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Plasma Catecholamine's: Blood Molecules Implicated in Alzheimer's Disease?

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Abstract

Current research highlighted a degeneration of the dopaminergic and noradrenergic systems in the brain, i.e. the ventral tegmental area (VTA) and the locus coeruleus (LC) at an early stage of Alzheimer's disease (AD), and alterations of catecholamines concentrations in different body fluids (CSF, plasma and urine) of AD patients and animal models. These findings imply a potential utility of catecholamines in the molecular and mechanistic AD comprehension. Following our previous work on plasma noradrenaline in the context of AD, this retrospective study includes a cohort of 105 patients (43 AD, 29 with other dementia and 32 without dementia) from the cognitive neurology center of Lariboisière (Paris) who consulted for memory complaints. We show for the first-time different relations between plasma catecholamines and AD biomarkers at cognitive (MMSE score) and molecular (CSF biomarkers concentrations) levels. Our ROC analyses illustrate the good potential of plasma catecholamines to discriminate AD from non-AD patients with a relatively low or high MMSE score. Taken together, our results support the idea that plasma catecholamines could be blood molecules implicated in AD physiopathology, opening new frontiers in the development of a blood-based AD diagnosis.

Keywords: Alzheimer's disease; Catecholamines; MMSE score; Cerebrospinal fluid biomarkers

Introduction

Challenges in the diagnosis: The need to identify new AD biomarkers

Alzheimer's disease (AD) is the most common cause of dementia [1]. Despite considering progress of AD research, the vast majority of clinical trials for therapies have failed to affect disease progression [2,3]. AD diagnosis is complex, the only gold standard being the direct observation of amyloid plaques and neurofibrillary tangles in postmortem brain tissue, which are specific features of AD. Due to the difficult accessibility of these observations, *in vivo* diagnosis requires a cluster of information describing the pathophysiological characters of the disease at a cognitive, morphological, and molecular levels. National Institute on Aging and Alzheimer's Association (NIA-AA) guidelines [4] recommend validated AD diagnostic criteria, which include imaging techniques and cerebrospinal fluid (CSF) biomarkers. These guidelines aim to capture neurofibrillary tangles formation and cortical amyloid plaques deposition, together with their physiological consequences. However, these recommended techniques have the disadvantage of being costly, as for brain imaging, or invasive and risky, as for lumbar puncture. In this context, researchers are seeking alternative methods of investigation such as i) quantification of known biomarkers of AD in more available body fluids (blood [5], saliva [6]) or sampling (eye observation [7], skin [8], EEG [9]), ii) identification of yet unknown and noninvasively accessible biomarkers [10] of AD early stage [11], which could take place decades before the apparition of the cognitive symptoms [12]. However, the utility in clinical practice of these diagnostic alternatives were not yet convincingly demonstrated as their results are often not reproductible due to technical and/or physiological reasons [13].

Identification of early pathophysiological mechanisms in AD

Until recently, the most credited idea was that the onset and the evolution of AD probably involve two interplaying phenomena [14], i.e. progressive cortical accumulation of amyloid plaques through amyloid biochemical cascade [15] associated with cholinergic synaptic dysfunction [16]. These hypotheses have been called into question since therapeutic molecules targeting amyloid metabolism or cholinergic pathway could not slow down disease evolution [2,3]. This suggests that those signaling events may be too advanced in AD pathophysiology. The observation of early alterations in subcortical brain nuclei, i.e. the locus coeruleus (LC) and the ventral tegmental area (VTA), at a prodromal stage of the disease [17-19] opened new perspectives in the pathophysiological description of AD. LC and VTA are among the main brain sources of noradrenergic and dopaminergic neurons, respectively [20,21]. With the improvement of imaging techniques focusing on LC [22], a decreased contrast and volume of the LC in MCI and AD patients [23-27] has been described, which is consistent with LC neuronal loss and deregulated NA levels observed in post-mortem brain tissue of AD patients [27-29]. Moreover, LC alterations seem to correlate with Braak stages [30] which describe the presence of neurofibrillary tangles in different brain regions during AD development [31]. Concerning the dopaminergic system, in MCI and advanced AD patients, VTA size and its connectivity with the hippocampus were associated with hippocampal size and memory competence [32,33]. In murine model of AD, VTA [34-36] and LC [37] damage and altered NA levels were observed [37]. Moreover, VTA neuronal death correlates with reduced D outflow in the hippocampus, synaptic plasticity in the CA1, memory performance and food-reward processing. In line with these observations, it has been observed that the VTA-hippocampus-NAc circuit, essential for spatial, novelty and persistent memory formation, is early impaired in AD mice. Similarly, it was also demonstrated that provoked lesions of the LC in mice impact working memory [38,39]. Further, pharmacological LC activation was able to rescue the main cognitive and behavioral deficits [37,40]. Finally, it has been shown that

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neurons from the LC can co-secrete dopamine (D) with noradrenaline (NA) in the hippocampus [41,42], indicating that NA and D might cooperate in learning and memory processes [43]. Taken together, those results support the idea that brain catecholamines circuits are impaired at an early phase of AD, with a loss of LC and VTA neurons correlating with memory, but also behavioral deficits (depression, anxiety, apathy, sleep-wake cycle disruption, etc.) usually observed in AD patients long before amyloid plaques and neurofibrillary tangles formation [44].

A renewed interest for plasma catecholamines in AD

Early studies on alterations in LC structure and in catecholamines levels within brain, CSF and plasma from AD patients, were followed by a long disinterest for this topic. A recent renewed attention for the role of catecholamines in the context of AD was motivated by the recent observations in MCI patients of noradrenergic and dopaminergic systems alterations before amyloid plaques deposition. NA, D and adrenaline (A) are the three main catecholamines. These molecules are linked through several enzymatic steps. In the brain, they act as neurotransmitters and locally as a hormone by diffusion. At the peripheral level, they are synthesized by adrenal medulla and sympathetic noradrenergic neurons, and act as hormones. The need to identify AD biomarkers in alternative more accessible body fluids motivated a large number of studies on catecholamines fluctuations in urine [45,46] and plasma [47-49] of AD patients and mouse model of AD. Despite conflicting, this field of research showed interesting results. Our retrospective study examined the relationship between plasma catecholamines concentrations and concomitant diagnostic criteria such as Mini Mental Sate Examination (MMSE) score and CSF biomarker profile ($A\beta_{1-42}$, Tau and p-Tau). As described in our previous report for plasma NA [50], we wanted to determine i) whether other plasma catecholamines concentrations could be correlated with clinical parameters which reflect disease stages at cognitive (MMSE score) and molecular ($A\beta_{1-42}$, Tau and p-Tau CSF biomarkers) levels, and ii) whether the combination of these putative biomarkers could be exploited for the early diagnosis of AD pathology.

Materials and Methods

Study population

All patients presented to the Cognitive Neurology Center of Lariboisière (Paris) for their first consultation between 2017 and 2019. Patients involved in this study were between 53 and 72-years old at the time of blood sampling. MMSE score and lumbar puncture were performed the day of blood sampling or less than one month later. More details on MMSE cutoffs are given in our previous article [50]. Sample size was calculated with the same method of our previous article [50]. In this retrospective study, 104 patients were included: 43 AD patients (diagnoses were performed according to NIA-AA guidelines [4]), 29 patients with other dementia (OD; frontotemporal dementia, vascular dementia or dementia with Lewy bodies), and 32 neurological control (NC) patients. NC patients were defined as those with memory complaints, mental depression, or anxiety but for whom no dementia was diagnosed. Two NC patients were removed from the study because of their extremely low MMSE score (3 and 8). As we previously described [50], cutoff values for $A\beta_{1-42}$ (<550 pg/mL), total-Tau (>400 pg/mL), and p-Tau (>50 pg/mL) were used to identify AD dementia. Table 1 synthetizes all information concerning demographic, cognitive, physiological, and co-medications data.

Total number of patients		NC	OD	AD	p-value
		32	29	43	-
Sex	% of female patients	40.63	41.38	58.14	0.2257
Age	Age median (IQR) in year	62.5 (59.25-69)	67 (61.50-69)	68 (63-70)	0.0619
MMSE	MMSE score median (IQR)	27 (26-28)	24 (19-26)	20 (15-26)	<0.0001
CSF $A\beta_{1-40}$ concentration[$]	CSF Aβ concentration median (IQR)	12449 (8961-14868)	10865 (8924-14561)	11878 (8707-15338)	0.8656
CSF $A\beta_{1-42}$ concentration	CSF Aβ concentration median (IQR)	1133 (938-1333)	1112 (976-1390)	575 (463-667)	<0.0001
CSF Tau concentration	CSF Tau concentration median (IQR)	195.5 (158.5-227)	227 (190.5-301)	492 (373-672)	<0.0001
CSF p-Tau concentration	CSF p-Tau concentration median (IQR)	34 (19.26-47.75)	39.5 (26.43-50.40)	77.7 (59-105)	<0.0001
Plasma NA concentration	Plasma NA concentration median (IQR)	2064 (1556-3117)	2295 (1855-3034)	2499 (1826-3086)	0.3809
Plasma A concentration	Plasma A concentration median (IQR)	276 (146-442)	222 (172-428)	318 (216-478)	0.2493
Plasma D concentration	Plasma D concentration	311 (110.5-494.8)	239 (206.5-370)	201 (100-363)	0.1216
median (IQR)	311 (110.5-494.8)	239 (206.5-370)	201 (100-363)	0.1216	1 month
	Anti-Alzheimer, neuroleptics, antidepressants	15.625	20.69	16.667	0.8595
	Lipid-lowering agents, oral antidiabetics	18.75	27.586	19.048	0.6273
	Anti-hypertensive agents	21.875	31.034	35.714	0.4338

[#]co-medication information are missing for 1 AD patient; [$]$A\beta_{1-40}$concentration is missing for 1 AD patient

Table 1: Demographic and physiologic data of studied cohort.

Quantification of NA, A and D in plasma

Blood sampling was performed on 12h-fasted patients in supine position, as described in our previous article [50]. Samples purification and analysis were performed with Chromsystems kit (order #5000) for plasma catecholamines high-performance liquid chromatography (HPLC) analysis. Briefly, after blood-stabilization with glutathione and direct centrifugation (less than 60 min after sampling) to isolate the plasma, samples were frozen and stored at-80°C. 1 mL of thawed plasma was used for catecholamines dosage by HPLC coupled with electrochemical detection. Quantification of catecholamines was made without knowing the patient group of samples. For plasma D concentration ($[D]_{plasma}$), values under the range of detection were considered as equal 100 pmol/L.

Quantification of $A\beta_{1-42}$, total Tau, and p-Tau in the CSF

As we previously described [50], CSF samples were obtained by lumbar punctures on fasted patient. Then, they underwent 10 min centrifugation (1 g, 4°C) within 4h after collection. 500 µL-polypropene tubes were used for aliquoting and -80°C storage. Sandwich ELISA INNOTEST® kit (Fujirebio Europe NV, formerly Innogenetics NV) was used for AD biomarkers quantification in the CSF ($A\beta_{1-42}$, total Tau, and p-Tau).

Data analysis and statistical tests

Similarly to what performed in our previous study [50], we first tested normality (D'Agostino-Pearson normality test) to determine results illustration and statistical test choice. For normally distribution, we presented mean with standard deviation (SD) in figures and used Student's t-test (two-tailed) to compare two groups. In the absence of normal distribution, we presented median with interquartile range (IQR: 25-75th percentiles) (95% confidence interval in figures) and used Mann-Whitney test (two-tailed) to compare two groups or Kruskal-Wallis test to compare distribution of their data. For multiple group comparisons we performed one-way ANOVA. Linear correlations were tested using Pearson's correlation test or Spearman's correlation test for normally or not-normally distributed data, respectively. All analyses and multiple logistic regressions were performed with GraphPad Prism 9.0.0 software. We used Medcalc software to apply the empirical nonparametric method from Delong et al. [51] to compare AUCs from ROC curves. No outliers were identified in our cohort (p-value>0.01) by performing Rosner's Extreme Studentized Deviate test for multiple outliers (using log-normal distribution and two-side test). AUC were compared using the empirical nonparametric method by Delong et al. [51] with Medcalc software. Cutoff for p-value was 0.05 to identify statistical significance.

Results

Cohort description

We found no difference concerning age, sex or pharmacological treatments between NC, OD and AD groups that differed by MMSE score and CSF biomarkers (one-way ANOVA, p-values are mentioned in Table 1). Patients' clinical diagnosis was established by the neurologist accordingly to NIA-AA guidelines [4].

Relations between catecholamines and AD CSF biomarkers

Consistent with our previous results in a different cohort [50], we found that AD patients with a MMSE score above 23 (the cutoff value for dementia in the old population [52]) (n=19) had a higher $[NA]_{plasma}$ than non-AD patients with similar MMSE score (n=49, 31 NC and 18 OD patients) (Mann Whitney test, p-value=0.0485) (Figure 1A). On the other hand, AD patient with a MMSE score under 23 (n=24) had a comparable $[NA]_{plasma}$ than non-AD patient (n=12, 11 OD and 1 NC patients) (Mann Whitney test, p-value=0.4969) (Figure 1B). Knowing that NA is the precursor of the other catecholamine adrenaline, we wanted to know if there was a correlation between $[NA]_{plasma}$ and $[A]_{plasma}$ in order to determine if A could potentially be associated with specific AD features, just as NA. Interestingly, we found a significant positive linear correlation between $[NA]_{plasma}$ and |A]plasma of AD patients (n=43) (Spearman's correlation, r=0.5130 (95% CI: 0.2428 to 0.7093), p-value=0.0004, equation: Y=0.1119*X +72.33) (Figure 1C), which was inexistent in OD (n=29) (Spearman's correlation, r=0.3153 (95% IC:-0.06925 to 0.6182, equation: Y=0.04465*X +217.6), p-value=0.0957) and NC (n=32) (Spearman's correlation, r=0.1881 (95% IC:-0.1823 to 0.5117), p-value=0.3026, equation: Y=0.01520*X +297.1) patients (Figure 1C). As we previously described for NA [50], we examined whether there was a correlation between $[A]_{plasma}$ distance from the NC patient median-defined as the absolute value of $[A]_{plasma}$-$<[A]_{plasma/NC}>$, with $<[A]_{plasma/NC>}$ the median value of $[A]_{plasma}$ from NC patients (276 pmol/L)- and the CSF biomarkers profile. Indeed, we observed that $|[A]_{plasma}-<[A]_{plasma/NC}>|$ for extreme $[A]_{plasma}$ values-meaning below 1st tercile value (33% percentile: 162.5 pmol/L) and above 3rd tercile value (67% percentile: 421 pmol/L) of $[A]_{plasma}$ from NC patients was significantly higher in AD patients (n=19) than in control patients (n=21) (Mann Whitney test, p-value=0.0096) (Figure 1D), which was not the case when comparing NC and OD patients (n=12) (Mann Whitney test, p-value=0.6118) (data not shown). Parallelly, concerning patients with an $[A]_{plasma}$ closer to the median control (meaning between 1st tercile value and 3rd tercile value of $[A]_{plasma}$ from NC patients), we found no significant difference between NC (n=11) and AD (n=24) (Mann Whitney test, p-value=0.9788) (Figure 1D) or NC and OD (n=17) (Mann Whitney test, p-value=0.6516) patients for $|[A]_{plasma}-<[A]_{plasma/NC}>|$ (data not shown). Taken together, this suggests that, unlike for OD patients, AD cohort presents more extreme high or low $[A]_{plasma}$ values than NC patients. We found a significant negative linear correlation between$|[A]_{plasma}-<[A]_{plasma/NC}>|$ and $[A\beta_{1-42}]_{CSF}$ in AD patients (n=43) (Spearman's correlation, r=-0.3895 (95% IC:-0.6232 to-0.09190), p-value=0.0098, equation: Y=-0.3186*X +655.4) (Figure 2A), which was not the case in OD (n=29) (Spearman's correlation, r=-0.08594 (95% IC:-0.4478 to 0.3001), p-value=0.6576, equation: Y=0.05645*X +1151) and NC patients (n=32) (Spearman's correlation, r=0.01148 (95% IC:-0.3481 to 0.3681), p-value=0.9503, equation: Y=0.03872*X + 1170) (Figure 2A). To understand the relation between raw $[A]_{plasma}$ values and CSF biomarkers concentrations, we looked at negative and positive relative distance from $<[A]_{plasma/NC}>$, i.e. when $[A]_{plasma}$ is respectively lower or higher than $<[A]_{plasma/NC}>$. We found opposite correlations between $[A]_{plasma}$ and $[A\beta_{1-42}]$ CSF when comparing AD cohort with negative and positive distance from $<[A]_{plasma/NC}>$. Indeed, in AD cohort with negative relative distance from $<[A]_{plasma/NC}>$, we observed a non-significant positive linear correlation for negative distance (n=19) (Spearman's correlation, r=0.2544 (95% IC:-0.2396 to 0.6438), p-value=0.2933, equation: Y=0.7615*X+464.4) and a significant negative correlation in AD for positive distance (Spearman's correlation, r=-0.4478 (95% IC:-0.7270 to-0.04161), p-value=0.0282, equation: Y=-0.3419*X +769.6) (Figure 2B). We found no significant linear correlation between $|[A]_{plasma}-<[A]_{plasma/NC}>|$ and $[p\text{-}Tau]_{CSF}$ and $[Tau]_{CSF}$ (data not shown). However, we found a significant positive linear correlation between $[A]_{plasma}$ and the ratio $(p\text{-}Tau/Tau)_{CSF}$ in AD patients (n=32) (Spearman's correlation, r=0.3293 (95% IC: 0.02300 to 0.5791), p-value=0.0311, equation: Y=(4.863*10-5)*X +0.1386)) (Figure 2C), a significant negative linear correlation in NC patients (n=32) (Spearman's correlation, r=-0.3984 (95% IC:-0.6621 to-0.04702), p-value=0.0239, equation: Y=(-2.439*10-5)*X +0.1781)), and no significant correlation in OD patients (n=29) (Spearman's correlation, r=0.09681 (95% IC:-0.2901 to 0.4565), p-value=0.6174, equation: Y=(2.716*10-5)*X + 0.1556)) (Figure 2C). We found no correlation between negative and positive distance with $(pTau/Tau)_{CSF}$ in AD patients (data not shown). However, $(pTau/Tau)_{CSF}$ tended to be lower in 2nd tercile (n=15) of $[A]_{plasma}$ from AD patients ($[A]_{plasma}$ 33% percentile: 235.1 pmol/L and 67% percentile: 432 pmol/L) in comparison with 1st tercile (n=14), without reaching significance (Student's

t-test, p-value=0.1894), and was significantly lower in comparison with 3rd tercile (n=14) (Student's t-test, p-value=0.0129), without difference between 1st and 3rd terciles (Student's t-test, p-value=0.2470) (Figure 2D). Taken together, those results suggest that, like $[NA]_{plasma}$, $[A]_{plasma}$ could be related to CSF biomarkers profile in AD patients. The recent studies emphasizing the implication of VTA and dopaminergic neurons in an early stage of AD evolution prompted us to determine whether $[D]_{plasma}$ could be related as well to CSF AD biomarkers. Interestingly, we found a significant positive linear correlation between $[D]_{plasma}$ and $|[A]_{plasma}-<[A]_{plasma/NC}>|$ in AD patients (Spearman's correlation, r=0.3057 (95% IC:-0.003251 to 0.5614), p-value=0.0462, equation: Y=0.3751*X + 189.6), which was not the case for NC (Spearman's correlation, r=-0.1198 (95% IC:-0.4582 to 0.2490), p-value=0.5137, equation: Y=0.2295*X + 309.5) or OD (Spearman's correlation, r=0.2691 (95% IC:-0.1193 to 0.5860), p-value=0.1581, equation: Y=0.1432*X+ 285.8) patients (Figure 3A). Moreover, we could identify that AD patients present a lower $[D]_{plasma}$ than non-AD (NC and OD) patients (Mann Whitney test, p-value=0.0435) (Figure 3B) with a significant different cumulative distribution (Kolmogorov-Smirnov test, p-value=0.0389) (Figure 3C). We found no significant correlation between $[D]_{plasma}$ and CSF biomarkers (data not shown). However, we found a significant difference of $(A\beta_{1-42}/A\beta_{1-40})$CSF ratio between AD patients under 1st tercile value of AD $[D]_{plasma}$ (168 pmol/L) (n=13) and AD patients above this value, i.e. 2nd and 3rd tercile (n=29) (Student's t-test, p-value=0.0201) (Figure 3D). In summary, we could identify in our cohort that $[D]_{plasma}$ seems altered in AD patients and that AD patients with the lowest values of $[D]_{plasma}$ differed from other AD patients with a significantly smaller $(A\beta_{1-42}/A\beta_{1-40})_{CSF}$ ratio. Altogether, our results strongly suggest that plasma catecholamines-NA, A and D-are potential informative molecules that could mirror CSF biomarkers alterations illustrating brain AD physiopathology.

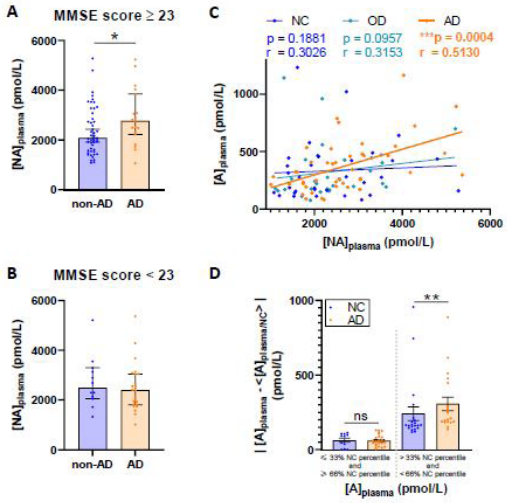

Figure 1: Plasma noradrenaline concentration in AD patients is related to MMSE score and plasma adrenaline concentration, which extreme high and low values are more distanced from median control, in AD patients. A-B: $[NA]_{plasma}$ in AD (orange) and non-AD (blue) patients with MMSE score above 23 (A) and below 23 (B). C: Correlation between $[NA]_{plasma}$ and $[A]_{plasma}$ in AD (orange) patients but not in NC (dark blue) and OD (light blue) patients. D: Distance $|[A]$ plasma-$[A]_{plasma/NC}|$ from patients with extreme $[A]_{plasma}$ values (right) and $[A]_{plasma}$ close to median control (left) in AD (orange) and NC (blue) patients.*means p-value<0.05; ***means p-value<0.001.

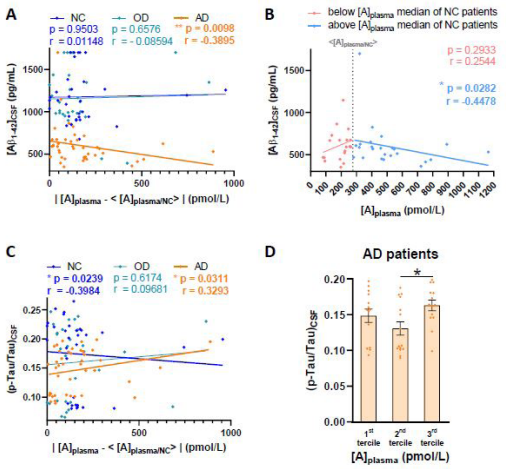

Figure 2: Plasma adrenaline concentration distance from median control correlates with CSF biomarkers $A\beta_{1-42}$ concentration and p-Tau/Tau ratio in AD patients. A: Correlation between $|[A]_{plasma}-<[A]_{plasma/NC}>|$and $[A\beta_{1-42}]_{CSF}$ in AD patients (orange) but not in NC (dark blue) and OD (light blue) patients. B: Linear correlations between negative (in pink) and positive (in blue) values of|A]plasma-$<[A]_{plasma/NC}>$ with $[A\beta1–42]_{CSF}$ in AD patients. C: Correlation between $|[A]_{plasma}-<[A]_{plasma/NC}>|$and $(p\text{-Tau/Tau})_{CSF}$ in AD patients (orange) but not in NC (dark blue) and OD (light blue) patients. D: $(p\text{-Tau/Tau})_{CSF}$ values in 1st, 2nd and 3rd $[A]_{plasma}$ terciles of AD patients.*means p-value<0.05 ; **means p-value<0.01.

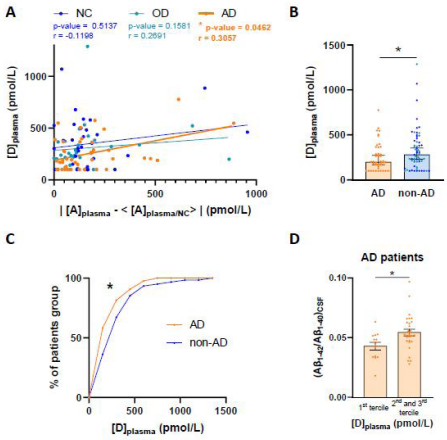

Figure 3: Plasma dopamine concentration in AD patient, related to plasma adrenaline concentration distance from median control and to CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio, is lower than in non-AD patient population. A: Correlation between $|[A]_{plasma}-<[A]_{plasma/NC}>|$and $[D]_{plasma}$ in AD patients (orange) but not in NC (dark blue) and OD (light blue) patients. B: $[D]_{plasma}$ in AD (orange) non-AD (blue) patients. C: Cumulative distribution of $[D]_{plasma}$ between AD (orange) and non-AD (blue) patients. D: $(A\beta_{1-42}/A\beta_{1-40})_{CSF}$ ratio values in 1st, 2nd and 3rd $[D]_{plasma}$ terciles of AD patients.*means p-value<0.05; **means p-value<0.01.

Utility of plasma catecholamines to discriminate AD from non-AD patients

We recently highlighted in our studies relations between plasma catecholamines concentrations and AD biomarkers at cognitive (MMSE score) and molecular (CSF biomarkers) levels. These results prompt us to test whether plasma catecholamines could help in AD diagnosis in the context of a first neurological consultation for memory complaints. We pooled the two cohorts of our previous [50] and present studies (75 AD, 51 OD and 49 NC patients) to perform multiple logistic regressions from two different models: a model based on one parameter $[A\beta_{1-42}]_{CSF}$ and another taking into account parameters obtained from plasma catecholamines concentrations, i.e. $[NA]_{plasma}$, $[A]_{plasma}$, $[D]_{plasma}$, $|[NA]_{plasma}-<[NA]_{plasma/NC}>|$, $|[A]_{plasma}-<[A]_{plasma/NC}>|$, $|[D]_{plasma}-<[D]_{plasma/NC}>|$, (NA/A)plasma ratio, (NA/D)plasma ratio, (A/D)plasma ratio, to discriminate AD from non-AD patients. Considering the whole cohort, we found that AUCs from the two different ROC curves were significantly different (Delong's methodology, difference between areas=0.252, p-value<0.0001) (Figure 4A). However, knowing that $[NA]_{plasma}$ is related to MMSE score, we performed multiple logistic regression in three different groups based on 33% and 64% percentiles of MMSE score in the cohort (<22, between 22 and 26, >26). The different multiple logistic regressions models use the same parameters, but the model adapts to the cohort defined by the MMSE score. We found that AUCs from two different ROC curves were not different in groups with extreme MMSE scores, meaning under 22 (Delong's methodology, difference between areas=0.110, p-value=0.2999) and above 26 (Delong's methodology, difference between areas=0.0342, p-value=0.6741), and were significantly different in the middle group (Delong's methodology, difference between area=0.235, p-value=0.0022) (Figures 4B-D). This implies that plasma catecholamines parameters could help to discriminate AD patients with similar performances as $[A\beta_{1-42}]_{LCR}$ in an advanced or early stage of AD evolution from a cognitive view point. In other words, plasma catecholamines parameters could potentially help to distinguish advanced AD patients from other demented patients with a relatively low MMSE score and identify mild AD patients among non-demented patients with a relatively high MMSE score. Taken together, our observations open the road for the use of plasma catecholamines in the diagnosis of AD.

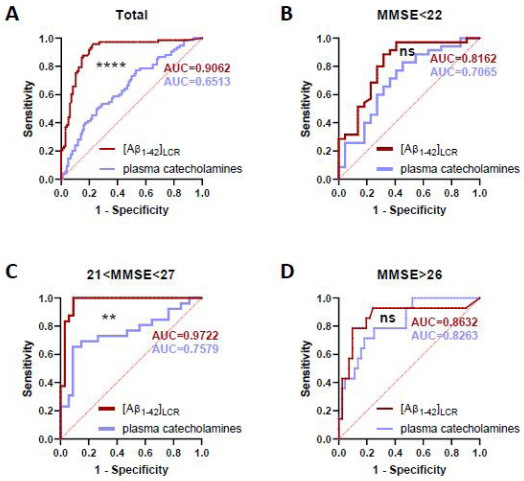

Figure 4: ROC curves from multiple logistic regressions reveal the utility of plasma catecholamines to discriminates AD from non-AD patients with similar MMSE score. A-D: ROC curves of multiple logistic regressions discriminating AD from non-AD patients. ROC curves were generated from two models for all patients (A), patients with MMSE score under 22 (35 AD and 22 non-AD patients) (B), between 22 and 26 (26 AD and 34 non-AD patients) (C) and above 26 (14 AD and 44 non-AD patients) (D)-model 1 (red): $[A\beta_{1-42}]_{LCR}$; model 2 (blue): plasma catecholamines concentrations, their distance from NC median, $[NA]_{plasma}/[A]_{plasma}$, $[NA]_{plasma}/[D]_{plasma}$, $[A]_{plasma}/[D]_{plasma}$ ratios. Comparison of AUC with Delong's method: p-value<0.0001****, p-value<0.01:**, not significant ns.

Discussion

In this retrospective study, we showed for the first-time relations between LCR AD biomarkers and $[A]_{plasma}$ and $[D]_{plasma}$. We also found a higher concentration of $[NA]_{plasma}$ in AD patients with a high MMSE score in comparison with other non-AD patients with a similar MMSE score, which is consistent with our previous independent study [50-53]. Moreover, we observed that $[A]_{plasma}$ correlated with $[NA]_{plasma}$ and was related to $[D]_{plasma}$, significantly lower in AD cohort in comparison with other patients. We could then highlight the potential discrimination power of plasma catecholamines by comparing ROC analysis based on $A\beta_{1-42}$ or plasma catecholamines signature to differenciate AD from non-AD patients. We observed that AUC from the two models were similar for patients with extreme MMSE scores (<22 or >26), suggesting that the catecholamines signature is informative in cognitively advanced or early stage of AD cognitive evolution.

AD early mechanisms linked to catecholaminergic system?

As mentioned in the introduction, many evidences show that subcortical catecholaminergic nuclei, i.e. LC and VTA, are among the first affected by tau protein abnormalities [53]. This, together with the fact that noradrenergic and dopaminergic neurons form a vast and complex network throughout the brain, contribute to the hypothesis of an early "prion-like" spreading of Tau pathology, from subcortical nuclei to cortex and other brain regions [54]. A reasons explaining why catecholaminergic neurons would be first impacted during the disease is their strong vulnerability in comparison with other CNS neurons [55]. Anatomically, their projections to other brain regions are long and poorly myelinated, which make them fragile. They contain neuromelanin, a heavy metal chelator resulting of catecholamine oxidation, whose accumulation becomes toxic. Moreover, they have a stronger energetic demand which could expose them to cellular and oxidative stress. Lastly, their large contact surface with blood vessels and ventricles exposes them to a high amount of toxins and pathogens. Such phenomenon could then indirectly participate in amyloid plaques formation and inflammation in AD. Moreover, NA system deregulation in the CNS has a role in amyloid pathology [56], neuronal metabolism [57], and neuroinflammation [58]. It is probable that NA system and those mechanisms interact in a synergic vicious-circle manner during AD. These features make catecholaminergic neurons the first potential actors in AD onset. Finally, it is important to mention that imaging of human brain showed an early disconnection of VTA, but not LC, with other brain structures [32]. This evidence suggests that D circuits might also participate in the onset of early non-cognitive behavioral and psychological symptoms of AD, such as irritability, or sleep disorders.

Peripheral catecholamines in AD

Adolfsson et al. first identified an altered concentration of D and NA in human postmortem brain tissue of AD patient in comparison with age-matched control, which correlated with dementia score in some brain regions [59]. This is consistent with a recent article reporting on similar results, and also including a correlation between MMSE score and cortical NA level (BA22) [60]. Other studies also show reduced cerebral NA [61,62] and D concentration in AD patients [63,64], with sometimes no change in D [65], which could depend of the observed brain regions. Parallelly, despite conflicting results showing both increased [66,67] and decreased [68] levels, NA CSF concentrations in AD patients seem to be altered. Moreover, Yohimbine-induced increase of CSF NA levels was greater in AD patients in comparison with their age-matched controls [69]. Interestingly, it was also demonstrated that cognitive performances and CSF NA concentration do correlate [70]. Finally, higher CSF concentrations of D [66] and A [71] have been measured in AD patients, and it seemed that CSF A increased with disease severity. Parallely, in other peripheral body fluids, it was previously shown that urine catecholamines concentrations in AD patients [45] and rat model of AD [46] are decreased. On the other hand, previous studies showed altered [47,48] or unchanged [49] NA and A plasma concentrations in AD patients. Conflicting results could be explained by different disease stages, as well as age and gender ratio presented in those articles. The correlations that we found between plasma catecholamines in AD patients were not observed in non-AD patients from our cohort. Correlations between $[A]_{plasma}$ and $[NA]_{plasma}$ were previously described in horses during and after intense physical exercises [72]. The coefficient of correlation between $[NA]_{plasma}$ and $[A]_{plasma}$ decreased as the intensity of exercise decreased, suggesting a different process in release, distribution and clearance of these molecules and/or a different proportion in sympathetic nervous system and surrenal medulla involvement. Interestingly, LC and VTA are involved in the regulation of sympathetic system activity [20]. Hence, it is tempting to speculate that the different correlations between plasma catecholamines concentrations measured in AD patients, but not in non-AD subjects, could be due to an alteration of the sympathetic system caused by LC and VTA dysregulation. Moreover, autonomic dysfunction seems to be implicated in AD [73].

The potential role of plasma catecholamines in the understanding of AD pathology

According to our observations, plasma catecholamines should not be considered as classical biomarkers with a defined cutoff helping in AD identification, but rather as an additional information to the clinical picture (MMSE score, memory complaints, education, age, etc.) of the patient. It is also important to precise that LC neurodegeneration is not specific to AD, as it also occurs during aging and other dementia. However, AD-related neuronal loss in the LC follows a rostro-caudal gradient, unlike for other neurodegenerative diseases where neuronal loss is scattered in the LC [23,27,30,37,55]. Knowing that LC neurons are regionalized depending on the targeted brain region [20], we could imagine that some loss of function and compensation mechanisms (such as oversecretion of catecholamines or network reorganization) in LC and VTA brain areas are specific to AD [19]. These potential compensation mechanisms due to a defined neuronal loss pattern, could explain, for example, the lower concentration of NA observed in cortical postmortem tissue of AD patients in comparison with age-matched controls without dementia or with other dementia [60,62]. Moreover, VTA deregulation seems to occur before LC degeneration [35,74], suggesting that D might participate in a specific physiological response, relative to other dementia, during AD evolution.

Study limitations

The first limitation that we identified in our work is the relatively small size of our cohort. Future studies in larger cohorts will allow to validate the observed relations between plasma catecholamines and CSF biomarkers, and the good potential of those molecules in discriminating AD from non-AD patients, as shown by multiple logistic regression analysis. Secondly, our study lacks longitudinal observations to investigate the dynamics of plasma catecholamines concentrations overtime during disease evolution. This would help understanding their potential utility as predictors of MCI to AD conversion. Finally, the relation between brain catecholamines dysregulation and plasma catecholamines alteration during AD is currently not clear. Hitherto, it is still difficult to assess whether plasma catecholamines could be a good mirror of catecholaminergic dysregulation in the brain. It is important to notice that Raskind et al. found a linear correlation between $[NA]_{CSF}$ and $[NA]_{plasma}$ in their study (AD and controls subjects), which is consistent with the correlation that we found between plasma and CSF

concentration for NA and D in 10 AD patients from a cohort of our previous article [50]. This result supports the idea that catecholamines alterations in the brain and CSF could be also observed in the plasma. However, further studies are necessary to assess the relation between brain and plasma catecholamines in the context of AD. More details on our hypothesis linking $[NA]_{plasma}$ alterations and brain dysregulation in AD, that could be extend to A and D, are discussed in our previous article [50]. The dysregulation of plasma catecholamines concentrations during AD is a complex phenomenon as it might be the consequence of multiple interconnected physiological events (stage of the disease, compensation phenomenon, cognitive reserve effect, sympathetic system activity, etc.).

Conclusion

Based on our results and on previous literature, catecholamines seem to be good candidate to ameliorate early diagnosis. However, further investigations are needed to define a specific use of plasma catecholamines within the diagnostic pathway of AD. Our results open the possibility to explore new molecular mechanisms in order to better understand the physiopathology of this complex disease, and, by extension, to improve AD diagnosis, potentially helping the development of new drugs aimed at slowing down and/or stopping disease evolution.

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Competing Interests Statement

Alzohis is a company that has activities related to the submitted work. This study and this publication were produced and written in a responsible and ethical manner.

Ethics Approval and Consent to Participate

Patients gave their informed and written consent to have their samples stored in an officially registered and ethically approved biological collection that has been approved by the Ethics Committee of Paris University Hospitals (CEERB [Comité d'Ethique En Recherche Biomédicale], Bichat University Hospital, Paris, France).

Data Availability

The data that support the findings of this study are available from the corresponding author, Romain Verpillot, upon reasonable request.

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