.

Patient Selection

From November 2023 to April 2024, 59 patients with AIS caused by LVO in the anterior or posterior circulation who underwent MT were recruited for this pilot evaluation. Clot samples from intracranial LVO were collected during the procedure. The inclusion criteria were as follows: (1) adults (18 years or older), (2) AIS due to LVO in the anterior or posterior circulation, (3) pre-stroke modified Rankin Scale score 0–2, (4) time from onset to groin puncture <24 hours, and (5) a known onset time of AIS. Exclusion criteria included concomitant intracranial hemorrhage before MT.

Study Variables

Baseline clinical data included age, sex, cardiovascular risk factors (e.g., arterial hypertension, diabetes mellitus, dyslipidemia, atrial fibrillation), history of coronary artery disease, history of transient ischemic attack (TIA) or stroke, smoking habits, drinking habits, baseline National Institutes of Health Stroke Scale (NIHSS), and time from onset to reperfusion. Baseline radiological data included the Alberta Stroke Program Early CT Score (ASPECTS) using non-contrast CT (NCCT-ASPECT) for patients with anterior circulation stroke. Collateral flow on CTA (sec. Tan et al.,[12]) was evaluated with a triphasic CT angiogram. Stroke etiology was determined according to the Trial of Org 10,172 in Acute Stroke Treatment (TOAST) classification.[13]

Study Endpoints

The primary endpoint was to detect and quantify Al, Cd, Ni, and Pb in thrombi retrieved MT.

Secondary endpoints included assessing the relationship between thrombus metal concentrations and clinical, radiological, and procedural variables, as well as comparing thrombus concentrations with published blood and plasma values to contextualize local metal accumulation.

Sample Collection

The retrieved thrombi were Formalin fixed and paraffin embedded (FFPE) immediately after MT was performed. Three-µm FFPE serial sections were used for both morphological and immunohistochemical evaluations. The cellularity (number of cells per field at 40X magnification, eosinophils count per field at 40X

magnification) and morphological characteristics of thrombi were evaluated on Haematoxylin/eosin-stained sections.

Immunohistochemistry Analysis

Immunohistochemical analyses were conducted to investigate the expression of molecules related to immunity (CD68, CD20). Briefly, FFPE sections underwent antigen retrieval with EDTA citrate pH 7.8 buffer using a pressure cooker system (TintoRetriever Pressure Cooker, Bio SB). Thereafter, sections were incubated for 1 h at room temperature with the following primary antibodies: a mouse monoclonal anti-CD68 antibody (pre-diluted; clone CD68-L-CE, Leica Biosystems, Wetzlar, Germany) and a mouse monoclonal anti-CD3 antibody (pre-diluted; clone CD3-565-L-CE, Leica Biosystems, Wetzlar, Germany). Washing was performed using PBS/Tween20 pH 7.6 and the reactions were revealed by the HRP-DAB Detection Kit (UCS Diagnostic, Rome, Italy). The immunoreaction was assessed by quantifying the number of positive cells per field at 40X magnification in 2 independent areas of each section.

Inductively Coupled Plasma—Mass Spectrometry (ICP-MS) Analysis

Four FFPE serial sections of 20 μm each were obtained from each sample. Sections were stored in 1,5 ml Eppendorf. Xylene was added and left overnight to allow paraffin melting. After its evaporation, xylene was added, and the process was repeated twice. Finally, several changes of pure ethanol were performed to remove paraffin residues and dry the samples. Dried tissues were weighed (mean dry weight: 0.0014 mg) and subjected to oxidative acid digestion using a 1:10 (v/v) mixture of hydrogen peroxide (H_2O_2) and nitric acid (HNO_3). Digests were brought to a final volume of 10 mL with ultrapure water. ICP-MS analysis was performed using an Agilent 7700 ICP-MS system (Agri-Bio-Eco Laboratori Riuniti S.R.L., in service). Quantitative analysis focused on Al, Cd, Ni, and Pb, selected based on their established relevance in cardiovascular and cerebrovascular pathology and their proven detectability in FFPE tissues using ICP-MS.[6,7,14,15] Metal concentrations were determined using the standard calibration curve method. Calibration curves were generated from certified standard solutions over concentration ranges of 0.01—50 μg/L for Pb, Cd, and Al, and 0.05—50 μg/L for Ni.

Analytical validation included the assessment of linearity, precision, and limits of quantification (LOQ). The LOQ, defined as the lowest analyte concentration quantified with a signal-to-noise ratio ≥10, was 0.071 μg/kg for Pb, Cd, Al, and 0.36 μg/kg for Ni. All calibration curves exhibited excellent linearity, with coefficients of determination (R^2) greater than 0.998.

Statistical Analysis

Bivariate correlations among all variables were assessed using Pearson's or Spearman's correlation coefficients, with Spearman's applied for non-normally distributed data as determined by the Shapiro-Wilk test. Analyses were conducted across the entire sample and within discrete groups characterized by specific combinations of metals (Al-Pb, Al-Pb-Cd, Al-Pb-Cd-Ni) to explore potential associations with clinical outcomes, cardiovascular risk factors, stroke etiology and occlusion site. To control for Type I error, the false discovery rate (FDR) was managed using the Benjamini-Hochberg procedure. Statistical significance was defined as a *P*-value <0.05, provided it fell below the critical value established by the procedure.

Comparative Analysis with Published Blood Concentrations

To contextualize the thrombus concentrations of Al, Cd, Ni, and Pb, we performed a descriptive comparison using reference blood and plasma values reported in the literature. These included a population-wide nutritional study for Cd, Pb,[16] a cohort study assessing plasma Al and Cd in AIS patients[17] and a recent profiling study of plasma toxic metals, including Al, Ni, and Pb.[18] As no matched peripheral blood samples were available from our cohort, direct statistical correlation was not feasible. Instead, a descriptive comparison was conducted, whereby thrombus concentrations (expressed in μg/g of dry tissue) were qualitatively compared with reported systemic levels (μg/L), using mean or median values as appropriate. To ensure a valid comparison, the plasma and whole-blood concentrations from the literature, originally reported in μg/L, were converted to μg/g. This conversion was performed using the standard densities of 1.025 g/mL for plasma and 1.06 g/mL for whole blood.

RESULTS

Study Population

The characteristics of the study population are summarized in **Table 1**.

The distribution of stroke etiology according to the TOAST classification and anatomical site of occlusion in the study population are summarized in **Table 2**.

Histological Analysis

Morphological and immunohistochemical investigations showed a great variability in thrombi composition particularly in terms of cellularity (**Figure 1**), namely the number of neutrophils (**Figure1A,B**), eosinophils (**Figure 1B,C**), lymphocytes (**Figure 1D,E**), and macrophages (**Figure 1F,G**), as well as the presence and distribution of fibrin. No association between histological features and clinical outcomes was observed,

Table 1. Clinical Features of the Study Population

Clinical Data	
Age (y.o.)	73,43 ± 13,25
Sex (F), n (%)	31 (52.5%)
History of Atrial Fibrillation, n (%)	14 (23.7%)
History of Arterial Hypertension, n (%)	47 (79.7%)
Alcohol abuse, n (%)	4 (6.8%)
Smoking >10y, n (%)	15 (25.4%)
Diabetes, n (%)	18 (30.5%)
Hypercholesterolemia, n (%)	18 (30.5%)
Coronary artery disease (CAD), n (%)	11 (18.6%)
History of TIA or stroke, n (%)	13 (22%)

Table 2. Stroke Etiology (TOAST) and Site of Occlusion of the Study Population

TOAST	
Large-artery atherosclerosis (LAA)	6 (10.2%)
Cardioembolism (CE)	28 (47.5%)
Other (dissection)	6 (10.2%)
Other (neoplasia)	1 (1.7%)
Other (aortic plaque ulcerated)	1 (1.7%)
ESUS	9 (15.3%)
UD (Incomplete follow up)	8 (13.6%)
Site of occlusion	
Middle cerebral artery (MCA)	29 (49.2%)
Anterior cerebral artery (ACA)	1 (1.7%)
Internal Carotid Artery intra and extra-cranial tract (ICA)	2 (3.4%)
Tandem occlusion (ICA extra-cranial + MCA)	8 (13.6%)
T-Occlusion	6 (10.2%)
L-Occlusion (ICA i.c. + MCA)	6 (10.2%)
Basilar artery (BA)	6 (10.2%)
Posterior cerebral artery (PCA)	1 (1.7%)

although this may be attributed to the limited number of samples collected.

Metal Concentrations in Retrieved Thrombi

Quantitative analysis using ICP-MS detected Al, Cd, Ni, and Pb in all thrombi analyzed (N = 59). Concentrations are reported as micrograms per gram (μg/g) of dry thrombus tissue. Summary statistics are presented in Table 3. Aluminum concentrations showed the highest overall levels, with a mean of 201.4 μg/g and values ranging from 14.0 to 771.0 μg/g. Cd concentrations were markedly lower, with a median of 0.23 μg/g and a maximum of 1.6 μg/g. Nickel displayed considerable variability, including a right-skewed distribution with outlier values up to 715.0 μg/g (median: 2.00 μg/g). Lead concentrations ranged from 1.0 to 180.0 μg/g, with a median of 19.0 μg/g.

Correlations Between Metals

Aluminum showed a significant correlation with Cd ($r = 0.733$; $P < 0.001$) and Ni ($r = 0.558$; $P = 0.001$); Cd exhibited strong correlations with Pb ($r = 0.784$; $P < 0.001$) and Al; Ni correlated significantly with Al; Pb showed strong correlations with Cd ($r = 0.784$; $P < 0.001$).

Correlations Between Outcomes and Metals

When considering the correlation between the presence of toxic metals in thrombi and clinical data, Cd was positively correlated with first-pass recanalization ($r = 0.648$, $P < 0.001$). Lead demonstrated a significant relationship with the NIHSS scores at onset ($r = 0.502$, $P < 0.001$), at 12 hours ($r = 0.502$, $P = 0.007$), and at 24 hours ($r = 0.349$, $P = 0.012$). Nickel was associated with ASPECTS on NCCT at 24 hours ($r = 0.525$, $P = 0.003$). No significant correlations were found between metals (singularly or in combination) and the modified Rankin Scale at 3 months.

Thrombi Containing Four Metals (Al, Cd, Ni, Pb)

When the results were categorized by subgroups based on the combination of metals detected in thrombi, all 4 investigated metals (Al, Cd, Ni, Pb) were simultaneously detected in the thrombi of 17 patients (29%). In this subgroup, Al negatively correlated with NIHSS scores at 12 hours ($r = -0.775$, $P < 0.001$) and positively correlated with Cd ($r = 0.724$, $P = 0.001$). Cadmium demonstrated strong correlations with Al ($r = 0.724$, $P = 0.001$) and Pb ($r = 0.805$, $P < 0.001$).

Thrombi Containing Three Metals (Al, Cd, Pb)

In the subgroup of 12 patients (20%) whose thrombi contained 3 metals (Al, Cd, and Pb), strong intercorrelations were observed among all 3 elements. Al correlated significantly with both Cd ($r = 0.880$, $P < 0.001$) and Pb ($r = 0.790$, $P = 0.002$), while Cd showed an even stronger correlation with Pb ($r = 0.963$, $P < 0.001$). Cadmium levels were also significantly associated with several clinical variables, including time from onset to recanalization ($r = 0.806$, $P = 0.002$), FPR success ($r = 0.803$, $P = 0.002$), and circulating monocyte count ($r = 0.875$, $P < 0.001$). Similarly, Pb concentrations correlated with onset-to-recanalization time ($r = 0.773$, $P = 0.003$), FPR ($r = 0.839$, $P = 0.001$), and monocyte levels ($r = 0.870$, $P = 0.001$).

Thrombi Containing 2 Metals (Al, Pb)

Last, in the thrombi of 14 patients (24%), 2 metals (Al, Pb) were simultaneously detected. In this subgroup, Al correlated strongly with Pb ($r = 0.780$, $P = 0.001$) and negatively with collateral circulation scores ($r = -0.781$, $P = 0.001$). Lead had a negative correlation with collateral circulation scores ($r = -0.973$, $P < 0.001$) and positive association with NIHSS scores at onset ($r = 0.823$, $P < 0.001$) and at 12 hours ($r = 0.762$, $P = 0.002$).

Comparative Analysis with Published Blood Concentrations

To contextualize thrombus metal concentrations, we performed a descriptive comparison with blood and plasma values reported in the stroke case groups of relevant literature sources (Table 4).[16-18] These included plasma and whole-blood concentrations of Al, Cd, Ni, and Pb from case data, excluding control populations. Plasma and whole-blood values, originally expressed in μg/L, were converted to μg/g using standard densities of 1.025 g/mL for plasma and 1.06 g/mL for whole blood. After conversion, thrombus concentrations (reported as mean and median) of all measured metals were markedly higher than the corresponding blood/plasma values. For example, thrombus Al levels (mean 201.35 μg/g; median 180.50 μg/g) exceeded plasma levels reported by Wen et al. (0.049 μg/g)[17] and Yuan et al. (0.038 μg/g)[18] by more than 3 orders of magnitude. Cadmium levels in thrombi (mean 0.38 μg/g; median 0.23 μg/g) were substantially higher than plasma values from Wen et al. (0.000088 μg/g)[17] and whole-blood values from Huang et al. (0.00037 μg/g).[16] Similarly, thrombus Nickel concentrations (mean 15.92 μg/g; median 2.00 μg/g) far exceeded the plasma value reported by Yuan

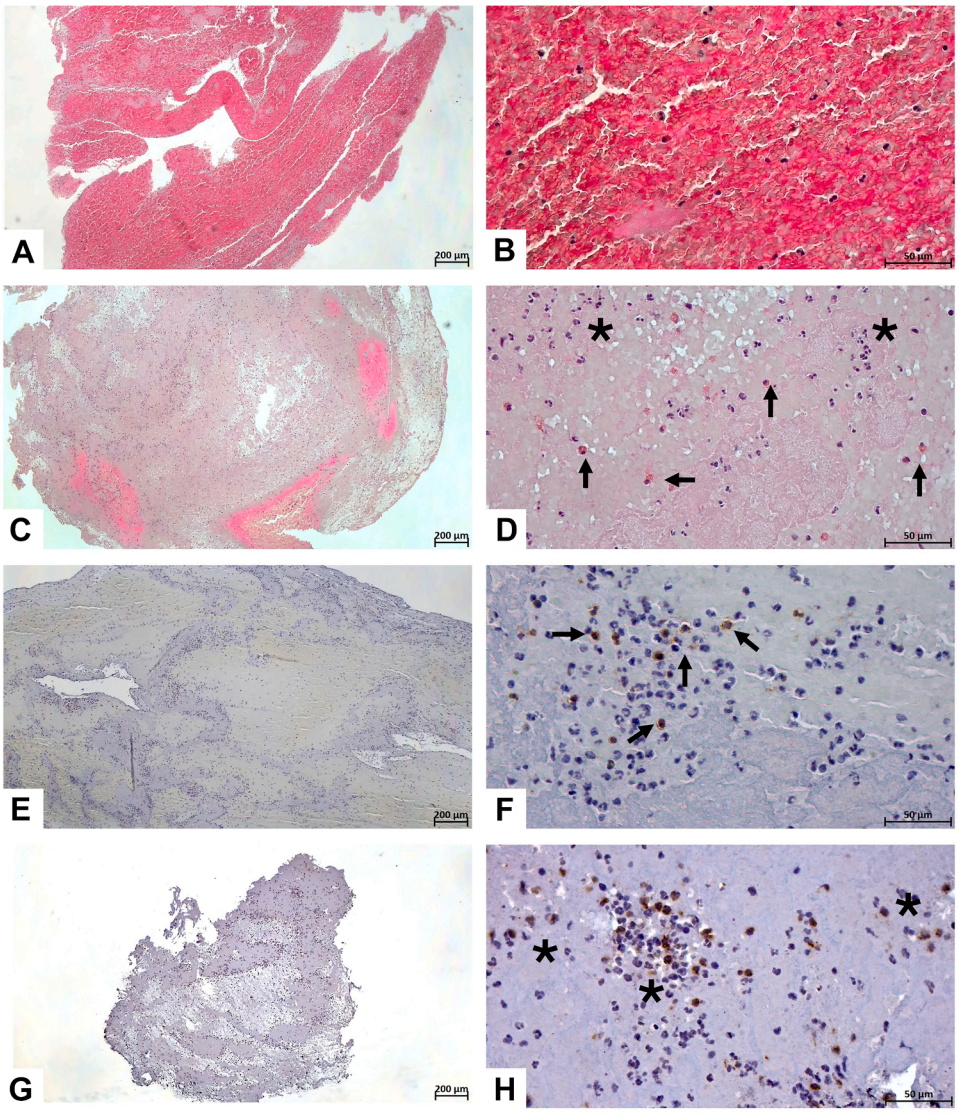

Figure 1. Histological and immunohistochemical analysis. (**A**) Image shows a thrombus characterized by low cellularity. (**B**) High-magnification view of panel A reveals the presence of neutrophils placed among red blood cells. (**C**) A thrombus with high cellularity. (**D**) High magnification of panel C shows the presence of both neutrophils (asterisks) and eosinophils (arrows). (**E**) A thrombus with moderate cellularity. (**F**) High magnification of panel E diplays numerous CD3 positive lymphocytes (arrows). (**G**) A thrombus with areas of high cellularity. (**H**) High magnification of panel G shows numerous CD68 positive macrophages (asterisks). A,C,E,G (Magnification 5x – scale bar 200 µm) B,D,F,H (Magnification 40x – scale bar 50 µm).

et al. (0.00081 µg/g).[18] Lead levels in thrombi (mean 32.19 µg/g; median 19.00 µg/g) were also several orders of magnitude higher than plasma (0.00100 µg/g)[18] and whole-blood values (0.00113 µg/g)[16] from stroke patients.

DISCUSSION

Endovascular procedures have enabled detailed analyses of thrombi retrieved during MT, and ICP-MS provides insights into thrombus composition, including toxic metal detection. To our knowledge, this is the first study to examine the presence of toxic metals in thrombi with clinical and radiological outcomes in AIS patients undergoing MT. The correlations found between metal concentrations in thrombi of AIS patients included clinical, radiological, and interventional parameters such as AIS severity at presentation, leptmeningeal collateral scores and FPR success rate.

The growing evidence that toxic metals exposure represents a critical issue for human health[19–21] has led to increased efforts to

Table 3. Descriptive Statistics for Metal Concentrations in Cerebral Thrombi (μg/G dry Weight)

Parameter	Al	Cd	Ni	Pb
Number of samples (n)	59	59	59	59
Mean concentration	201.35	0.38	15.92	32.19
Standard deviation (SD)	161.43	0.34	91.94	38.21
Minimum	14.00	0.10	0.10	1.00
Median (50th percentile)	180.50	0.22	2.00	19.00
Maximum	771.00	1.60	715.00	180.00

investigate how pollutants impact processes involved in cardiovascular diseases.[6,15] Several studies previously demonstrated an association between Al, Pb and Cd serum levels and increased AIS risk.[2,17] However, while those studies explored serum levels, our investigation uniquely addressed the presence of toxic metals within the thrombi retrieved during MT and demonstrating their concentration inside the clot.

In recent investigations, ICP-MS analysis from FFPE has been associated with the occurrence of several diseases including non-communicable ones such as atherosclerosis and cancer. This technique is known to be a highly sensitive technology, useful in the detection of trace elements into histological specimens.[22,23] The application of ICP-MS on FFPE samples has been recently validated in the study of Coyte et al.[24] In fact, by comparing the ICP-MS results from FFPE and fresh tissues, the authors showed that the histological preparation of samples does not significantly change the concentration of metals, including Al, Pb, Cd, and Ni, in the tissues. From a pathophysiological perspective, all metals are known to exert neurovascular toxicity through mechanisms including oxidative stress, endothelial dysfunction, and pro-thrombotic effects. Lead has been consistently associated with increased risk of stroke and vascular inflammation through disruption of calcium-dependent processes in endothelial cells and platelets.[25,26] Cadmium is implicated in promoting atherosclerosis and microvascular damage, particularly via its long biological half-life and accumulation in vascular tissues.[27,28] Nickel exposure has been linked to systemic inflammation, endothelial activation, and modulation of lipid metabolism, all of which are relevant to cerebrovascular pathogenesis.[29,30] Lastly, Al, although less studied in CVDs, has neurotoxic properties and has been associated with blood-brain barrier disruption and neuronal degeneration, making it of particular interest in brain pathology.[31]

Table 4. Comparison Between Thrombus Toxic Metal Concentrations and Blood/Plasma Levels From the Literature. Reference Values are Expressed in μg/g

Metal	Thrombus, Mean[15]	Thrombus, Median[15]	Plasma, Mean[17]	Plasma, Median[18]	Blood, Mean[16]
Al	201.35	180.50	4.9×10^{-2}	3.8×10^{-2}	/
Cd	0.38	0.23	8.8×10^{-5}	/	3.7×10^{-4}
Ni	15.92	2.00	/	8.1×10^{-4}	/
Pb	32.19	19.00	/	1×10^{-3}	1.13×10^{-3}

This Comparison is Descriptive and not Adjusted for Matrix Differences.

Notheworty, these metals were also selected based on their proven detectability in FFPE tissue using ICP-MS, as demonstrated in the work of Coyte et al. [19], thus ensuring technical feasibility and reliability of the measurements. While other environmental metals may also play a role in vascular disease, our choice was narrowed to these 4 metals due to their strong prior association with stroke-related mechanisms and their established analytical performance in fFFPE material.

Moreover, the presence of toxic metals in thrombi suggests that they may serve as carriers, transporting these elements to the brain at high concentrations. Notably, the concentration of Al in thrombi was more than ten times higher than that recently observed in our previous study on colon cancer tissues,[20,23] suggesting that, once the thrombus reaches the brain, it could maintain this metal into the local microenvironment. This is particularly concerning given the well-established neurotoxicity of Al and its association with neurodegenerative diseases such as Parkinson's and Alzheimer's.[32,33] These findings point to a potentially underrecognized role of toxic metals in AIS outcomes.

In this study, Cd and Pb, in particular, appeared to impact recanalization efficacy and stroke progression, as reflected in correlations with FPR and NIHSS scores at onset and early follow-up. Both metals are known to exert pro-thrombotic, pro-inflammatory, and neurotoxic effects. Cadmium has been shown to promote oxidative stress by generating reactive oxygen species (ROS) and depleting intracellular antioxidants such as glutathione, leading to endothelial dysfunction, increased vascular permeability, and enhanced platelet activation.[34,35] These alterations may contribute to the formation of more resistant thrombi and impact microcirculatory reperfusion even after successful MT. Lead, similarly, interferes with calcium-dependent processes in vascular smooth muscle and platelets, disrupting vascular tone and promoting vasoconstriction.[36,37] It also impairs nitric oxide signaling, contributing to endothelial dysfunction, and induces a low-grade chronic inflammatory state that supports thrombus stability and impairs endogenous fibrinolysis.[38]

Clots rich in platelet and fibrin are more difficult to retrieve during mechanical thrombectomy procedures compared to platelet-poor clots as they exhibit a higher stiffness, defined as resistance to deformation in response to an applied force; stiffness is highly related to the fibrin and platelet content of thrombi.[39] Similarly, every material (including metals) exhibits a certain stiffness. Whether the amount of metal in a clot can influence its elastic properties and stiffness is beyond the scope of this preliminary work; however, comparing the stiffness of clots with similar cellular composition and different heavy metals content might be a topic for future research and have translational implications on the identification of metal-rich clots on computed tomography and the choice of proper thrombectomy strategies.

Strong correlations among metals (e.g., Al-Cd, Ni-Al, Cd-Pb) suggest a common origin, most probably linked to

environmental, occupational or dietary exposures. Indeed, smoking and alcohol consumption, major cardiovascular risk factors, also correlated with metal concentrations.[2] Therefore, to further contextualize our findings, we compared thrombus metal concentrations with plasma and whole-blood values reported in AIS patients from previous studies, converting published data from μg/L to μg/g using standard densities (1.025 g/mL for plasma and 1.06 g/mL for whole blood). This descriptive comparison revealed that in all cases, concentrations of Al, Cd, Ni, and Pb in thrombi were markedly higher—often by several orders of magnitude—than corresponding circulating levels.

This observation suggests that toxic metals not only circulate systemically but may also actively accumulate within the thrombus microenvironment. The enrichment of metals within thrombi raises the hypothesis that they may contribute directly to thrombus formation or stabilization, rather than being mere passive markers of environmental exposure. Their elevated presence within the clot compared to blood supports a potential mechanistic role in thrombogenesis, perhaps by promoting local oxidative stress, endothelial injury, or enhanced platelet activation at the site of thrombus formation.

Moreover, this finding underscores the added value of thrombus analysis over traditional blood-based toxicological approaches. While circulating metal levels provide insight into systemic exposure, the direct quantification of metals within thrombi offers a novel and potentially more relevant measure of their involvement in AIS. The possible link between the presence of toxic metals in thrombi and their clinical outcome could be the capability of these metals to dysregulate biological processes such as oxidative stress[14,40] and inflammation.[41,42] It has been demonstrated that oxidative stress can impair the function of cells involved in thrombus formation, including vascular endothelial cells, platelets, and red blood cells, thereby triggering a cascade of events leading to thrombogenesis.[43] Similarly, inflammation plays a crucial role in promoting a pro-thrombotic environment. The synergy between inflammation and thrombosis drives thrombotic diseases, as inflammatory mediators such as cytokines and adhesion molecules enhance platelet activation and endothelial dysfunction, further amplifying thrombus formation and stability.[44]

Patients whose thrombi are included in this paper come from the same metropolitan area and therefore we assume a similar exposition to these specific metals; at the same time, it is extremely difficult to investigate retrospectively the environmental exposure to certain substances such as heavy metals in this specific case. Studies have shown that 14 to 17% of cropland is affected by toxic metal pollution globally and up to 1.4 billion people are potentially daily-exposed to metals like cadmium, nickel and lead[45]; the relevance of this topic and the insight of metals accumulation in intracranial clots further emphasize the impact of heavy metals on human health and paves the way for further research.

LIMITS

Our study has several limitations that should be considered. The relatively small cohort of 59 patients, limits generalizability and statistical power. However, it offers initial insight into a previously unexplored aspect of stroke biology as the presence of toxic metals within intracranial thrombi. The associations found between these metals and clinical/radiological variables suggest that metal bioaccumulation could influence thrombus behavior, recanalization efficacy, and early stroke severity. The absence of a control group on toxic metal circulating concertation restricts comparisons and broader implications regarding toxic metals in thrombi. Additionally, individual exposure levels (e.g., blood or urine measurements) were not assessed, and potentially relevant metals like Hg and As were not analyzed, despite their known links to AIS. Moreover, functional assays were not performed to demonstrate how toxic metals promote thrombus formation or affect outcomes, and other cardiovascular risk factors or comorbidities that might influence metal levels were not fully accounted for. The lack of correlation between the metal burden and the histological composition of the thrombus in fibrin, platelet and red blood cells is a further limitation and might be addressed in future research. Lastly, our findings highlight the relevance of toxic metals in AIS outcome addressing environmental, occupational, and dietary sources of metal exposure may be crucial for mitigating their contribution to AIS pathogenesis. However, the potential sources of toxic metals, along with the numerous ways through which they enter the body, make it challenging to identify specific contaminants that can definitively explain their presence in thrombi. Future research should include larger cohorts, detailed exposure assessments, and functional analyses to address these limitations and advance our understanding of toxic metals in AIS pathophysiology.

CONCLUSION

Our analysis suggests that multiple concurrent metal exposures may exert cumulative or even synergistic effects on thrombus structure and clinical outcome. The observation that clinical associations varied depending on the specific metal combinations supports the hypothesis that not all exposures carry equal biological weight, and that their interactive effects may be more relevant than their individual concentrations. Preliminary findings suggest a correlation of metal burden in clots with different clinical and radiological features of AIS and need to be further investigated in larger cohorts. The early identification of metal-rich thrombi on computed tomography and the development of dedicated thrombectomy devices are just some possible research scenarios: the translational impact of the research is promising, with potential effects on both the diagnostic and interventional steps of AIS.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Manuel Scimeca: Funding acquisition, Investigation, Methodology, Project administration, Validation, Writing — original draft, Writing — review & editing. **Alessandro Mauriello:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing — original draft, Writing — review & editing. **Thanh N. Nguyen:** Methodology, Validation, Writing — review & editing. **Marco Nezzo:** Investigation. **Federico Sabuzi:** Investigation, Writing — review & editing. **Renato Argirò:** Investigation. **Daniele Morosetti:** Investigation. **Mariafrancesca Trulli:** Data curation, Investigation, Writing — review & editing. **Lorenzo Rocchi:** Data curation, Formal analysis, Investigation, Methodology, Validation, Writing — original draft. **Ilaria Maestrini:** Data curation, Investigation. **Valeria Palumbo:** Data curation, Investigation, Writing — review & editing. **Giovanni Maria**

D'Amico: Data curation, Investigation. **Michele Treglia:** Data curation, Investigation. **Francesca Di Giuliano:** Investigation. **Jacopo Troisi:** Data curation, Investigation, Validation. **Francesco Garaci:** Investigation, Supervision, Validation. **Valerio Da Ros:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing — original draft, Writing — review & editing.

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