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Association between Homocysteine, Frailty and Biomechanical Response of the CNS in NPH-suspected Patients.

Sophie Guillotin, MSc, ^{1,2}, Alexandra Vallet, PhD, ³, Sylvie Lorthois, PhD, ⁴, Philippe Cestac, PharmD, PhD, ¹, Eric Schmidt, MD, PhD, ^{5,6}, Nicolas Delcourt, PharmD, PhD, ^{2,5*}

Affiliations

1 Center for Epidemiology and Research in POPulation health (CERPOP), University of Toulouse, Toulouse, France.

2 Poison Control Center, Toulouse-Purpan University Hospital, Toulouse, France.

3 Department of Mathematics, Faculty of Mathematics and Natural Sciences, University of Oslo, Oslo, Norway

4 Institut de Mécanique des Fluides de Toulouse (IMFT), University of Toulouse, CNRS, Toulouse, France.

5 Toulouse NeuroImaging Center (ToNIC), University of Toulouse, INSERM, UPS, 31024, Toulouse Cedex 3, France.

6 Department of Neurosurgery, Toulouse-Purpan University Hospital, Toulouse, France.

*Correspondance : Dr Nicolas Delcourt, Centre antipoison et de toxicovigilance, Pavillon Louis Lareng, CHU Purpan – TSA 40031, 31059 Toulouse, France. Tel: +33(0)567691644.

Mail: delcourt.n@chu-toulouse.fr

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Abstract

Frailty is a geriatric syndrome that combines physiological decline, disruptions of homeostatic mechanisms across multiple physiologic systems and thus, strong vulnerability to further pathological stress. Previously, we provided the first evidence that increased risk of poor health outcomes, as quantified by a frailty index, is associated with an alteration of the central nervous system (CNS) biomechanical response to blood pulsatility. In this study, we explored correlation between fourteen biological parameters, the CNS elastance coefficient and frailty index. We included 60 adults (52-92 years) suspected of normal pressure hydrocephalus (NPH) and presenting with markers of multiple co-existing brain pathologies, including Parkinson disease (PD), Alzheimer disease (AD) and vascular dementia. We showed that the homocysteine (Hcy) level was independently and positively associated with both frailty index and the CNS elastance coefficient (adjusted R^2 of 10% and 6%). We also demonstrated that creatinine clearance and folate level were independently associated with Hcy level. Based on previous literature results describing the involvement of Hcy in endothelial dysfunction, glial activation and neurodegeneration, we discuss how Hcy could contribute to the altered biomechanical response of the CNS and frailty.

Keywords: biochemical parameters, biomechanic, brain, aging

Introduction

Frailty is a clinical syndrome characterized by a decrease of the physiological reserve capacities with failure of homeostatic mechanisms and vulnerability to stressors (1,2). Frailty is also a marker of risk for mortality and adverse events, including disabilities, falls, hospitalizations and institutionalizations (1). Notably, age is considered as a determinant of frailty but does not alone explain this syndrome. Its clinical expression is indeed modulated by comorbidities and psychological, social, economic and behavioral factors (3).

With regard to pathologies of the central nervous system (CNS), it is well known that frailty is associated with an increased risk of cognitive decline and dementia (4–6). Interestingly, few studies also suggested that neurodegenerative or neurovascular changes are substrates of the frailty phenotype. Notably, it was shown that the presence of macro infarcts, Alzheimer disease (AD), Lewy body pathology, and nigral neuronal loss were associated with a more rapid progression of frailty (7). In the same manner, reduced total brain or gray matter volume and increased cortical brain infarcts could be part of the structural substrate of the frailty phenotype. Frailty is indeed associated with the presence, degree, and some specific localizations of cerebral atrophy in a population of cognitive-decline patients (8). Frailty is also associated with arterial stiffness (9), failure of homeostatic mechanisms and vulnerability to stressors (10).

The research of circulating frailty biomarkers that reflect the physiological state of individuals and the underlying mechanisms related to homeostatic changes throughout an individual lifespan remains a challenge. Among them, inflammatory, nutritional or metabolic risk markers are of the most important targets due to their potential modifiable characteristics (11–18). In parallel, several pathophysiological mechanisms have been proposed to result in frailty, such as chronic, low-grade inflammation related to aging (19). Homocysteine (Hcy), known to be related to inflammation and oxidative stress, is associated with frailty but also with physical

and cognitive decline (19–21), and patients with hyperhomocysteinemia (HHcy) ($>15 \mu\text{mol/L}$) are at higher risk for decline of both physical and cognitive functions (22).

In line with the demonstration that frailty is associated with arterial stiffness (9), we recently provided the first evidence that frailty index (FI) is also associated with the CNS elastance coefficient (23). An increased CNS elastance coefficient induces larger intracranial pressure rises for given intracranial blood volume change. Such an abnormal transmission of mechanical stresses from blood to intracranial tissues at each cardiac cycle could lead to brain damage. Similar to frailty but based on the physical behavior of the CNS rather than on biomedical markers, the CNS elastance coefficient could be considered as an index reflecting a decrease in mechanical reserve capacities with failure of homeostatic mechanisms and vulnerability to stressors.

We seek for plasma parameters statistically correlated to both FI and the CNS elastance coefficient in an observational cohort of patients suspected of normal pressure hydrocephalus (NPH) and presenting markers of multiple co-existing pathologies, including AD, Parkinson disease (PD) and vascular dementia. We integrate several plasma parameters divided into biological profiles: renal profile, metabolic profile, lipid profile and inflammatory markers. These plasma parameters make it possible to describe the body as a whole by integrating risk factors related to frailty, vascular and cognitive risks. Then, based on previous results from the literature, we discuss several hypothesis underlying Hcy based molecular mechanisms by which increased CNS elastance coefficient at the macroscopic scale could possibly contribute to frailty.

Methods

Study design and participants

One hundred patients suspected of NPH (46 women and 54 men aged 52–92 years) were prospectively included in the PROLYPHIC cohort between 2013 and 2015 at Toulouse University Hospital. The suspicion of NPH was based on established guidelines, i.e. the presence of (1) clinical symptoms of gait or balance disturbance, cognitive impairment or urinary disorders, (2) neuroimaging evidence of ventriculomegaly (i.e. Evans Index > 0.3) and (3) narrow callosal angle, temporal horn enlargement or periventricular signal changes (24). The study was approved by the competent authority and registered at ClinicalTrials.gov under no NCT02016352. The local ethics committee approved the study. All patients gave their written informed consent.

Clinical characterization of the population

NPH is an important differential diagnosis of neurodegenerative diseases (25). It is important to define these different diseases within our cohort. The increased risk of AD was examined based on the ratio between cerebrospinal fluid (CSF) tau and amyloid beta 42 (A β 42) peptides using a cut-off of 0.215 (26). Presynaptic dopamine deficiency, which is a characteristic of PD, was examined visually by an expert neurologist using [123 I]FP-CIT DATSPECT. The presence of white matter lesions, which is a characteristic of increased risk of vascular dementia, was ascertained on FLAIR MRI images using the Fazekas scale with a cut-off of \geq grade 2 (27). Finally, abnormal CSF dynamics was quantified via the CSF outflow resistance R_0 (28) derived from the lumbar infusion test (23). A threshold of $R_0 > 12$ mmHg/mL/min was chosen as a marker of altered CSF dynamics and increased risk of NPH. Indeed, the symptoms mentioned above are not specific to NPH and may be associated with other neurodegenerative diseases. That is why the infusion test is recommended in the clinical practice in order to characterize CSF dynamic alterations by a quantitative measurement of the resistance to CSF outflow R_0 . Although the above in vivo markers do not, by themselves, corroborate the clinical diagnosis of neurodegenerative diseases, they do flag an increased risk of having or developing them. For

each marker, the prevalence of patients at risk was calculated only in patients with available data.

Data collection

Comorbidities and lifestyles were recorded using a registration questionnaire for admission: tobacco, alcohol, diabetes mellitus, high blood pressure and dyslipidemia as qualitative variables. Questioning about alcohol and tobacco consumption were based on the current use. Lipid profile with Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Apolipoprotein A1 and B, triglycerids and total cholesterol, inflammatory profile with C reactive protein (CRP) and Hcy, renal profile with creatinine, uric acid, blood urea nitrogen (BUN), and metabolic profile with cobalamin and folates were measured by the hospital laboratory according to routine procedures. The creatinine clearance was estimated with the Cockcroft-Gault equation.

Frailty assessment

Frailty was quantified by means of a FI based on the deficit accumulation approach, which has been widely validated across many settings and populations (29,30). The FI was defined as the ratio, ranging from 0 (not frail) to 1 (very frail), between the number of health deficits presented by the individual and the total number of deficits considered. A total of 40 variables were selected to measure the deficits in (1) executive functioning, (2) mentation, behaviour and mood, (3) gait, (4) rigidity and agility of extremities, (5) blood laboratory tests of markers related to kidney function, metabolic function and inflammatory conditions, which are of particular importance in adults over 50 years old (31), (6) urinary symptoms, (7) nutrition and (8) history including medication intake and the presence of previously diagnosed hypertension or diabetes mellitus. Supplementary table 1 presents the variables and thresholds for deficit status. Following the guidelines (29) for creating a FI, we did not include variables that had

more than 5% missing data and less than 1% of participants meeting criteria for the deficit. Patients presenting more than 10% of missing data on the 40 health deficits considered were excluded.

CNS mechanical response assessment

Infusion tests were performed in patients according to standard infusion test protocol (32). Lumbar CSF pressure, a surrogate marker of intracranial pressure (ICP), was measured; then a constant rate lumbar saline injection was performed in order to gauge CNS mechanical properties. Patients also underwent phase contrast magnetic resonance imaging (PCMRI) (see below) in order to gauge intracranial fluids dynamics. The biomechanical response of the CNS was characterized by the CNS elastance coefficient inferred *in vivo* from PCMRI and ICP monitoring during lumbar infusion test. This latter parameter characterizes the biomechanical response of the CNS to stress and measures its ability to adapt to a change of volume. It results from a complex interplay between the global stiffness of the brain parenchyma (homogeneous to a Young modulus E [Pa]), and the volume V [ml] of the compressible volumes within the CNS, and dimensionally scales as $E/(V \cdot p)$, where p is still to be identified but must be homogeneous to a pressure or stress [Pa] (for details, see (23)).

PCMRI protocol and image analysis

The 2D PCMRI was used as a non-invasive technique to quantify intracranial blood flows as reported elsewhere (33). Patients who could not undergo PCMRI were excluded from the study. All other participants were imaged in a 3.0 T magnetic resonance (MR) scanner (Philips Achieva, Philips, Best, Netherlands) with a commercial 32-channel head coil. Retrospective cardiac-gated PCMRI sequences were performed with a plethysmograph set on the subject's finger to synchronize the acquired images with the subject's heartbeat. The selected segment was perpendicular to the intracranial internal carotids, basilar artery, straight sinus and sagittal sinus vessels. The acquisitions were made with an encoding velocity of 80 cm/s and 32 time

points were sampled for each cardiac cycle. Preprocessing steps, including velocity aliasing correction, noise reduction and vessel segmentation, were performed by a person blinded to the biomechanical data and using the dedicated Flow Analysis software, version 2018-12-12. Data identified by an independent reviewer as non-reliable due to poor image or segmentation quality were excluded from the study. The instantaneous intracranial arterial flow $Qa(t)$ was defined as the sum of blood flows in the intracranial internal carotid and basilar arteries. The instantaneous intracranial sinus flow $Qs(t)$ was defined as the sum of blood flows in the straight and sagittal sinuses. It was assumed that during the whole cardiac cycle, the blood volume entering the cranial cavity through arteries was equal to the blood volume exiting it through veins. However, the straight and sagittal sinuses are not the only exit routes for blood. A correction factor was therefore defined to take the undetected blood volume exiting the cranial cavity into account: $acorr = \int_0^T Qa(t)dt / \int_0^T Qs(t)dt$, where T is the cardiac cycle period. The total instantaneous venous flow was then estimated as $Qv(t) = acorr Qs(t)$. The instantaneous blood volume change was $V(t) = \int_0^t Qa(t)dt - \int_0^t Qv(t)dt$. The maximal blood volume change was defined as $\Delta V = \max\{V(t)\}_{t \in [0, T]} - \min\{V(t)\}_{t \in [0, T]}$.

Statistical analysis

Descriptive analysis of quantitative parameters was performed with mean \pm standard deviation (SD) and qualitative parameters with percentage.

The associations between quantitative independent and dependent variables were assessed by univariate and multivariate linear regressions. In univariate regression analysis, the associations were evaluated and any independent variate that displayed p-value < 0.05 in a given univariate analysis model was considered as a potential independent variable. In multivariate regression analysis, the independent variable selection was based on the preliminary univariate analysis. Three models were tested with Hcy as the dependent variable. For each model and its variables,

β -estimate, regression coefficients, adjusted regression coefficients, and p-values were reported. Multicollinearity was checked using the variance inflation factor.

The associations between qualitative independent variables and Hcy as the dependent variable were assessed by logistic regressions. To illustrate the logistic correlation, a forest plot was built from odds ratio.

To ascertain normality, continuous variables were inspected through histograms and Lilliefors tests.

Statistical analyses were performed using R version 4.0.2.

Results

Clinical and biochemical characteristics of the study population

After careful evaluation, 40 patients were excluded from the initial cohort of 100 patients, for low quality of PCMRI data, FI reliability criterion or missing data (23). This resulted in a final analytical sample of 60 patients, with statistical characteristics of demographic variables, FI and biomechanical parameters (Table 1). Concerning markers of neurodegenerative pathologies, 57% of patients had abnormal CSF dynamics and increased risk of NPH, 75% had increased risk of AD, 25% (of 53 patients) had increased risk of PD, and 62% (of 53 patients) had increased risk of vascular dementia. In addition, among 52 patients without missing markers, 4% presented no risks of any of the four diseases considered, 19% presented risks of one disease, 38% presented risks of two diseases, 35% presented risks of three diseases, and 4% presented risks of the four diseases (data not shown). In this cohort, there were 48.3% of women. Concerning environmental factors, among the patients, 24.6% and 26.3% respectively smoked and drunk alcohol. Regarding vascular risk factors, 43.3% were hypertensive, 28.3% diabetic and 28.3% had dyslipidemia. The CSF outflow resistance R_0 was observed to be higher than average due to the specific cohort suspected of NPH. For the metabolism profile, folates and cobalamins levels were in normal ranges. Despite the prevalence of dyslipidemia, the mean

of different parameters in the lipid profile were in the norms. Moreover, the renal profile is relatively conserved for this cohort with multiple comorbidities. Finally, the inflammatory markers were also in normal ranges.

Hcy plasma level is independently associated with frailty index, the CNS elastance coefficient and age

Table 2 displays univariate regression analysis between all measured biochemical parameters and two factors, the CNS elastance coefficient and FI. CRP, Hcy and folates were associated with FI (p-value < 0.05) (Table 2). Hcy, LDL, total cholesterol and apolipoprotein B were associated with the CNS elastance coefficient (p < 0.05). Among all measured biochemical parameters, Hcy plasma level was the sole to be correlated with both the CNS elastance coefficient (Adjusted R² 6% and p-value <0.05) and FI (Adjusted R² 12% and p-value <0.05). These associations are represented in Figure 1, where an increase in Hcy is associated with an increase in both the CNS elastance coefficient and FI. An increase in Hcy is also associated with an increase in age (Adjusted R² 23% and p-value <0.001).

Hcy plasma level is not associated with comorbidities and lifestyles

The association between Hcy plasma level with qualitative lifestyle parameters and comorbidities was measured by logistic regression analysis (Supplementary Figure 1). In this cohort of patients with NPH, Hcy plasma level was not different between males and females (OR = 0.989; 95% CI: 0.881 – 1.105). Moreover, with the high frequency of cardiovascular risk factors in this cohort, links between high blood pressure (OR = 0.888; 95% CI: 0.755 – 1.01), diabetes mellitus (OR = 0.96; 95% CI: 0.831 – 1.082) and presence of dyslipidemia (OR = 0.968 ; 95% CI: 0.835 – 1.094) with Hcy plasma level were measured and no link was found. Concerning lifestyles with alcohol (OR = 1.057; 95% CI: 0.928 – 1.2) and tobacco (OR = 1.091; 95% CI: 0.965 – 1.246), Hcy plasma level was also independent of these two features. Finally,

the Hcy plasma level was also independent on comorbidity risks, including increased risk of AD (OR = 1.11; 95% CI: 0.961-1.34), of PD (OR = 1.08; 95% CI: 0.936-1.246), of vascular dementia (OR = 1; 95% CI: 0.891-1.132) and of NPH (OR = 0.971; 95% CI: 0.866-1.088). Moreover, Supplementary table 2 displays the univariate regression analysis between R_0 with CRP, FI and the CNS elastance coefficient. No linear correlation was measured with R_0 (p-value > 0.05). No parameters depend on R_0 despite the patients' suspicion of NPH. We checked that the main confounder related to our NPH suspected population, R_0 , is not such a confounder variable.

Hcy plasma level is independently associated with creatinine clearance and folate

Associations between Hcy plasma level and plasmatic biochemical parameters were measured by Pearson univariate linear regression (Supplementary Table 3 and Table 4). Concerning the serum lipid profile, no correlation was found (p-value > 0.05). For the metabolic profile, our data showed a link between folate level (p-value < 0.05) and Hcy plasma level contrary to cobalamin level (p-value > 0.05). In the renal profile, only creatinine clearance was correlated with homocysteine plasma level (p-value < 0.001). Finally, for the inflammatory profile, Hcy and CRP were not associated (p-value > 0.05). Then, only folates levels (figure 2A) and creatinine clearance (figure 2B) were associated with Hcy plasma level. As shown in figure 2C, three models of multivariate regression analysis were performed from the significant biochemical parameters in figure 2A and 2B, the biomechanical response and the clinical response. The model A associated Hcy plasma level with the CNS elastance coefficient (p-value < 0.05). The model B added on top the folates and the creatinine clearance (p-value < 0.001). Finally, the model C added on the top the age and the FI. The model C was the most representative model of the variability of Hcy (p-value < 0.001), with an adjusted R^2 of 56%.

Discussion

Our cohort is a convenient model of pathological brain aging with a wide range of degrees of frailty, suitable to assess biomechanical and biological parameters. Indeed, the NPH suspicion provides access to various parameters (CSF sample, ICP measure, PCMRI, ...), especially in relation to neurodegenerative diseases. Moreover, with 43% of patients having an unconfirmed diagnosis of NPH, we are in the context of non-specific disease cohort. This fact is reinforced by the lack of correlation between R_0 and all parameters tested.

Here, we took a step back and focused on a potential biological parameter that might link frailty and CNS biomechanics in a group of patients presenting markers of multiple co-existing pathologies and suspected of NPH. Among the various biochemical parameters tested, Hcy was the sole parameter independently associated with both FI and biomechanical response of the CNS. Thus, our data suggest that the importance of Hcy may be a parameter involved in the shift from robust brain aging to pathological brain aging (Figure 3).

The first part of our work aimed to identify a potential plasma parameter associated with frailty. Among all the tested plasmatic biological parameters, we showed that FI is associated with Hcy, folates and CRP (Table 2). Our data are in concordance with the general view that clinical frailty is associated with inflammation (17), as suggested from previous cross-sectional studies (19,21) and meta-analysis (34,35). Among inflammatory parameters, CRP, hsCRP, interleukin-6 (IL-6) and Tumor necrosis factor- α (TNF- α) were shown to be significantly higher in frail subjects. Nevertheless, a recent study including 1394 adults aged 70 years and more showed that Hcy and CRP levels were not able to predict frailty for a period of 5 years, in an adjusted model (18), and several studies observe different results on a possible correlation between CRP and frailty (11,13). Differences in study designs, such as the use of different frailty scores or techniques to measure parameters, the recruitment of only men or women or differences in participants' ages, might explain these differences. Thus, further investigations would be needed to complete these latter studies in terms of biomarkers such as interleukins.

Then, we showed that impaired creatinine clearance, a marker of renal dysfunction, and folate are independently associated with Hcy. Concerning the association with folates, these results are in accordance with previous literature showing that a lack of folate can lead to HHcy (36) and may be related to cognitive impairment (37). Deficiencies in vitamin B12 and folates are nutritional deficiencies that contribute to variations in Hcy metabolism, as these molecules are involved in the de novo synthesis of the methionine methyl group. Interestingly, it was demonstrated that folate and vitamin B12 supplementation reduce the level of Hcy in the serum of patients with mild cognitive impairment and improve cognitive function (38). Moreover, we found that Hcy is independently associated with creatinine clearance. These results are of interest as HHcy has been recognized to be associated with chronic kidney disease (CKD) (39). It was also recently demonstrated that Hcy is positively and independently associated with serum creatinine levels in male patients with hypertension (40). Interestingly, a recent meta-analysis confirmed that frail individuals had higher plasmatic creatinine levels than robust individuals (35).

Finally, we aimed to determine whether some biochemical parameters measured in plasma could be associated with the CNS elastance coefficient. In a recent work, we found a significant association between the CNS elastance coefficient and FI (23). Noteworthy, Phillip et al. already suggested that aging was not only accompanied by major biochemical changes, but also biomechanical alterations, including increased stiffness, at macroscopic and microscopic length scales (41). Consistently, as illustrated in Table 2, we showed that the CNS elastance coefficient is associated with biochemical parameters, including Hcy, LDL-Cholesterol, total-Cholesterol and Apolipoprotein B, but not CRP. Thus, we demonstrated that Hcy is independently associated with both FI and CNS biomechanics, as quantified by the CNS elastance coefficient.

Then, by integrating all the biochemical and biomechanical parameters, we proposed a representative model of the variability of Hcy (adjusted R^2 56%) that is composed of the CNS

elastance coefficient, age, FI, creatinine clearance and folate level (Figure 2C). Interestingly, this model does not include CRP, a parameter that is only associated with FI in our cohort. These results suggest that whereas low-grade inflammation could be associated with frailty, only Hcy seems to be linked with both renal and brain disorders.

While these correlations are not sufficient to prove a causal relationship between Hcy, FI and the CNS elastance coefficient, they nevertheless raise the question of the possible underlying pathways biological plausibility by which Hcy could result in increased vulnerability of these organs. In the brain, Hcy is linked to neurodegeneration, notably by modulating PP2A activity and tau phosphorylation, and to neuroinflammation by inducing inflammatory cytokine such as TNF- α and IL-1 β (42,43). The neurotoxicity of Hcy was also shown to be mediated by the NMDA and metabotropic glutamate receptors (44–46). It is also well known as a cardiovascular risk factor, in particular involved in kidney microvascular dysfunction.

It can thus be hypothesized that Hcy induces damage of small blood vessels. Thus, high Hcy levels is now studied as a possible contributing factor to microvascular dysfunction and cerebral small vessel disease (43) by modulating inflammation, oxidative stress, immune activation and endothelial dysfunctions (43,47,48). Notably, HHcy was well described to induce oxidative damage by ROS accumulation (43). Indeed, longer HHcy exposure induces a downregulation of endothelial nitric oxide synthase (eNOS), leading to the decrease in L-citrulline and nitrite formation, and then to oxidative damages (49). Another mechanism that contributes to vascular inflammation mediated by Hcy concerns the homocysteinylation process (50). N-homocysteinylation is a post-translational modification that alters protein structure, function, causes protein aggregation, as well as protein misfolding, and induces apoptosis (50). Interestingly, it was also shown that treatment of microvascular endothelial cells derived from mouse brains with Hcy leads to the decrease of growth factor such as VEGF, FGF α and leptin, and increase of the matrix metalloproteinase MMP-9 (51). Finally, in this same study, by

modulating $\beta 1$ integrin abundance at the plasma membrane, Hcy also disturbed the extracellular matrix integrity in the intimal layers around endothelial cells, and potentially their biomechanical properties.

Moreover, microvascular dysfunction is a leading cause for renal dysfunction, which may thus explain the association between Hcy level and creatinine clearance. It was indeed previously shown that impaired kidney function, as measured by decreased glomerular filtration rate, is related to subclinical markers of cerebral small vessel disease, independent of cardiovascular risk factors (52). From a biomechanical point of view, we can hypothesize that the consequences of increased Hcy described above, such as the loss of neural cells, misfolded protein aggregation, microvascular dysfunction or vessel wall alteration could modify the mechanical properties of both the brain parenchyma and the cerebro-vascular system, resulting in an increased CNS elastance coefficient (23).

All together, we hypothesize from the statistically significant results that pathological brain aging, pathological kidney aging and frailty share common underlying biological processes, which could involve microvascular dysfunction. Consistently, CRP, which is not associated with the CNS elastance coefficient nor creatinine clearance, was not previously found to play a role neither in brain nor in renal microvascular dysfunctions.

The included patients were suspected of NPH. However, 43% of patients had normal CSF dynamics, demonstrating that about half of our population was not hydrocephalic. Moreover, 75% had increased risk of AD, 25% of PD and 62% of vascular dementia, demonstrating that our population was wider than NPH. Hence, the associations between Hcy, FI and the CNS elastance coefficient seems not to be specific to NPH and the other neurological diseases and makes acceptable the absence of a non NPH control group (23,53).

Despite convincing results, the study showed some limitations. First, our study did not provide information on the relevance of our approach in the preclinical stage as all patients presented clinical symptoms. Second as the number of cases is small, it will be necessary to confirm our results in a larger cohort. Third, in the statistical study, the cross-sectional design of the study did not allow resolving the directionality of the association between Hcy, FI and the CNS elastance coefficient. Fourth, concerning the biochemical exploration of plasma, we did not analyze other inflammatory parameters such as IL-6 and TNF- α , we cannot conclude on a link between the inflammasome and the biomechanical response of CNS in NPH suspected patients. Then, further investigations are needed to argue in favor of a causality according to Bradford Hill criteria (54).

In conclusion, our exploratory study demonstrated that Hcy level is positively associated with FI and the CNS elastance coefficient, providing a potential biological link between pathological brain aging and a biomechanical response of the CNS. The biological plausibility at the origin of the different correlations described above are reported (Figure 3). In a recent perspective article, Fried and Coll. raised the question of whether there is a shared biological driver of multi system dysregulation in parallel in physical frailty (3). Our results reinforce the interest to work on the role of Hcy at the mechanistic level in order to understand whether Hcy could be one of these drivers. Notably it will be interesting to explore how modulation of Hcy homeostasis supports a possible causality in the frailty-related increase in the CNS elastance coefficient. Future works could use global approaches such as OMICs or experimental models in order to generate comprehensive data that help to understand the biological mechanisms underlying the transition from normal to pathological aging of the brain.

Conflict of interest

The authors declare no conflict of interest.

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Quantitative parameters		Qualitative parameters				
Parameter (unit)	Mean (Standard deviation)	Normal range	Parameter	% score = 1	Score	
Clinical features	Age (years)	74.22 (6.58)	-	Sex	48.3	0: man / 1: woman
	Frailty index	0.324 (0.142)	-	Tobacco	24.6	0: no / 1: yes
Biochemical parameters	Creatinine clearance (mL/min)	75.93 (25.38)	[90-140]	Alcohol	26.3	0: no / 1: yes
	Plasmatic creatinine (μ M)	77.83 (20.91)	[60-115] M ^c / [45-105] W ^d	High blood pressure	43.3	0: no / 1: yes
	Blood urea nitrogen (mM)	6.85 (2.59)	[2.5-7.5]	Diabetes mellitus	28.3	0: no / 1: yes
	Uric acid (μ M)	313.03 (10.21)	[180-420] M ^c / [150-360] W ^d	Dyslipidemia	28.3	0: no / 1: yes
	Apolipoprotein A1 (g/L)	1.42 (0.34)	[1.1-2] M ^c / [1.2-2.22] W ^d	Alzheimer disease	75	0: no/ 1: yes
	Apolipoprotein B (g/L)	0.96 (0.22)	[0.55-1.35] M ^c / [0.55-1.25] W ^d	Parkinson disease	25	0: no/ 1:yes
	Very Low Density Lipoprotein (mM)	0.61 (0.26)	[0.1-1.7]	Vascular dementia	62	0: no/ 1:yes
	Low Density Lipoprotein (mM)	2.83 (0.92)	<4.1	Normal pressure hydrocephalus	57	0: no/1: yes
	High Density Lipoprotein (mM)	1.43 (0.51)	>1.0			
	Total cholesterol (mM)	4.87 (1.04)	[4.1-5.2]			
	Triglycerides (mM)	1.37 (0.64)	[0.4-1.7]			
Plasmatic homocysteine (μ M)	13.61 (5)	<15				
C reactive protein (mg/L)	3.01 (3.77)	<5				
Cobalamins (pg/mL)	402.02 (163.81)	[200-900]				
Folates (ng/mL)	8.6 (12.87)	[5-15]				
Biomechanical parameters	CNS ^a elastance coefficient (1/mL)	0.511 (0.129)	-			
	CSF ^b outflow resistance (R0)	14.92	-			

(mmHg/mL/min)

(7.73)

Note: ^aCNS: central nervous system ; ^bCSF: cerebrospinal fluid ; ^cM : man ; ^dW : woman

Table 1: Characteristics of subjects. Continuous variables were presented with mean, standard deviation and normal range and categorical data were presented as the percentage of deficit.

		CNS elastance coefficient			Frailty Index		
		R ²	Adjusted R ²	P-value	R ²	Adjusted R ²	P-value
Renal profile	Creatinine clearance	0.013	-0.049	0.398	0.012	-6.59E-03	0.423
	Plasmatic creatinine	1.33E-03	-0.016	0.782	4.07E-03	-0.015	0.644
	Blood urea nitrogen	2.60E-03	-0.015	0.699	0.024	5.55E-03	0.259
	Uric acid	0.026	0.010	0.215	0.030	0.012	0.205
Lipid profile	Triglycerides	0.089	-8.32E-03	0.477	3.03E-03	-0.016	0.690
	Apolipoprotein A1	7.26E-05	-0.017	0.949	4.55E-03	-0.014	0.625
	Apolipoprotein B	0.070	0.053	0.043*	2.76E-03	-0.016	0.703
	Very Low Density Lipoprotein (VLDL)	0.011	-6.41E-03	0.430	4.03E-03	-0.015	0.645
	Low Density Lipoprotein (LDL)	0.127	0.111	0.006*	0.010	-8.38E-03	0.461
	High Density Lipoprotein (HDL)	0.011	-5.96E-03	0.421	0.030	0.012	0.204
	Total cholesterol	0.106	0.091	0.011*	0.025	6.30E-03	0.252
Metabolism profile	Cobalamins	5.62E-03	-0.012	0.579	0.045	0.026	0.131
	Folates	4.12E-03	-0.019	0.675	0.104	0.081	0.040*
Inflammatory	Homocysteine	0.077	0.059	0.044*	0.123	0.104	0.014*

markers	C reactive protein	0.002	-0.016	0.765	0.106	0.089	0.015*
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Table 2: Biochemical parameters associated with the CNS elastance coefficient and frailty

index. Parameters associated were described by Pearson univariate correlation with R^2 ,

adjusted R^2 and p-value

p-value < 0.05: *

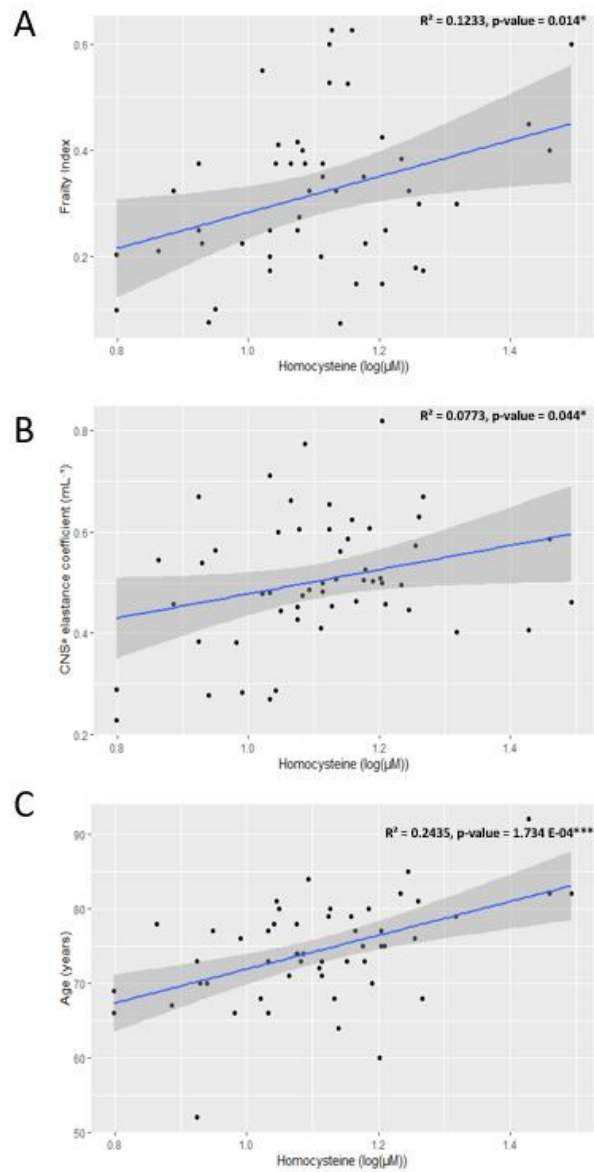


Figure 1: (A) Linear relations between FI and homocysteine ($R^2 = 0.1233$; p -value = 0.014*), between (B) the CNSa elastance coefficient and homocysteine ($R^2 = 0.0773$; p -value = 0.044*) and (C) between age and homocysteine ($R^2 = 0.2435$; p -value = 1.734 E-04***). R^2 and p -value lead to describe the Pearson univariate regression analysis. Linear regressions (blue lines) and 95% confidence intervals (grey bands) are indicated. Homocysteine level is expressed in logarithmic form to respect the normality condition. aCNS: Central Nervous System p -value < 0.05: * ; p -value < 0.01: **; p -value < 0.001: ***

33x63mm (300 x 300 DPI)

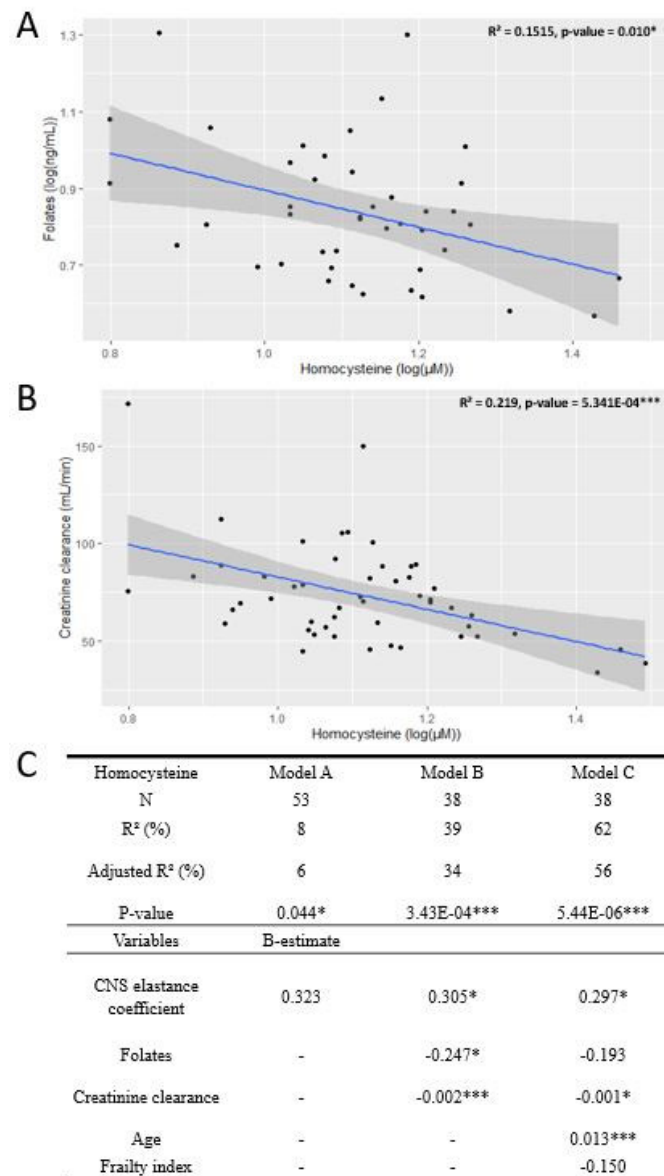


Figure 2: Correlation between homocysteine plasma level and biochemical, clinical and CNS biomechanical parameters. (A) Hcy plasma level is associated with folates ($R^2 = 0.1515$; p -value = 0.010^*) and (B) creatinine clearance ($R^2 = 0.219$; p -value = $5.341E-04^{***}$). Linear regressions (blue lines) and 95% confidence intervals (grey bands) are indicated. Homocysteine and folates are expressed in logarithmic form to respect the normality condition. Associated biochemical factors with homocysteine plasma level in the Pearson univariate and multivariate regression analysis with B-estimate (C). Model A is composed by the CNS elastance coefficient. Model B is composed by the CNS elastance coefficient, folates and creatinine clearance. Model C is composed by the CNS elastance coefficient, folates, creatinine clearance, age and frailty index.

p -value < 0.05: * ; p -value < 0.01: ** ; p -value < 0.001: ***
aCNS: Central Nervous System

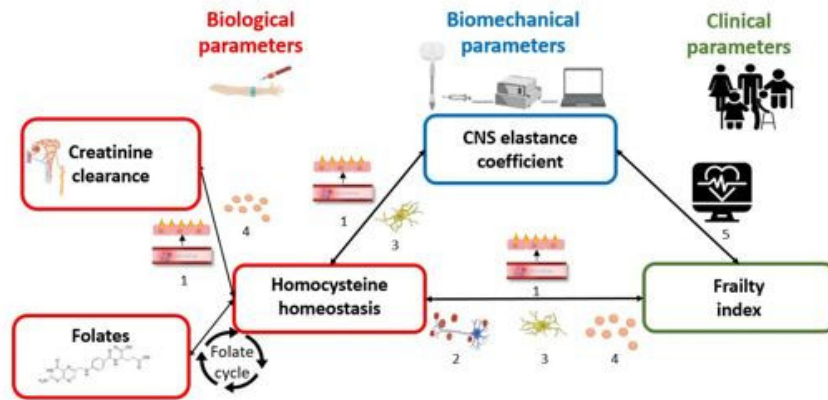


Figure 3: Known associations between molecular and cellular roles of homocysteine and their interplay with the biomechanical response of the central nervous system (CNS) and frailty index
 1 vascular inflammation/endothelial dysfunction; 2 neurotoxicity; 3 glial activation; 4 endoplasmic reticulum / oxidative stress; 5 blood pulsatility; <-----> correlation

50x24mm (300 x 300 DPI)

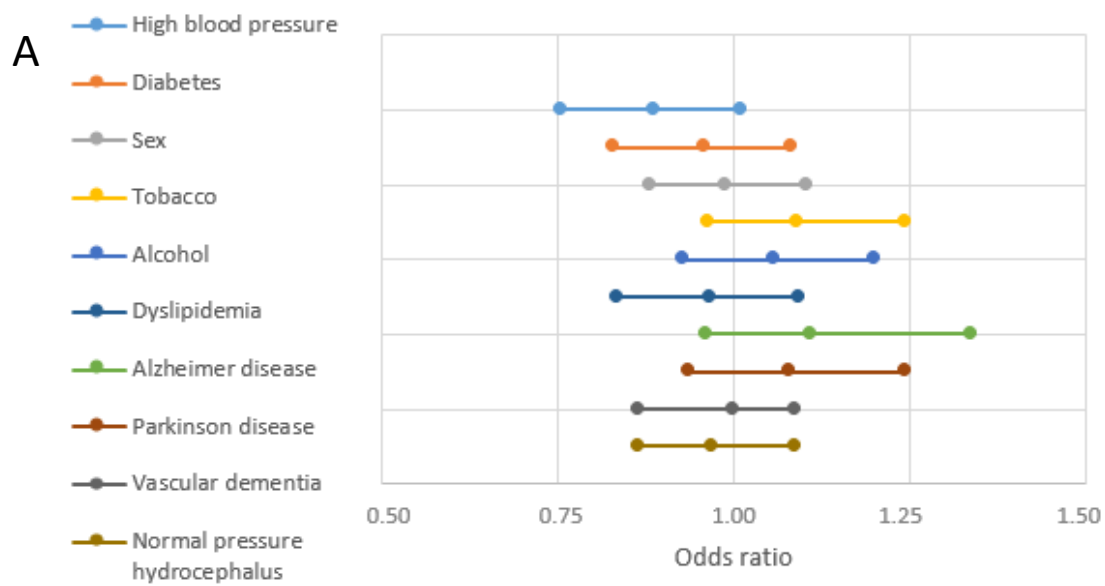
Supplementary data

Number	Parameters	Conditions for deficit
Executives functions		
1	Dependence for dressing	UPDRS ^a item score ≥ 2
2	Dependence for hygiene	
3	Handwriting	
4	Speech	
5	Transferring	
6	Chewing and swallowing	
7	Doing hobbies and other activities	
Mood and cognition		
8	Cognitive impairment	UPDRS ^a item score ≥ 2
9	Depressed mood	SEGA ^b item > 1
10	Apathy	UPDRS ^a item score ≥ 2
11	Self-reported health compared to others	SEGA ^b item > 1
12	Night-time sleep problems	UPDRS ^a item score ≥ 2
13	Anxious mood	
Gait		
14	Walking and balance	UPDRS ^a item score ≥ 2
15	Freezing	UPDRS ^a item score ≥ 2
16	Gait	
17	Global spontaneity of movement	
Rigidity and agility of extremities		
18	Tapping fingers	UPDRS ^a item score > 2 left + right
19	Tapping toes	
20	Rigidity upper extremities	
21	Rigidity lower extremities	
22	Hand movements	

23	Leg agility	
Laboratory blood test		
24	Urea blood level	< 2.9 mM or > 8.2 mM
25	Creatinine blood level	Cockcroft-Gault formula < 60 mL/min/1.73 m ²
26	C-reactive protein blood level	> 3 mg/L
27	Triglyceride blood level	> 2.25 mM
28	Glycated hemoglobin	> 5.7%
29	Cobalamin	< 300 pg/mL
30	Sodium	< 136 mM or > 142 mM
Urinary symptoms		
31	Need to urinate every 2 h	IPSS ^c item ≥2
32	Nocturia	
33	Leakage	
34	Sensation of bladder not empty	
Nutrition		
35	Body mass index	< 18.5 or > 25
36	Loss of appetite	MNA ^d ≤2
37	Loss of weight	MNA ^d ≤2
History		
38	Drugs history	More than four drugs
39	Hypertension	Present
40	Diabetes mellitus	Present

Supplementary Table 1: Variables used in the construction of the frailty index

^aUPDRS: Unified Parkinson's Disease Rating Scale / ^bSEGA: short emergency geriatric assessment / ^cIPSS: International Prostate Score Symptom / ^dMNA: Mini Nutritional Assessment



B

Variables	Odds ratio	95% CI	p-value
High blood pressure	0.888	(0.755 - 1.01)	0.106
Diabetes	0.96	(0.831 - 1.082)	0.534
Sex	0.989	(0.881 - 1.105)	0.837
Tobacco	1.091	(0.965 - 1.246)	0.161
Alcohol	1.057	(0.928 - 1.2)	0.375
Dyslipidemia	0.968	(0.835 - 1.094)	0.623
Alzheimer disease	1.110	(0.961 - 1.340)	0.213
Parkinson disease	1.080	(0.936 - 1.246)	0.276
Vascular dementia	1.000	(0.866 - 1.088)	0.606
Normal pressure hydrocephalus	0.971	(0.866 - 1.088)	0.606

Supplementary Figure 1: Independence of homocysteine plasma level for comorbidities and lifestyles. (A) Forrest plot of parameters illustrating the logistic regressions by the odds ratio and (B) description in the table with the quantification of the associated odds ratio, 95% confidence intervals (95%) and p-value.

p-value < 0.05: *

	R ²	Adjusted R ²	p-value
CRP ^a	0.015	0.002	0.348
FI ^b	0.022	0.003	0.286
CNS ^c elastance coefficient	5.90E-04	-0.02	0.854

Supplementary Table 2: Correlation between R₀^d with CRP, FI^b and the CNS elastance coefficient. Factors were correlated by Pearson univariate regression analysis with 3 parameters: R², adjusted R² and p-value.

p-value < 0.05: *

^aCRP: C-reactive protein

^bFI: Frailty Index

^cCNS: Central Nervous System

^dR₀: CSF outflow resistance

	CRP^a		
	R²	Adjusted R²	p-value
Creatinine clearance	0.002447	-0.01603	0.7173
Folates	0.003359	-0.01982	0.7053

Supplementary Table 3: Correlation between CRP with creatinine clearance and folates.

Associated factors were described by Pearson univariate correlation with R², adjusted R² and p-value.

p-value < 0.05 : *

^aCRP: C-reactive protein

	Homocysteine		
	R²	Adjusted R²	P-value
Creatinine clearance	0.219	0.203	5.34E-04***
Blood urea nitrogen	0.036	0.017	0.176
Uric acid	0.014	-0.005	0.392
Triglycerides	0.023	0.004	0.2783
Apolipoprotein A1	1.19E-04	-0.020	0.939
Apolipoprotein B	2.318E-06	-0.02	0.992
Very Low Density Lipoprotein (VLDL)	0.025	0.006	0.262
Low Density Lipoprotein (LDL)	0.008	-0.012	0.541
High Density Lipoprotein (HDL)	4.35E-05	-0.021	0.964
Total cholesterol	0.023	0.003	0.283
Cobalamins	0.055	0.035	0.102
Folates	0.152	0.131	0.010*
C reactive protein	0.019	-0.001	0.330

Supplementary Table 4: Correlation between homocysteine plasma level and biological parameters. Associated factors were described by Pearson univariate correlation with R², adjusted R² and p-value.

p-value < 0.05: * ; p-value < 0.01: ** ; p-value < 0.001: ***