New Biomarkers in Predicting Intracerebral Hemorrhage Expansion: Cell-Free DNA Levels and Noncontrast Computed Tomography Markers

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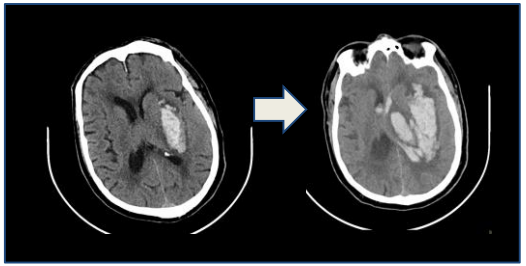
HEMATOMA EXPANSION

- **Hematoma Expansion (HE):**
  - Occurs in 20-30% of patients with intracerebral hemorrhage (ICH) within 24h of the onset

  Mostly within the first 3 h after onset

  - A major determinant of poor functional outcomes and increased mortality.



Baseline hematoma volume, NCCT markers and spot signs are associated with increased **risk of HE**

Intracerebral haemorrhage expansion: definitions, predictors, and prevention. Morotti, Andrea et al. The Lancet Neurology
Greenberg SM, Ziai WC, Cordonnier C, et al. 2022 Guideline for the Management of Patients With Spontaneous Intracerebral Hemorrhage: A Guideline From the American Heart Association/American Stroke Association.
Davis SM, Broderick J, Hennerici M, et al. Hematoma growth is a determinant of mortality and poor outcome after intracerebral hemorrhage. Neurology. 2006

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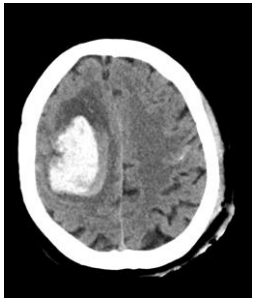
NCCT MARKERS AS PREDICTORS OF HEMATOMA EXPANSION

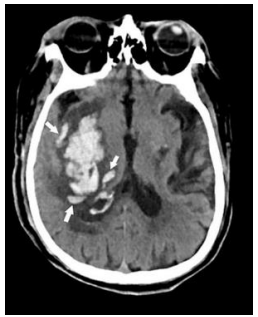
Blend sign

Mixed-density hematoma with two well-defined components (a relatively hypoattenuating area and an adjacent hyperattenuating region)



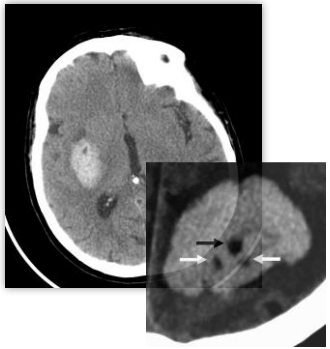
Island Sign

≥3 scattered small hematomas all separate from the main hematoma or (2) ≥4 small hematomas some or all of which may connect with the main hematoma.



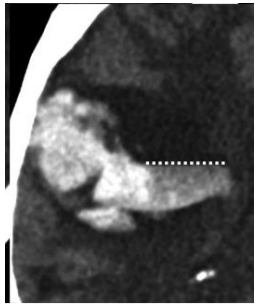
Black hole

Hypoattenuating area encapsulated within the hyperattenuating hematoma with a clearly defined border



Fluid Level

presence of 1 distinct hypoattenuating area (hypodense to the brain) above 1 hyperattenuating area (hyperdense to the brain), below a discrete straight line of separation



Standards for Detecting, Interpreting, and Reporting Noncontrast Computed Tomographic Markers of Intracerebral Hemorrhage Expansion. Morotti A et al Ann Neurol. 2019
NCCT Markers of Intracerebral Hemorrhage Expansion Using Revised Criteria: An External Validation of Their Predictive Accuracy. C. Ducroux et al. AJNR Am J Neuroradiol. 2023

Island Sign: An Imaging Predictor for Early Hematoma Expansion and Poor Outcome in Patients With Intracerebral

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Background of Cell-Free DNA (cfDNA) in Stroke

Fragments of DNA released into the bloodstream from dying or damaged cells. cfDNA levels rise due to: **Cell death (necrosis or apoptosis)** in brain tissue. **Blood-brain barrier disruption**, allowing cfDNA to enter the circulation.

- **Stroke**: Both **ischemic** and **hemorrhagic strokes** lead to significant cell damage, contributing to elevated cfDNA levels in the blood.
- **Clinical Significance**:
  - Elevated cfDNA has been associated with **stroke severity**, **infarct size**, and **poor prognosis**.
  - cfDNA can serve as a **biomarker** for monitoring tissue damage and predicting outcomes like **hematoma expansion** in hemorrhagic stroke.
- **Emerging Use**:
  - Potential for **early detection**, prognosis, and guiding therapeutic interventions.

Cell-free DNA as a biomarker in stroke: Current status, problems and perspectives Kristina V. Glebova et al. Critical Reviews in Clinical Laboratory Sciences 2018

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Objectives

- To assess the predictive value of Non-Contrast CT (NCCT) markers and cf-DNA levels for HE in ICH patients.
- Determine the role of these biomarkers in early risk stratification for HE.

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Methods and Materials

Study Design

- **Prospective single-center study** involving **consecutive patients** with intracerebral hemorrhage (ICH) within **<12 hours of onset** and follow up CT scan
- **Participants**: 182 consecutive patients (mean age 71.6 ±12.89 years, 39% female).
- **Definition of Hematoma Expansion (HE)**: Increase in ICH volume **>6mL** and/or **>33%** between baseline and 24-hour follow-up CT scans.

Neuroimaging (NCCT Markers)

- **Evaluated by vascular neurologists** blinded to patient outcomes.
- **Non-contrast CT (NCCT) markers** assessed:
  - **Island Sign**
  - **Black Hole Sign**
  - **Fluid Level**
  - **Blend Sign**
- NCCT markers were evaluated in admission cranial CT scans to assess their predictive value for HE.

Laboratory Measures (Blood Samples)

- **Plasma cf-DNA concentration** at admission was measured using the **Quant-iT PicoGreen dsDNA kit**.
- **cf-DNA levels** were correlated with hematoma expansion and compared between patients with and without HE.

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Hematoma Expansion was observed in 27.5% of the patients

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Results: Demographic Characteristics, Comorbidities

	No Hematoma Expansion (N: 132)	Hematoma Expansion (N: 50)	p-value
Age (years) n %	70.5 (61.0-79.0)	81.0 (71.9-85.0)	0.013*
Sex (Female) n %	46 (34.8%)	25 (50.0%)	0.062

	No Hematoma Expansion (N: 132)	Hematoma Expansion (N: 50)	p-value
Hypertension History n %	102 (77.3%)	34 (68.0%)	0.202
Diabetes Mellitus n %	29 (22.0%)	11 (22.0%)	1.000
Smoking (Current) n %	30 (23.6%)	6 (12.0%)	0.222

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Results: Clinical and prognosis data

		No Hematoma Expansion (N: 132)	Hematoma Expansion (N: 50)	p-value
NIHSS Baseline median IQR		10.7 (5-16)	14.5 (9-20)	0.002*
NIHSS at 24h median IQR		9.89 (3-16)	17.8 (13-22)	<0.01*
In-hospital Mortality n %		11 (8.3%)	12 (24.0%)	0.004*
90-day Mortality n %		17 (13.2%)	18 (39.1%)	<0.01*
mRS 90d (Score 4-6) n %		33 (25%)	23 (46%)	0.020*

HE significantly increases both short-term (in-hospital) and long-term (90-day) mortality.

Worse functional outcomes (mRS scores) at 90 days.

Neuroimaging Characteristics		No Hematoma Expansion	Hematoma Expansion	p-value
Spot Sign on CT n %		12 (10.5%)	16 (39.0%)	<0.01*
Lobar Hemorrhage Location n %		34 (26.0%)	22 (44.9%)	0.01*
Ventricular Invasion n %		35 (26.5%)	13 (26.5%)	1.00
Volume of Hematoma (Baseline) Median SD		14.8 (23.5)	32.2 (26.2)	<0.01*
Blend Sign n %		17(12.9%)	16 (32.0%)	<0.01*
Black Hole Sign n %		25 (18.9%)	14 (28.0%)	0.18
Island Sign n %		10 (7.6%)	14 (28.0%)	<0.01*
Fluid Level n %		2 (1.5%)	0 (0%)	0.38
Volume of Hematoma (24h) Median SD		14.3 (20.2)	48.8 (34.8)	<0.01*

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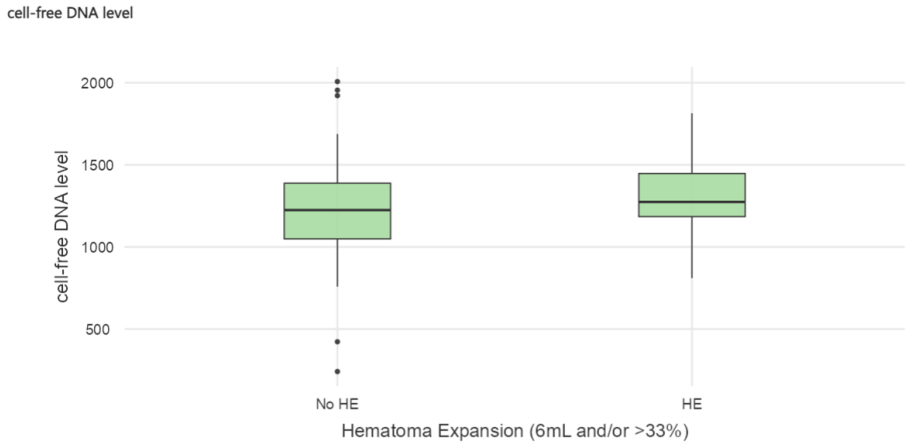
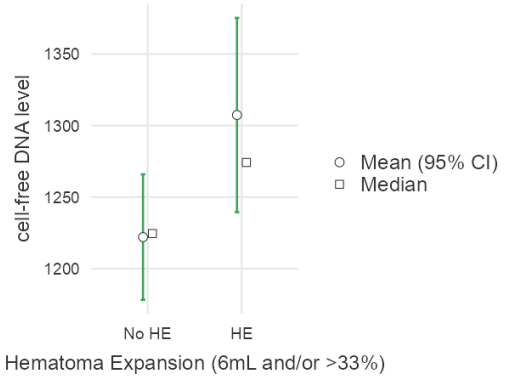
Results cf-DNA levels





Descriptives

	Hematoma Expansion (6mL and/or >33%)	N	Mean	Median	SD	Minimum	Maximum
cell-free DNA level	No HE	132	1222	1225	257	241	2007
	HE	50	1307	1274	245	810	1814

Figure 1. Cell-free DNA levels stratified by hematoma expansion status. The mean and median levels are displayed along with their 95% confidence intervals. The cell-free DNA level is higher in the hematoma expansion group. **A Mann-Whitney U test was used, yielding U = 2698 and p = 0.029.**

Association between NCCT Markers and Hematoma Expansion (HE)

Hematoma Expansion (6mL and/or >33%)	No HE	HE	Total
NCCT markers Absence	92	18	110
NCCT markers Presence	40	32	72
Total	132	50	182

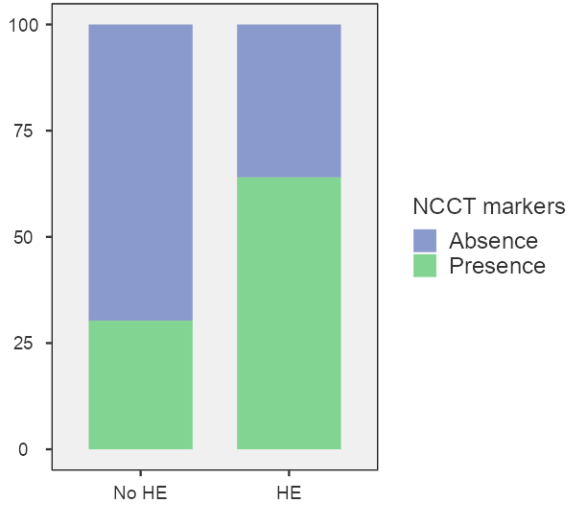
$\chi^2 = 17.2$, df = 1, $p < 0.001$

	Value	95% Confidence Interval
Odds Ratio (HE vs No HE)	4.09	(2.06 - 8.12)

There is a significant association between NCCT markers and hematoma expansion.

Patients with NCCT markers are 4 times more likely to experience HE.

Distribution of NCCT Markers by HE



The **presence** of NCCT markers is much higher in the group with **HE** compared to the group without HE.

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Multivariate Analysis for Hematoma Expansion Predictors

Predictor	OR	95% CI (Lower)	95% CI (Upper)	p-value
Cell-free DNA level	1.0017	1.00025	1.0032	0.021*
NCCT markers	2.6836	1.21508	5.9268	0.015*
ICH Basal Volume	1.0138	0.99684	1.0311	0.111
NIHSS	1.0383	0.98023	1.0997	0.201
Sex	1.6805	0.80978	3.4876	0.163

Predicting Hematoma Expansion using cf DNA levels and NCCT markers. The two most important predictors in this model are cell-free DNA levels and the presence of NCCT markers, both showing a statistically significant association with hematoma expansion. Other factors like ICH basal volume, stroke severity (NIHSS), and sex showed trends but were not statistically significant.

Cell-free DNA levels independent predictors of HE (p = 0.021, OR = 1.0017).

Presence of NCCT markers is a strong predictor for hematoma expansion OR of 2.68 (p = 0.015).

ICH Basal Volume, NIHSS Score, and Sex were not significant predictors in this model (p > 0.05).

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Conclusions

- Elevated **cf-DNA levels** and the presence of **NCCT markers** are significantly associated with **hematoma expansion (HE)** in patients with intracerebral hemorrhage (ICH).

- **NCCT markers** (Island Sign, Black Hole, Fluid Level, Blend Sign) and **cf-DNA levels** serve as **independent predictors** of HE.

- Combining both **radiological** and **biomolecular** markers improves the ability to predict HE, aiding in **early risk stratification** and potentially guiding therapeutic decisions.

- Early identification of patients at risk for HE is crucial for **improving outcomes** and reducing mortality in ICH.

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Discussion

The combination of **NCCT markers** and **cf-DNA levels** offers an approach to predict **HE**, which could lead to earlier and more targeted interventions.

Strengths:

- Prospective design with **blinded vascular neurologists**.
- Integration of **imaging** and **biomarker** data to enhances predictive accuracy.

Limitations:

- Single-center study, limiting **generalizability**.
- Further validation needed in **multi-center** and **larger population** studies.

Future Directions:

- Exploring the **synergistic role** of other biomarkers.
- Investigating interventions to **prevent HE** in patients identified at high risk through these markers.

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