Diagnostic Accuracy of Combined Measurement of Serum Homocysteine, C-Reactive Protein, and Serum Ferritin in Mild Cognitive Impairments and Alzheimer's Disease

Yiyang Li[1], Lin Duan[1], Yankui Shi[1], Ji Qi[1] and Tongtong Li[2]

[1]*Department of Clinical Laboratory, Affiliated Hospital of Hebei University, Baoding, China*
[2]*Department of Radiology, Affiliated Hospital of Hebei University, Baoding, China*

ABSTRACT

Objective: Evaluation of the diagnostic accuracy of combined detection of serum homocysteine (Hcy), C-reactive protein (CRP), and serum ferritin (SF) levels in patients with Alzheimer's disease (AD) and mild cognitive impairment (MCI).

Study Design: Descriptive study.

Place and Duration of the Study: Clinical Laboratory Section, Affiliated Hospital of Hebei University, Baoding, Heibei, China, from May 2022 to June 2023.

Methodology: Data of 120 patients with memory decline were retrospectively collected. They were divided into an MCI group and an AD group. A further 50 healthy participants were used as a normal control (NC) group. Differences in the Hcy, CRP, and SF levels between the three groups were evaluated. The specificity, accuracy, and sensitivity of these three indices in the combined or single diagnosis of AD and MCI were compared. Their associations with the severity of AD and MCI were also compared.

Results: The AD group had the highest levels of Hcy, CRP, and SF (18.79 ± 4.50, 6.35 ± 2.04, and 355.69 ± 120.36), followed by MCI (16.75 ± 3.06, 4.58 ± 2.31, and 203.48 ± 12.76), and NC group (14.32 ± 2.06, 2.06 ± 0.76, and 98.46 ± 5.06), with statistically significant differences (all $p < 0.001$). The diagnostic efficacy of AD for CRP was 98.50%, sensitivity was 96.00%, and specificity was 94.00%, which was higher than Hcy and SF. Tested together, the area under the ROC curve was 99.90%, specificity was 98.00%, and sensitivity was 98.00%. The diagnostic efficacy of SF for MCI had sensitivity of 100.00%, and specificity of 100.00%, which was higher than that of Hcy and CRP. When the three were combined for detection, the area under the curve of SF was 100.00%, sensitivity of 100.00%, and specificity of 100.00%. The levels of Hcy, CRP, and SF were positively correlated with the severity of AD ($p < 0.01$), while negatively correlated with the mini-mental state examination (MMSE) score ($p < 0.01$).

Conclusion: The combined detection of Hcy, CRP, and SF improved the diagnostic accuracy of comorbid AD and MCI.

Key Words: *Homocysteine, C-reactive protein, Cognitive impairment, Serum ferritin, Alzheimer's disease.*

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INTRODUCTION

Marked by a gradual decline in cognitive function, Alzheimer's disease (AD) is a neurodegenerative disease that affects the central nervous system (CNS).[1] Its pathology is defined by the development of intracellular neurofibrillary tangles (NFTs) and the build-up of amyloid plaques. Clinically, AD is characterised by manifestations of dementia, such as memory impairment, aphasia, apraxia, agnosia, and personality and behavioural disorders. There is a rising prevalence of this disorder as a result of the ageing of the worldwide population and changing lifestyle habits and dietary patterns.

Correspondence to: Dr. Lin Duan, Department of Clinical Laboratory, Affiliated Hospital of Hebei University, Baoding 071000, Heibei, China
E-mail: duanlin1030@163.com

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Currently, approximately 47 million people across the globe are affected by AD, and projections suggest that this number will exceed 131 million by the year 2050.[2] AD is a major disorder and a serious public health issue that profoundly affects the physical and mental health of older adults. The condition primarily affects older individuals and is the most prevalent form of dementia, representing approximately 60-80% of all cases.[3]

Globally, there has been a research emphasis on understanding, managing, and treating AD. Mild cognitive impairment (MCI) may arise for various reasons. However, in older adults, it may represent a transitional phase between the cognitive alterations associated with typical ageing and AD. Approximately 10% of those diagnosed with MCI progress to AD. Hence, timely MCI identification and intervention can be crucial in mitigating AD development.[4] As a trace element required by CNS, iron plays a crucial role in maintaining normal physiological functioning. Recent studies have shown a significant relationship between iron metabolism disorders and the development of AD.[5] Ferritin, a soluble tissue protein responsible for storing iron

in the body, has also been closely linked to neurodegenerative disorders.[6] High homocysteine (Hcy) levels have been identified as an AD risk factor and can worsen existing cognitive impairment.[7]

Accumulating evidence suggests that inflammation is heavily involved in brain ageing and neurodegeneration. Pathological investigations have revealed the presence of C-reactive protein (CRP) in amyloid plaques and NFTs in the brain tissue of patients with AD, indicating a possible role of CRP in the neuropathological process of AD. The objective of this study was to analyse and compare the levels of serum Hcy, CRP, and SF in patients with AD and / or MCI and healthy controls to evaluate the diagnostic accuracy of these three factors in patients with AD and MCI.

METHODOLOGY

Patients admitted to the Affiliated Hospital of Hebei University between May 2022 and June 2023 due to memory deficits were selected for inclusion in this descriptive study using the random number table method. They were categorised into two cohorts: An MCI cohort (n = 70) and an AD cohort (n = 50), based on specific diagnostic criteria and their scores on the mini-mental state examination (MMSE). A further 50 healthy individuals who had undergone physical examinations at the same facility between May 2022 and June 2023 were randomly selected for inclusion as a normal control (NC) cohort. This study was conducted in accordance with the tenets of the 2013 revision of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Affiliated Hospital of University (Approval No.: HDFYLL-KY-2022-020, approval date: December 30, 2022) and written informed consent to study participation was obtained from all participants. All data were collected from the hospital's outpatient and inpatient medical record systems.

Patients in the AD cohort fulfilled the diagnostic criteria for AD.[8] Patients in the MCI cohort fulfilled the diagnostic criteria for MCI.[9] Patients in the NC cohort were healthy individuals who had undergone physical examinations at the hospital during the study period, had no complaints of memory deterioration or other cognitive impairments, and had MMSE scores ≥27. All included patients were under the age of 70 years. Patients with speech, consciousness, and vision disorders that prevented physical examination, medical history taking, and/or full symptom evaluation were excluded. Patients with progressive neurological conditions such as Parkinson's disease were excluded. Patients with a history or current clinical manifestations of mental health conditions such as depression were excluded. Patients with blood or circulatory diseases, autoimmune diseases, and tumours were excluded. Patients with a history of traumatic brain injury or epilepsy were excluded. Patients with incomplete information on their medical records were also excluded.

After admission to the hospital, all participants had 3-4 ml of peripheral venous blood drawn on the first morning under fasting conditions as per the specimen collection requirements. This was then centrifuged at 3000 r/min for 10 min to separate the serum. The serum levels of Hcy, CRP, and SF were then measured using a fully automatic biochemical analyser. Hcy levels were measured by enzymatic cycling assay, CRP levels were determined by latex immunoturbidimetry, and SF levels were determined by chemiluminescence. All experiments were performed as per the instructions given on the instruments and reagents used, and the relevant experimental data were gathered for analysis.

MMSE was used to evaluate the degree of cognitive impairment and dementia in all participants. MMSE scores of 27-30 are considered within the normal range, scores of 21-27 indicate MCI, and the scores <21 indicate cognitive impairment.[10]

Mild AD manifests primarily in impaired language functioning, obvious memory impairments, reduced motivation for general activities, and either symptoms of depression or aggressive behaviour. Moderate AD manifests as increased forgetfulness, an inability to cook and clean independently, a gradual decline in self-care ability, gradually worsening aphasia, frequent aimless wandering, and other abnormal behaviours. Severe AD manifests as an inability to eat independently, inability to recognise family members or close friends, incontinence, difficulty in expressing thoughts verbally, and difficulty in walking. Patients at this stage are usually either dependent on a wheelchair for mobility or bedridden.[11]

Hcy, CRP, and SF levels of participants in the three cohorts were analysed and compared. The precision, accuracy, and sensitivity of MCI and AD detection based on increased levels of Hcy, SF, and CRP, and increased levels of all three combined were determined and compared. An analysis of the correlations between Hcy, CRP and, SF levels; MMSE scores and AD severity was performed.

All statistical analyses of the collected data were performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Measurement data were presented as mean ± standard deviation, while count data were presented as n (%). One-way ANOVA were used for intergroup comparisons. Ratio comparisons were performed using Chi-square tests, and ROC curve analysis was used to determine the area under the curve (AUC); to calculate sensitivity, specificity, and misdiagnosis rates; to determine the optimal diagnostic thresholds for Hcy, CRP, SF, and combined AD detection. The correlations between these biomarkers were examined using Pearson's correlation coefficient. A p-value of <0.05 were considered statistically significant.

RESULTS

No statistically significant differences were found between the socio-demographic characteristics of the three cohorts, and they could, therefore, be considered comparable.

Differences in the serum levels of Hcy, CRP, and SF were found between the three cohorts. Significant increases in all three indices were observed in the AD cohort and the MCI cohort compared to the NC cohort ($p<0.001$ for both, Table I).

Table I: Analysis of the differences in Hcy, CRP, and SF levels between patients with AD, patients with MCI and NC patients ($\bar{x}\pm s$).

Measures	AD group	MCI group	NC group	F	p-value
n	50	70	50		
Hcy (μmol/L)	18.79 ± 4.50	16.75 ± 3.06	14.32 ± 2.06	22.694	<0.001[△]
CRP (mg/L)	6.35 ± 2.04	4.58 ± 2.31	2.06 ± 0.76	64.78	<0.001[△]
SF (μg/L)	355.69 ± 120.36	203.48 ± 12.76	98.46 ± 5.06	193.86	<0.001[△]

[△]*One-way ANOVA. AD, Alzheimer's disease; CRP, C-reactive protein; Hcy, Homocysteine; MCI, Mild cognitive impairment; NC, Normal control; SF, Serum ferritin.*

Table II: Analysis of the diagnostic efficacy of Hcy, CRP, and SF levels, separately and in combination, for the detection of AD.

Measures		Cut-off	Sensitivity %	Specificity %	Yoden index rate %	AUC	95% CI	p-value
Hcy (μmol/L)	AD	16.50	82.00	90.00	0.720	0.843	0.756 - 0.930	<0.001
	MCI	16.50	45.70	90.00	35.70	0.717	0.626 - 0.808	<0.001
CRP (mg/L)	AD	3.30	96.00	94.00	0.900	0.985	0.956 - 1.000	<0.001
	MCI	2.70	84.30	84.00	68.30	0.864	0.793 - 0.936	<0.001
SF (μg/L)	AD	136.55	94.00	94.00	0.940	0.940	0.865 - 1.000	<0.001
	MCI	132.25	1.00	1.00	1.00	1.000	1.000 - 1.000	<0.001
Combined	AD	-	98.00	98.00	0.960	0.999	0.000 - 1.000	<0.001
detection	MCI	-	1.00	1.00	1.00	1.000	1.000 - 1.000	<0.001

AUC, Area under the curve; AD, Alzheimer's disease; CI, Confidence interval; CRP, C-reactive protein; Hcy, Homocysteine; SF, Serum ferritin.

Table III: Analysis of correlations between serum Hcy, CRP, and SF levels; AD severity and MMSE scores in patients with AD.

		Hcy (μmol/L)	CRP (mg/L)	SF (μg/L)
AD severity	r	0.850	0.505	0.468
	p	<0.01	<0.01	0.01
MMSE scores	r	-0.927	-0.684	-0.284
	p	<0.01	<0.01	0.02

AD, Alzheimer's disease; CRP, C-reactive protein; Hcy, Homocysteine; MMSE, Mini-Mental State Examination; SF, Serum ferritin.

Analysis of the diagnostic efficacy of the three biomarkers, both individually and in combination, for the detection of AD (Table II) found that the CRP exhibited an AUC of 98.50%, a sensitivity of 96.00%, and a specificity of 94.00%, outperforming SF and Hcy. Thus, CRP exhibited superior diagnostic efficacy for AD to SF and Hcy. Moreover, when the three were tested together, the AUC was 99.90%, the specificity was 98.00%, and the sensitivity was 98.00%. This was higher than the values obtained for Hcy, CRP, and SF alone. This indicates that the combination of the three markers has the best diagnostic efficacy.

Table II shows the diagnostic efficacy of single and integrated detection of MCI using the three variables. SF had an AUC of 100.00%, sensitivity of 100.00%, and specificity of 100.00%. These values were higher than those obtained for Hcy and CRP, suggesting that SF provides superior diagnostic efficacy for MCI to Hcy and CRP. When the three were tested together, the AUC was 100.00%, the sensitivity was 100.00%, and the specificity was 100.00%. These values were the same as those for SF and higher than those for Hcy and CRP alone, indicating that either the combined application of the three or SF has the best diagnostic efficacy.

Correlation analysis found a positive association between Hcy, CRP, and SF levels and AD severity, with the levels of all three increasing as the disease severity escalated ($p < 0.01$, Table III).

The authors found negative correlations between the Hcy, CRP, and SF levels and MMSE scores, with serum levels of all three decreasing as the MMSE scores increased ($p < 0.01$, Table III).

DISCUSSION

MCI and AD are pervasive clinical conditions characterised by psycho-behavioural symptoms. Approximately 90% of people diagnosed with AD and between 35-85% of individuals with MCI demonstrate at least one psycho-behavioural symptom.[12] AD is characterised pathologically by amyloid plaques, neuronal loss, amyloid angiopathy, and neurofibrillary tangles. Patients afflicted with AD exhibit persistent and progressive memory, speech and visuospatial deficits, and personality changes. However, the aetiology and pathogenesis of AD have not been fully elucidated. Several theories have been proposed to explain AD, including the β-amyloid cascade hypothesis, the Tau protein hypothesis, the false neurotransmitter hypothesis, the mitochondrial dysfunction hypothesis, and the inflammatory response hypothesis.[13]

MCI can serve as a transitional stage between typical cognitive abilities and the emergence of AD. The annual rate of MCI to AD conversion is between 10-15%. In a longitudinal study with a follow-up period of 6 years, an estimated 80% of MCI patients transitioned to AD.[14] The considerable diversity in the clinical presentations of MCI and AD makes the calculation of their respective risks and preliminary detection challenging. Therefore, identifying risk factors and biomarkers for MCI and AD is of great importance.

Homocysteine is an amino acid, generated through the demethylation of methionine. It is intricately linked to the onset of cerebrovascular diseases and correlated with AD. This is possibly related to the increased apoptosis and higher sensitivity to excitotoxicity that results from homocys-

teine-induced DNA damage.[15] Patients with either AD or vascular dementia have higher plasma homocysteine levels than normal controls. Analysis of the diagnostic accuracy of Hcy for MCI revealed an AUC of 83.1%, sensitivity of 84.870%, and specificity of 83.592%. This outcome validates elevated homocysteine as a risk factor for vascular dementia and AD and implies possible shared pathogenic mechanisms between the two conditions. It also offers new avenues for the treatment and prevention of dementia, as the reduction of plasma Hcy levels may potentially delay the progression of AD and vascular dementia. A previous study confirmed that patients with elevated serum Hcy demonstrate a progressive decrease in MMSE scores.[16] From this, it might be inferred that an association exists between serum Hcy levels and the onset of cognitive dysfunction. In concordance with previous findings, this research confirmed a direct association between Hcy concentrations and AD progression ($p < 0.01$), along with an inverse relationship between Hcy levels and MMSE scores ($p < 0.01$). Serum Hcy generates oxygen-free radicals *via* the activation of certain receptors. This leads to calcium ion overload, with consequent toxic effects on neurons. In turn, this impairs the hippocampal neuronal DNA repair function and simultaneously enhances neuronal sensitivity to the toxic effects of amyloid β-protein.

Ferritin is an organismal soluble tissue protein that plays a significant role in maintaining iron homeostasis. By binding to excess-free iron ions in the body, it effectively prevents iron overload due to degenerative changes, infections, tumours, metabolic disorders, and trauma. In addition, metabolic irregularities in ferritin are closely correlated with the onset of neurodegenerative disorders of the central nervous system.[17] The large increases in iron ions in the frontal cortices and basal ganglia of AD patients suggest that their binding proteins are involved in the pathologic process of AD. Serum ferritin (SF) levels are also significantly elevated in AD patients, suggesting abnormal intracellular ion channel status, impaired metabolic pathways, and increased production of endogenous iron ions. This leads to iron overload in neuronal organelles, contributing to neuron degeneration and necrosis and impaired cognitive function.[18] Increased SF levels are associated with more pronounced cognitive impairments and a greater likelihood of their progression to AD. The present study found SF to have diagnostic accuracy for MCI of 100.00%, with a sensitivity of 100.00%, and a specificity of 100.00%, surpassing the values obtained for Hcy and CRP. SF levels were positively correlated with MCI severity ($p < 0.01$) and inversely correlated with MMSE scores ($p < 0.01$). This was because SF levels in MCI patients gradually increase as the disease progresses, which is a protective mechanism to counteract the iron overload resulting from degenerative changes in the CNS. The mechanism prevents the deposition of iron ions in brain tissue while maintaining the iron ion production and metabolism homeostasis and generating more ferritin to bind with iron ions in the serum and cerebrospinal fluid. This helps reduce neuron degeneration, necrosis, and cerebral atrophy, while also delaying further cognitive impairments and progression to AD.

Serum CRP is an important biological marker of inflammation, with elevated CRP levels indicating an inflammatory response in the body. Studies have shown elevated CRP expression to be associated with atherosclerosis and cerebrovascular disease; it may, therefore, also be relevant to vascular dementia.[19] The CRP levels in the AD cohort of the present study were significantly higher than those in both the MCI cohort and the NC cohort ($p = 0.001$). Additionally, CRP levels were positively correlated with AD severity ($p < 0.01$) and negatively correlated with MMSE scores ($p < 0.01$). These findings align with previous research outcomes. They also suggest that a preliminary diagnosis of AD and MCI may be made using serum CRP levels. It has been posited that the inflammatory response is a vital contributor to the onset and progression of AD.[20] Natale *et al.* have suggested that CRP detection may be a means of differentiating AD from other dementias.[21] Studies suggest that the markedly elevated CRP levels in patients with AD are probably due to vascular endothelial damage and endothelial dysfunction.[22] However, the specific mechanisms of action of AD remain undetermined. Given the multifactorial aetiology of cognitive impairment and AD, a single test would likely be flawed. The present study utilises the AUC, specificity, and sensitivity to confirm the obvious advantages of the integration of the above three measures for AD detection.

This study had some limitations. First, the sample size was relatively small, and the present study's results need to be verified with a larger sample. Second, the authors did not conduct a correlation analysis of serological tests and imaging indices. In future, the authors intend to incorporate the findings of other relevant studies to gain deeper insights into the correlations between serologic tests and pathological neurological changes. By making such improvements, the authors hope to better serve both clinicians and patients.

CONCLUSION

Hcy, CRP, and SF are useful tools in the preliminary differential diagnosis of cognitive impairment and AD. The severity of AD patients was higher than that of the MCI patients. The integrated use of all three for AD detection was found to result in higher diagnostic efficacy. As serum levels of Hcy, CRP, and SF were found to correspond to the severity of the disease, they are of clinical value in predicting disease severity.

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ETHICAL APPROVAL:
This study was conducted in accordance with the tenets of the 2013 revision of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Affiliated Hospital of Hebei University (Approval No.: HDFYL-L-KY-2022-020, Approval Date: December 30, 2022).

PATIENTS' CONSENT:
Written informed consent to study participation was obtained from all participants.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
YL, LD: Carried out the studies, participated in collecting data, and drafted the manuscript.
YS: Participated in the design of the study and performed the statistical analysis.
JQ, TL: Participated in acquisition, analysis, and interpretation of data, and drafted the manuscript.
All authors approved the final version of the manuscript to be published.

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