Circulating miRNAs as potential biomarkers of haematoma growth in intracerebral haemorrhage: a pilot study

Maria Lucas Parra, Carme Gubern Mérida, Carla Vera Cáceres, Laia Carballo Perich, Mikel Terceño Izaga, Saima Bashir Viturro, Tomàs Xuclà Ferrarons, Juan Álvarez Cienfuegos Rodriguez, Joaquín Serena Leal, Yolanda Silva Blas.

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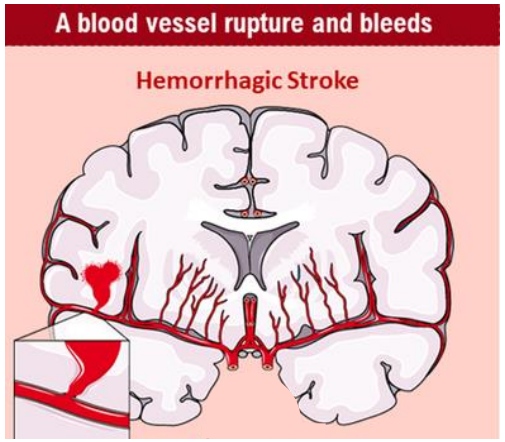
Haemorrhagic stroke

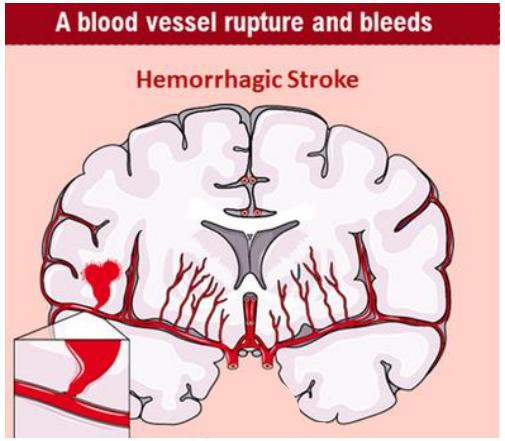


Modified from Tartari Neves et al., 2023.

Haemorrhagic stroke represents the highest mortality and morbidity rate:

- Mortality of **40%** of patients die in the **first month.**
- Only **1/3** of patients are **independent** after **one year.**

Haemorrhagic stroke



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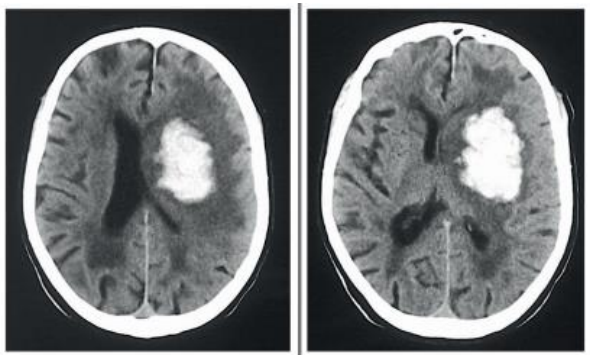

Extracted from Silva-Candal et al., 2021.

Haemorrhagic stroke represents the highest mortality and morbidity rate:

- Mortality of **40%** of patients die in the **first month.**
- Only **1/3** of patients are **independent** after **one year.**

HAEMATOMA GROWTH

Main cause of early neurological deterioration and poor clinical outcome.

Haematoma growth

- Occurs in 38% of patients.
- Onset within 24 hours (occasionally beyond 72h).
- Unknown cause.

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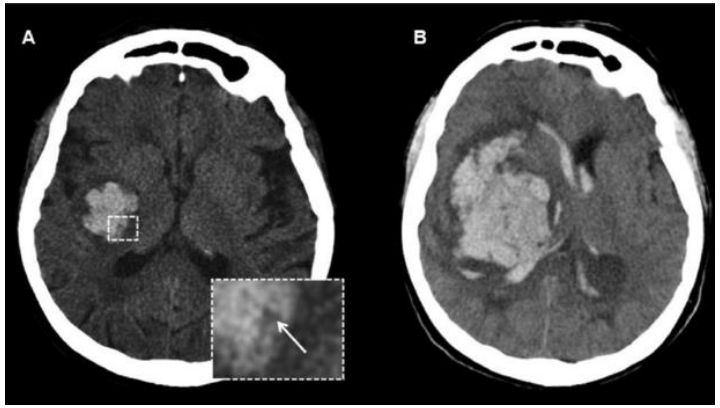
BIOMARKERS

Helpful in predicting patient's prognosis and optimising acute phase management.

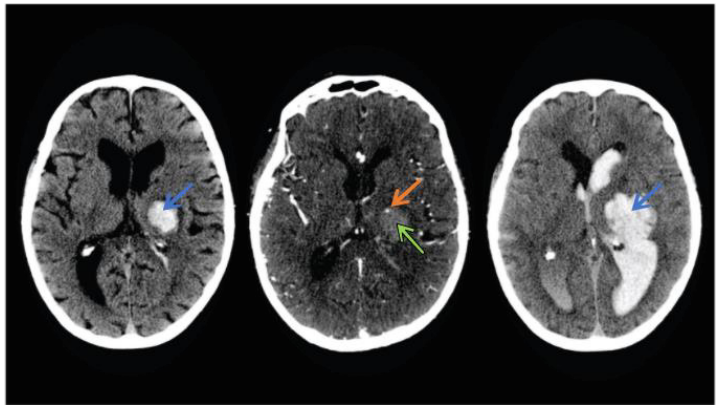
Predictive radiomarkers of haematoma growth

Hypodensity (CT)



Extracted from Morotti & Goldstein, 2020.

Spot Sign (CTA)



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Predictive radiomarkers of haematoma growth

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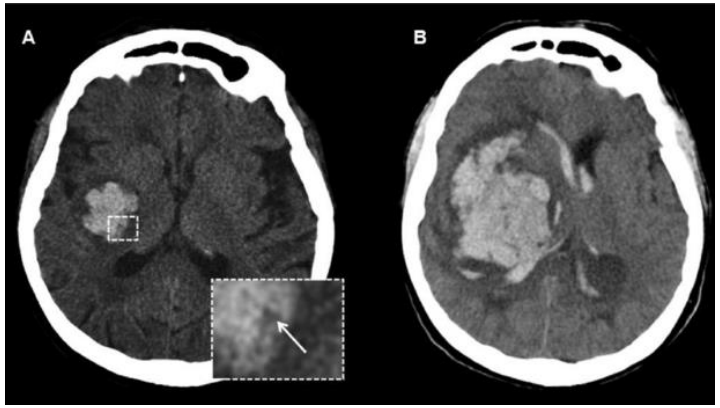

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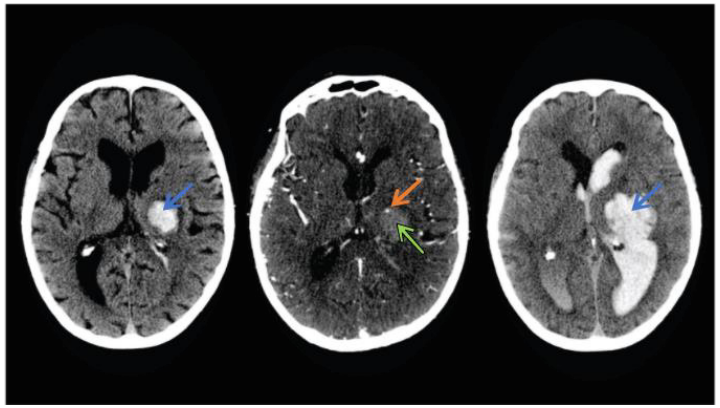

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- Measurement subject to **human variability**
- **Limited** sensitivity and/or specificity
- CTA **not available** in all centres

Predictive radiomarkers of haematoma growth

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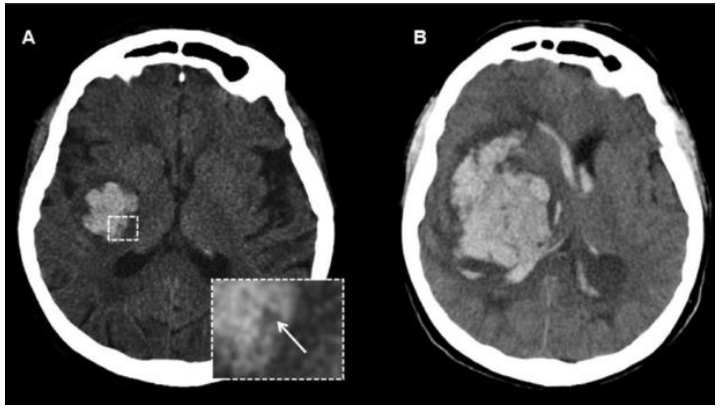

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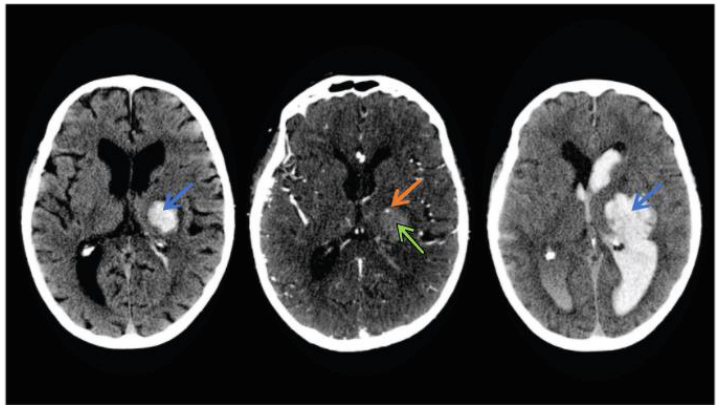

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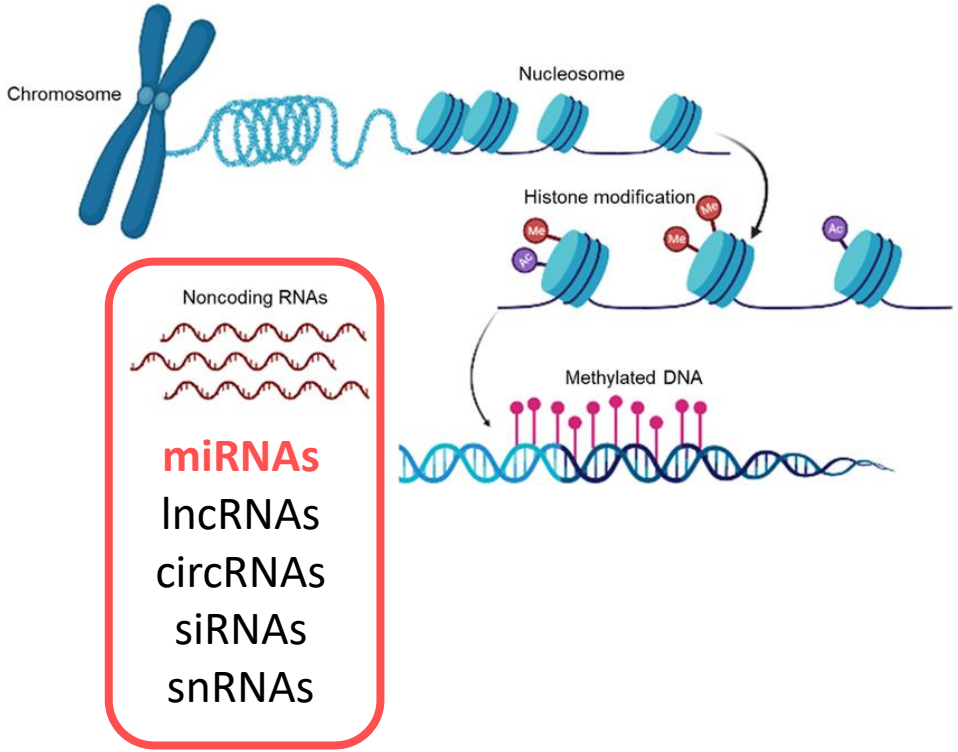
Molecular Biomarkers

Molecular biomarkers: miRNAs

Epigenetics mechanisms



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Molecular biomarkers: miRNAs

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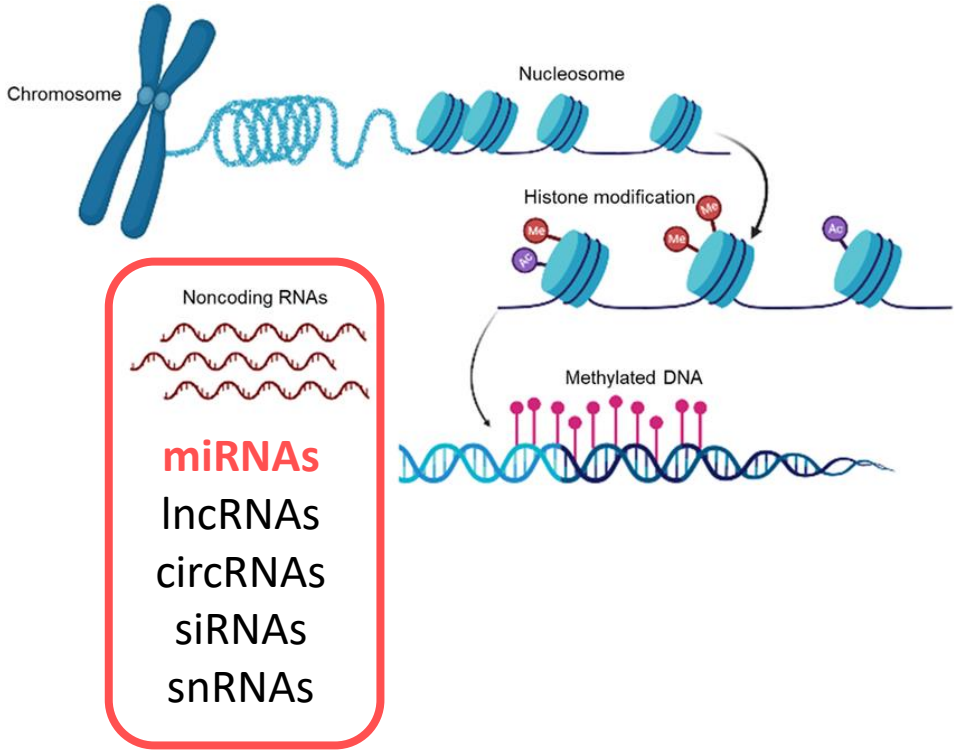

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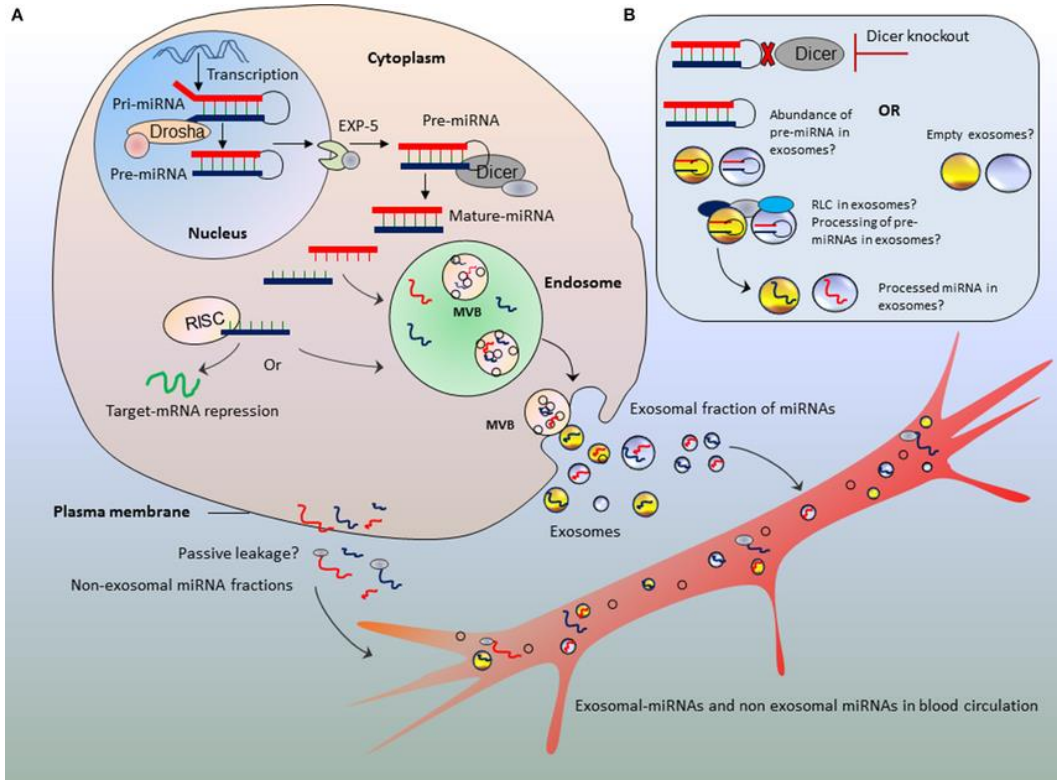
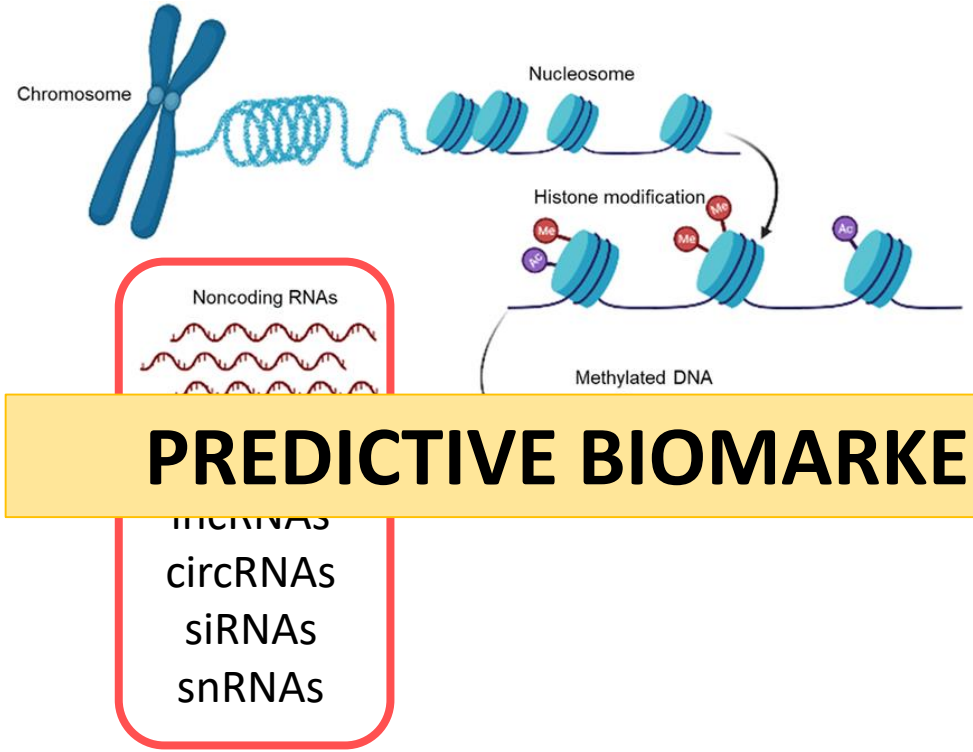
miRNAs are released to circulation



Extracted from Fatima & Nawaz, 2017.

Molecular biomarkers: miRNAs

Epigenetics mechanisms



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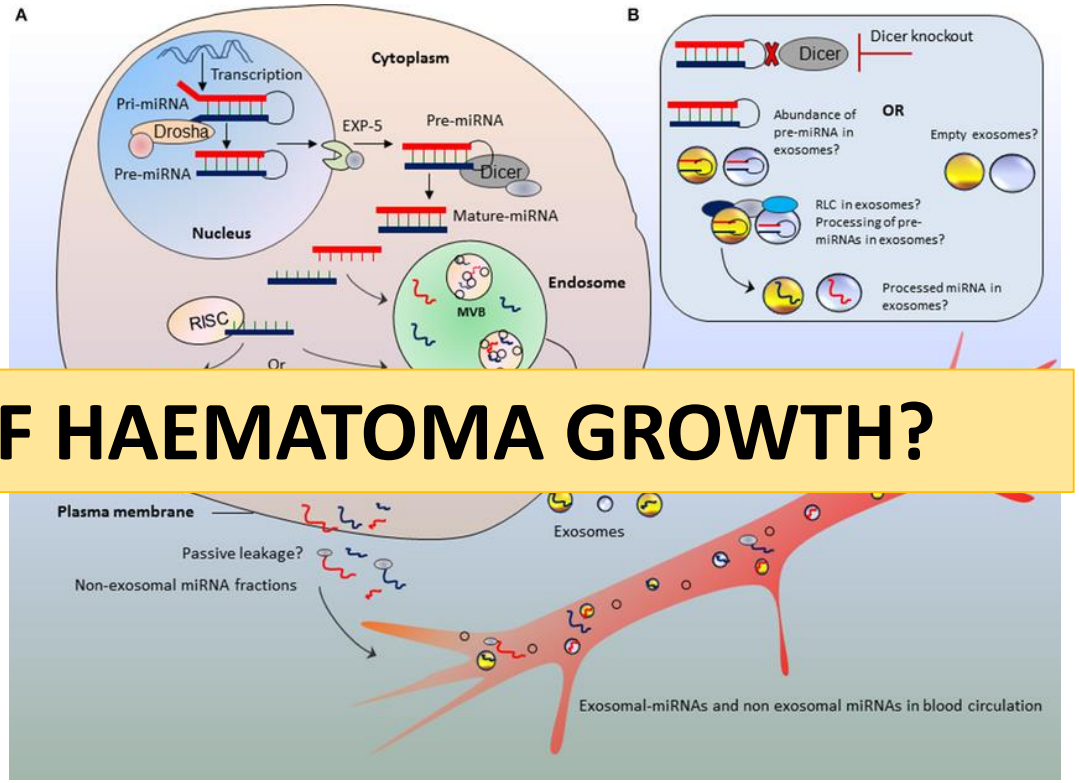

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PREDICTIVE BIOMARKERS OF HAEMATOMA GROWTH?

HYPOTHESIS AND OBJECTIVES

Hypothesis

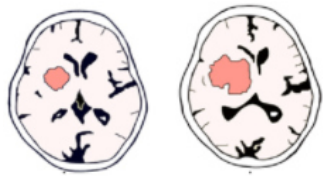
Plasma-circulating miRNAs analysis will permit the identification of a miRNA expression profile that is specific to haematoma growth.

Objective

To evaluate the expression of circulating miRNAs in plasma from ICH patients to identify a haematoma growth-specific miRNA profile by analysing plasma samples from a screening cohort.

Screening cohort

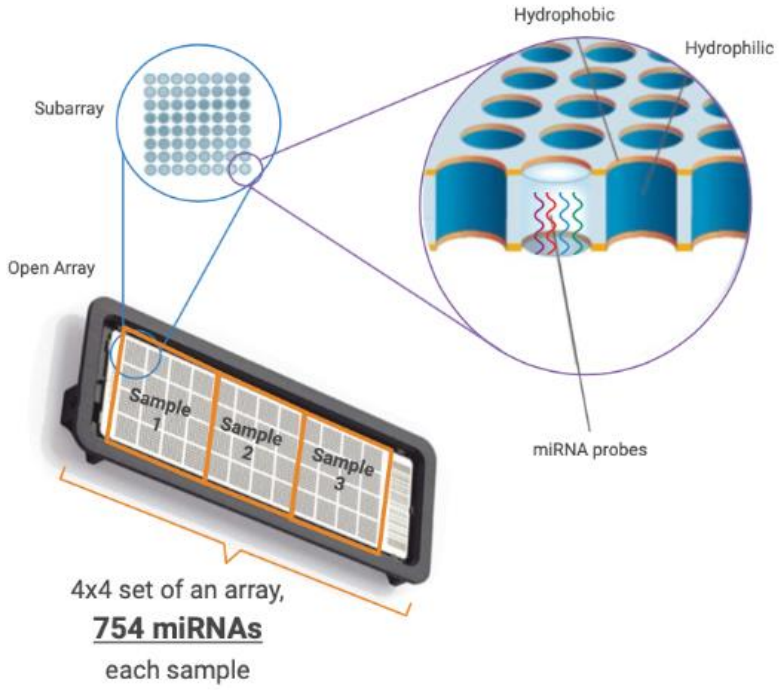
Patients diagnosed of **non-traumatic intracerebral haemorrhage** of less than **12 hours** from symptomatic onset.



	Haematoma growth (N=7)	Non-haematoma growth (N=13)	*p-value*
Age (years)	76.14 ± 13.17	67.23 ± 13.06	0.164
NIHSS	13 (7 - 22)	8 (5 - 21.5)	0.536
Female sex	3 (42.9)	7 (53.8)	1.000
Intraventricular blood	1 (14.3)	3 (23.1)	1.000
Arterial hypertension	6 (85.7)	11 (84.6)	1.000
Baseline haematoma volume (mL)	43.21 (8.89 - 51.05)	8.78 (4.59 - 17.39)	**0.046**

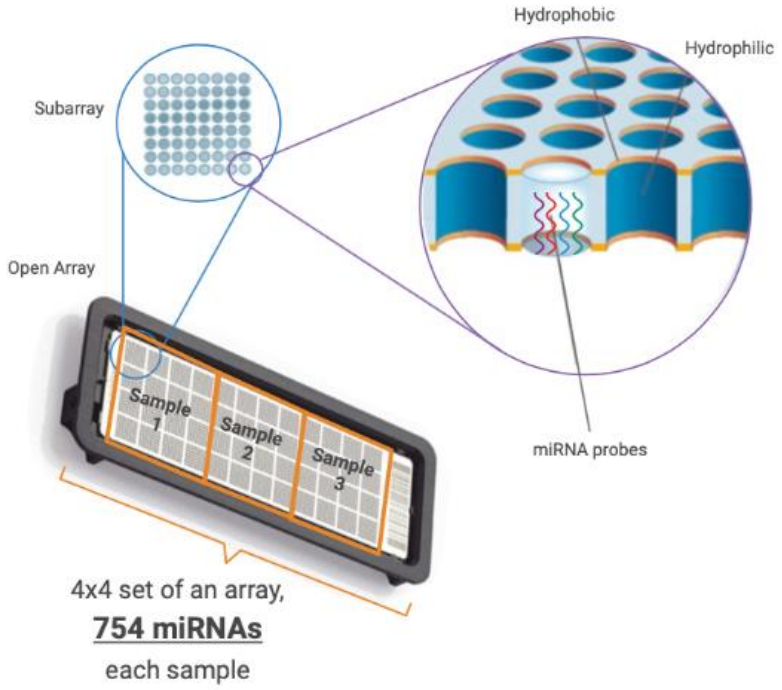
Haematoma growth: absolute growth of **6 mL / 33%** over initial volume during the first 24 hours.

miRNAs Screening



Basal Non-haemolised plasma samples

miRNAs Screening



Basal Non-haemolised plasma samples

Quality of the data

Amplification quality Cq confidence **>0.6,** AMP score **>1.1** & Cq **<28**

Sample quality Samples **> 100** miRNAs

Biogroups quality miRNAs expressed > 70% of each biogroup

180 miRNAs
20 samples

miRNAs expression normalisation

GLOBAL NORMALISATION

Haematoma growth vs. Non-haematoma growth

p-value<0.1 & FC±1.5

ENDOGENOUS NORMALISATION

Haematoma growth vs. Non-haematoma growth

p-value<0.1 & FC±1.5

miRNAs expression normalisation

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Optimal endogenous miRNAs

miRNAs expression normalisation

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Optimal endogenous miRNAs

Summarised Stability Score (SSS)

$$SSS = \sqrt{(Score_{geNorm})^2 + (Score_{NormFinder})^2 + (Score_{CV})^2}$$

miRNAs expression normalisation

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Optimal endogenous miRNAs

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$$SSS = \sqrt{(Score_{geNorm})^2 + (Score_{NormFinder})^2 + (Score_{CV})^2}$$

→

miR-425-5p

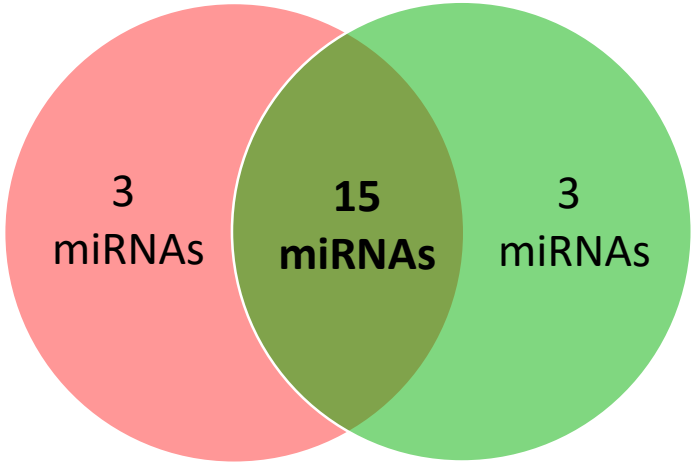
miR-484

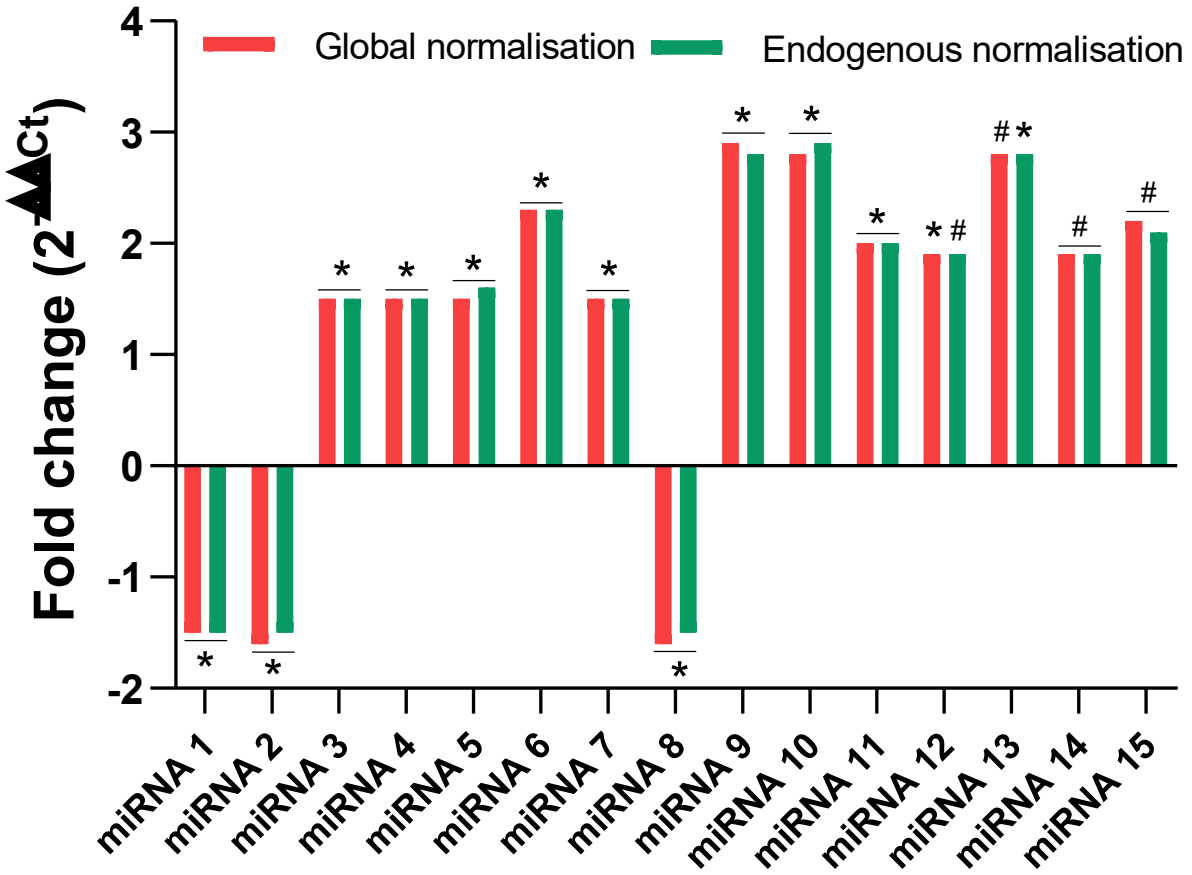
miRNAs as potential biomarkers of haematoma growth

GLOBAL NORMALISATION | **ENDOGENOUS NORMALISATION**

Haematoma growth vs. Non-haematoma growth

p-value<0.1 & FC±1.5





*#p-value<0.05, *p-value<0.1*

CONCLUSIONS

- miR-425-5p and miR-484 have been identified as the optimal endogenous miRNAs for normalisation of results.
- 15 miRNAs have been identified as potential predictive biomarkers of haematoma growth.
- These results need to be validated in an independent cohort of ICH patients with and without haematoma growth.

ONGOING EXPERIMENTS

SCREENING | **VALIDATION**

- 10 Endogenous miRNAs
- 15 potential biomarkers miRNAs

Selection of the validation cohort

N= 33 N= 33

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