Title: WOMEN ARE BIOLOGICALLY YOUNGER THAN MEN AT TIME OF INTRACEREBRAL HEMORRHAGE.

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Objective: To interrogate the sex dimorphism in epigenetic age acceleration of patients with spontaneous intracerebral hemorrhage (ICH).

Background: DNA methylation is an epigenetic modification that can influence gene expression. Epigenetic clocks are prediction method of chronological age based on the DNAm levels. Epigenetic age acceleration (EAA) is an estimation of an individual's biological age relative to their chronological age, implying that a person is older or younger than their years. Intrinsic epigenetic age acceleration (IEAA) is the EAA that is left unexplained by chronological age (Chron.Age) and blood cell composition. In patients with ischemic stroke, EAA has been associated with an increased risk, worse outcome and deacceleration in women. We hypothesize that sex dimorphism in EAA is also present in ICH patients.

Design / Methods: We evaluated clinical data and EAA estimates from two Spanish ICH cohorts (BasicMar and SantPau, n=200). DNAm was obtained using the Illumina EPIC BeadChip array. After appropriate quality controls and normalization steps, four epigenetic clocks were computed (Horvath, Hannum, Levine and Zhang). Intrinsic epigenetic age acceleration (IEAA) was estimated as the residuals by regressing epigenetic age on chronological age. Generalized linear models were used to identify acceleration differences between men and women, while accounting for smoking habit and monocytes count.

Results: Out of the 200 patents, 45% were women (n=90), who were chronologically older than men ($Chron.Age_{women}$ = 76.8±13.6; $Chron.Age_{men}$ = 68.0±14.4; p-value = $2.61x10^{-04}$). However, women had lower EAA on all measures after adjusting for multiple comparisons, except Levine's Pheno EAA (table 1). Women also had lower IEAA values than men for Hanum measure ($IEAA_{women}$ = -2.74; $IEAA_{men}$ = 1.56; bonf.p-value = $1.04x10^{-02}$)

Conclusions: Despite being older at the time of ICH, women are biologically younger than men, this difference seems to not be related with their chronological age or white blood cell counts. Further studies are needed to understand the role of these differences in stroke risk and recovery.

Disclosure and Study Support
No conflict of interest to disclosure.

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Table 1: The influence of sex on epigenetic age acceleration (EAA), measured by four epigenetic clocks.

	Estimate - mean female	Estimate - mean male	Standard Error	Z Statistic	P Value	Bonferroni P Value
Chronological Age						
Univariate	76.8	68.02	1.993	4.407	1.74E-05	**2.61E-04**
Multivariate 1 (smoke)	79.13	74.89	2.187	-1.941	5.38E-02	9.15E-01
Multivariate 2 (smoke + monocytes)	74.27	68.97	2.216	-2.393	1.77E-02	3.02E-01
Horvath EAA						
Univariate	-1.95	4.24	1.27	-4.871	2.31E-06	**3.70E-05**
Multivariate 1 (smoke)	-0.33	5.93	1.475	4.24	3.55E-05	**6.03E-04**
Multivariate 2 (smoke + monocytes)	-3.27	2.35	1.499	3.746	2.42E-04	**4.11E-03**
Hannum EAA						
Univariate	-9.86	-4.27	1.079	-5.183	5.67E-07	**9.63E-06**
Multivariate 1 (smoke)	-9.73	-3.67	1.242	4.88	2.31E-06	**3.93E-05**
Multivariate 2 (smoke + monocytes)	-10.97	-5.18	1.272	4.552	9.74E-06	**1.66E-04**
Levine's Pheno EAA						
Univariate	-4.87	-0.41	1.425	-3.131	2.02E-03	**2.63E-02**
Multivariate 1 (smoke)	-0.57	3.42	1.591	2.506	1.31E-02	2.22E-01
Multivariate 2 (smoke + monocytes)	-1.19	2.66	1.633	2.358	1.94E-02	3.30E-01
Zhang's BLUP EAA						
Univariate	-0.92	2.17	0.867	-3.562	4.95E-04	**6.93E-03**
Multivariate 1 (smoke)	1.43	4.93	0.946	3.696	2.89E-04	**4.92E-03**
Multivariate 2 (smoke + monocytes)	1.61	5.15	0.971	3.64	3.56E-04	**6.04E-03**