

Characteristic Biomarker and Cognitive Profile in Incipient Mixed Dementia

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Abstract.

Background: Research has shown that mixed dementia is more common than previously believed but little is known of its early stages.

Objective: To examine if incipient mixed dementia can be differentiated from incipient Alzheimer's disease (AD) and subcortical ischemic vascular dementia (SVD) using neuropsychological tests, cerebrospinal fluid (CSF) markers, and magnetic resonance imaging markers.

Methods: We included 493 patients and controls from the Gothenburg MCI study and used the dementia groups for marker selection (CSF total-tau (T-tau), phospho-tau (P-tau), and amyloid- β_{42} ($A\beta_{42}$), 11 neuropsychological tests, and 92 regional brain volumes) and to obtain cut-off values which were then applied to the MCI groups.

Results: Incipient mixed dementia was best differentiated from incipient AD by the Word fluency F-A-S test and the Trail making test A. CSF T-tau, P-tau, and $A\beta_{42}$ differentiated incipient mixed dementia from incipient SVD.

Conclusion: Incipient mixed dementia is characterized by an AD-like biomarker profile and an SVD-like cognitive profile. Incipient mixed dementia can be separated from incipient AD and incipient SVD using CSF markers and cognitive testing.

Keywords: Alzheimer's disease, cerebrospinal fluid, magnetic resonance imaging, mild cognitive impairment, neuropsychological test, vascular dementia

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia followed by vascular dementia, where subcortical ischemic vascular dementia (SVD) is the most common subtype [1, 2]. Mixed dementia,

i.e., AD with cerebrovascular disease, increases in prevalence in older patients and is generally regarded as the third most common cause of dementia but its prevalence may be underreported. [3, 4]. While the biomarker and clinical manifestations of the individual diseases AD and SVD are fairly well characterized, the presence of concomitant pathologies presents a clinical challenge. Although the clinical profile of mixed dementia has been described, its pattern of biomarker deviations and neuropsychological

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44 deficits have not been fully established, especially so
45 for the early phases of the disease [5].

46 Several studies have examined differential diag-
47 nostics in dementia patients using biomarkers and
48 cognitive testing [6]. Pathological levels of the cere-
49 brospinal fluid (CSF) markers amyloid- β ($A\beta$)₄₂,
50 total-tau (T-tau), and phosphorylated tau (P-tau) are
51 typical for AD but deviation from normal levels may
52 also be seen in SVD [7]. Although markers such
53 as neurofilament light protein and CSF/serum albu-
54 min ratio have been examined, there are currently no
55 reliable disease-specific CSF markers for SVD [8].
56 Explicit cognitive profiles have been established in
57 AD and SVD, and are reflected in the criteria used to
58 diagnose the diseases. SVD is further characterized
59 by the presence of white matter changes (WMC),
60 which can easily be detected using magnetic reso-
61 nance imaging (MRI).

62 Less is known, however, about differential diag-
63 nostics in the mild cognitive impairment (MCI) stage.
64 The many studies of incipient AD have encompassed
65 neuroimaging, cerebrospinal fluid (CSF) markers and
66 cognitive testing and have led to the discovery of
67 several important predictors of the risk of progres-
68 sion to AD [9]. In contrast, only a few studies have
69 included MCI patients with aetiologies other than
70 AD, and in most of these incipient SVD and mixed
71 dementia have shared the same biomarker and cog-
72 nitive characteristics as their manifest stages [10-12].
73 Furthermore, these studies have described deviations
74 in biomarkers and cognition in MCI subpopulations,
75 there is presently no study that has evaluated the re-
76 lative benefit of commonly used disease markers for
77 the differentiation of incipient mixed dementia from
78 incipient AD or SVD.

79 Moreover, in terms of mixed dementia, there is an
80 increasing interest and an ongoing debate whether
81 the AD-like and cerebrovascular pathological pro-
82 cesses have an independent or interactive effect
83 on the decline of cognitive functioning [13]. Some
84 researchers have found that AD and cerebrovascular
85 disease contribute independently to cognitive impair-
86 ment [14], but others have reported an interactive
87 effect [15].

88 Since the biomarker and cognitive profile of incip-
89 ient mixed dementia has not been determined, we
90 intend to investigate how incipient mixed dementia
91 could be best distinguished from AD and SVD, using
92 sequential CSF samples, structural imaging markers
93 and a neuropsychological test battery in consecutive
94 memory clinic patients and controls. Additionally,
95 in incipient mixed dementia, we examined if marker

96 levels suggested an independent or interactive effect
97 of AD and cerebrovascular pathology on cognitive
98 functioning.

99 MATERIAL AND METHODS

100 *The Gothenburg MCI study*

101 The Gothenburg MCI study is a clinically based
102 longitudinal study that aims at identifying neu-
103 rodegenerative and vascular disorders prior to the
104 development of dementia [16]. The Gothenburg MCI
105 study was approved by the local ethics committee
106 in Gothenburg (approval number: L091-99, 1999;
107 T479-11, 2011). Participants (or their close relatives)
108 gave their consensus for participating in the study
109 as well as for future results being published, which
110 was directed in accordance with the provisions of
111 the Helsinki Declaration. Inclusion required verifi-
112 cations of a progressive cognitive impairment for
113 more than 6 months, age ≥ 50 and ≤ 79 years, and
114 Mini-Mental State Examination (MMSE) score > 18 .
115 Exclusion criteria were acute/unstable somatic dis-
116 ease, severe psychiatric disorder, or substance abuse.
117 For an overview of the findings based on the Gothen-
118 burg MCI study, please see [17].

119 Healthy controls were primarily recruited from
120 senior citizens' organisations. Inclusion and exclu-
121 sion criteria were the same as for the patients with the
122 exception that controls were not included if they had
123 subjective or objective signs of cognitive disorders.

124 *Classification*

125 The patients' degree of cognitive decline was
126 staged according to the Global Deterioration Scale
127 (GDS) [18]. The GDS classifications were made
128 by means of the following instruments: Vari-
129 ables 13-20 of the Stepwise Comparative Status
130 Analysis (STEP) [19] (i.e., memory disturbance, dis-
131 orientation, reduced abstract thinking, visuospatial
132 disturbance, poverty of language, sensory apha-
133 sia, visual agnosia and apraxia); IFlex, which is a
134 short form of the Executive Interview (EXIT) [20]
135 (i.e., number-letter task, word fluency, anomalous
136 sentence repetition, interference task, Luria hand
137 sequences and counting task); MMSE [21]; and
138 Clinical Dementia Rating (CDR) [22]. The CDR
139 assessment was based on information from both the
140 patient and an informant. The guidelines for the
141 classification were as follows: For GDS 3 (MCI)
142 participants should have MMSE ≥ 26 , CDR > 0.5 ,

I-FLEX ≤ 3 and one or less positive outcomes on variables 13-20 of STEP, and GDS 4 (mild dementia) participants should have MMSE ≤ 25 , CDR > 1.0 , STEP > 1 , I-FLEX > 3 . When the guidelines were not applicable, a consensus decision among the physicians at the clinic was made to determine the appropriate GDS score. Participants classified as GDS 4 were further classified according to the following etiological dementia criteria; AD according to the NINCDS-ADRDA criteria [23] and SVD according to the Erkinjuntti criteria [24]. For mixed dementia, AD criteria had to be fulfilled as well as moderate/severe WMC (Fazekas score ≥ 2) on MRI or mild WMC in combination with a marked frontosubcortical-dysexecutive syndrome. The clinician who set the dementia diagnoses had access to MRI images but was blinded to volumetric data, neuropsychological test results and CSF biomarker data. The guidelines and diagnostic procedures have been described in detail previously [16]. Vascular burden was classified as two or more vascular risk factors (arterial hypertension, symptomatic arterial hypotension, congestive heart failure, angina pectoris, cardiac dysrhythmia, myocardial infarction, transient ischemic attack, stroke (also silent), hyperlipidemia, diabetes mellitus, and claudicatio intermittens) and the presence of not insignificant WMC.

The study population

The study population ($N=493$) consisted of 152 patients with dementia at baseline (83 with AD, 46 with mixed dementia, and 23 with SVD), 85 patients with MCI that converted to dementia at subsequent follow-ups (of which 43 converted to AD (MCI-AD), 26 converted to mixed dementia (MCI-mix), and 16 converted to SVD (MCI-SVD)), 130 MCI patients that remained cognitively stable until last follow-up (MCI-MCI), and 126 healthy controls. Study participants were included between 1999 and 2014. Patients with other forms of dementia than AD, SVD or mixed dementia at baseline or follow-up were not included in the study. The presented follow-up times are time until conversion for MCI patients subsequently converting to dementia, and time until last follow-up examination for the other groups.

Cerebrospinal fluid and genetic analysis

CSF samples were collected by lumbar puncture, which were performed in the morning to exclude influence on the results from possible diurnal

fluctuations in biomarker levels. Twenty milliliter (mL) of CSF were collected in a polypropylene tube and immediately transported to the local laboratory for centrifugation at $2\ 000\times g$ at room temperature for 10 minutes. The supernatant was collected, gently mixed to avoid possible gradient effects, and aliquoted in 0.5 mL aliquots in screw-cap polypropylene tubes that were stored at -80°C , without being thawed and refrozen, pending biochemical analyses. CSF levels of total (T)-tau, phosphorylated (P)-tau181, and amyloid- β amino acids 1 to 42 ($A\beta_{42}$) were determined using sandwich enzyme-linked immunosorbent assays (INNOTEST[®] hTau Ag, INNOTEST[®] PHOSPHO-TAU_(181P), and INNOTEST[®] β -AMYLOID₍₁₋₄₂₎, respectively) from Innogenetics, Gent [25]. The CSF samples were processed at the Clinical Neurochemical Laboratory at Gothenburg University as part of clinical routine on multiple occasions during the course of the study. The analytical variability was low [26]. To obtain apolipoprotein E (*APOE*) $\epsilon 4$ status, blood was drawn from all study participants and subsequently analysed using solid-phase minisequencing.

Neuroimaging

MRI data from a 1.5 T scanner (Siemens Symphony, Erlangen, Germany) was available for a subset of participants: Dementia group: 73 patients (i.e., AD: 41, mixed dementia: 25, SVD: 7), MCI converting to dementia: 31 patients (i.e., MCI-AD: 17, MCI-mix: 11, MCI-SVD: 3), stable MCI: 84 patients and controls: 55 participants.

Volumetry was performed on T1 3D IR/GR images (repetition time 1610 ms, echo time 2.38 ms, flip angle 15° , coronal slices, field of view 250×203 mm, slice thickness 1 mm, pixel spacing 0.49×0.49 mm, matrix size 512×416).

Brain volumes were measured using the automated segmentation software FreeSurfer version 5.3.0. The FreeSurfer analyses were performed on a computing cluster running 64 bit CentOS 6. These analyses were performed on nodes based on Supermicro X9DRT Intel E5-2670 (Sandy Bridge) running at 2.6 GHz. A few analyses were also performed using a MacPro 3.1 with 64bit 2x2.8 GHz quad-core Intel Xeon processors and Mac OSX 10.8.5.

Once the analysis process had finished, subcortical volumes were extracted using the `aparcstats2table` script. Quality control of the FreeSurfer output was performed using the FreeSurfer graphical user interface Freeview (<https://surfer.nmr.mgh.harvard.edu/fs>)

Table 1
Demographic data

Group	N	Age	MMSE score	Male sex, %	APOE ε4, %	Education, y	F-U time, months	WMC volume, cm ³	Vascular burden, %
AD	83	67.2 ± 7.1	23.5 ± 3.5 [†]	29	67 [†]	10.8 ± 3.5 [†]	15.5 ± 17.3	4.3 ± 2.9	13
Mixed	46	71.2 ± 6.0 [†]	23.8 ± 3.0 [†]	30 [†]	67 [†]	10.7 ± 3.4	11.5 ± 16.8	9.0 ± 6.1	46
SVD	23	70.3 ± 7.1 [†]	25.0 ± 2.3 [†]	74 [†]	39	11.4 ± 3.3	14.8 ± 17.2	17.0 ± 9.4 [†]	78 [†]
Controls	126	65.4 ± 7.5	29.4 ± 1.2	38	32	12.2 ± 2.9	10.2 ± 19.8	4.1 ± 6.1	19
MCI-AD	43	66.1 ± 6.4	27.8 ± 1.3	44	72*	11.8 ± 3.5	27.1 ± 12.3	4.3 ± 6.9	22
MCI-Mix	26	70.0 ± 6.3*	26.9 ± 2.2*	31	69*	11.2 ± 4.1	25.7 ± 9.7	8.0 ± 5.3	38
MCI-SVD	16	68.8 ± 7.5	28.3 ± 1.7	56	50	11.9 ± 3.8	38.7 ± 29.9	23.8 ± 7.8*	94*
MCI-MCI	130	65.4 ± 8.1	28.3 ± 1.5	44	38	11.3 ± 3.4	37.8 ± 25.3	5.4 ± 6.6	38

Dementia groups (AD, Mixed, and SVD) were compared with controls. Converting MCI subgroups (MCI-AD, MCI-Mix, and MCI-SVD) were compared with MCI-MCI. APOE ε4 %, Percentage of study participant with at least one APOE ε4 allele; F-U time, follow up time (time until conversion for converting MCI groups, total follow up time for the other groups); WMC volume, white matter changes volume; Vascular burden, Percentage of study participants with at least two vascular risk factors and not insignificant WMCs. [†]p-value<0.05 versus Controls after Bonferroni correction. *p-value<0.05 versus MCI-MCI after Bonferroni correction.

wiki/FreeviewGuide/FreeviewIntroduction). Minor errors were accepted but larger errors resulted in the exclusion of the affected region. The quality controller was blinded to group belonging and other patient data. Only a few patients had more than one region excluded from the analyses. The following data were excluded: temporal lobes: AD: 8 left, 12 right, mixed dementia: 3 left, 7 right, SVD: 1 left, 1 right, MCI-AD: 1 left, 3 right, MCI-mix: 2 left, 3 right, MCI-SVD: 1 right, MCI-MCI: 10 left, 19 right, controls 5 left, 14 right; frontal lobes: AD: 2 left, 1 right, mixed dementia: 1 left, MCI-AD: 1 left, MCI-mix: 1 left, 1 right, MCI-MCI: 5 right, controls: 1 left, 3 right.

FreeSurfer measurements for white matter “hypointensities” (i.e., WMC) are included in Table 1 but were excluded from further analysis to avoid circularity as visual assessment of WMC is included in the diagnostic procedure. All FreeSurfer volumes were normalized using FreeSurfer’s estimate of intracranial volume (eTIV). The residual normalization method has been described in a previous publication [27]. Briefly, a regression analysis was performed in the control group to obtain a regression coefficient (k) reflecting the association between eTIV and the region of interest, which was then applied to the study sample.

Neuropsychological assessment

The Gothenburg MCI study includes a comprehensive neuropsychological test battery. The tests and testing procedure have previously been described in detail [28]. The following tests were analysed in the present study: Trail Making Test (TMT) A and B, Rey

Auditory Verbal Learning Test (RAVLT, we applied the following measures: total learning (RAVLT TL), trials 1-5 and delayed recall, (RAVLT DR), Rey Complex Figure (RCFT) recall, Visual Object and Space Perception (VOSP) Silhouettes subtest, Rey Complex Figure copy and recall, Token test, Word fluency F-A-S, Parallel Serial Mental Operations (PASMO), and Stroop Test Victoria version. Not all study participants completed the neuropsychological test battery, i.e., 35 from the dementia groups, 44 from the control group, 20 from the MCI-MCI group, 4 from the MCI-AD group, 2 from the MCI-Mix group.

Statistical analyses

Demographic differences were analyzed using the unpaired *t*-test (age, MMSE, and years of education) and χ^2 (APOE ε4 status and sex). Patients with dementia at baseline were compared with healthy controls. Patients with MCI subsequently converting to dementia were compared to stable MCI patients. Group differences in biomarkers were evaluated using analysis of covariance (ANCOVA) with age as a covariate (age and years of education for neuropsychological tests). Significance levels presented in Tables 1 and 2 were corrected for multiple comparisons within each group using the Bonferroni method.

Variable selection and cut-off scores

Variable selection and generation of cut-off scores was performed using the dementia groups (AD, mixed dementia, and SVD) with the control group to derive the best discriminating variables and their cut-off values, which were then applied to the converting

Table 2
Baseline data in converting MCI groups (MCI-AD, MCI-Mix, and MCI-SVD)
compared to MCI-MCI for variables obtained from variable selection

	MCI-AD	MCI-Mix	MCI-SVD	MCI-MCI
N	43	26	16	130
A β ₄₂	447 ± 132*	408 ± 110*	529 ± 172	652 ± 239
T-tau	744 ± 451*	660 ± 304*	365 ± 156	356 ± 214
P-tau	91 ± 41*	76 ± 24*	51 ± 19	56 ± 23
3rd ventricle vol.	1.6 ± 0.7			1.7 ± 0.8
Left ERC	1.7 ± 0.1			1.9 ± 0.1
R. caudate vol.		3.0 ± 1.0		3.5 ± 0.8
R. paracentral vol.		3.2 ± 0.6		3.4 ± 0.7
RAVLT TL	30 ± 9*			39 ± 10
Token test		17 ± 4*		19 ± 2
F-A-S	36 ± 10			38 ± 13
RCF copy	29 ± 8*			32 ± 4
RAVLT DR		2.7 ± 3*		7.1 ± 4
TMT A			59 ± 17*	44 ± 15

CSF markers (ng/L), volumetric data (cm³) and neuropsychological test scores are presented as mean value ± SD. Data is only presented for the variables. Left ERC, left entorhinal cortex volume; R. caudate vol., right caudate volume; R. paracentral vol., right paracentral volume.

**p*-value < 0.05 versus MCI-MCI after Bonferroni correction.

MCI groups and the stable MCI group. Volumetric variables and test scores were analyzed separately, and only three variables from each modality were used to complement the three CSF markers available. For variable selection, we performed a forward stepwise logistical regression (likelihood ratio) to select the best discriminating variables in up to three steps. We used all volumes calculated by FreeSurfer except for “WM-hypointensities”, totaling 92 variables.

For AD, left entorhinal cortex volume, 3rd ventricle and left hippocampus had the highest explained variance with a Nagelkerke R^2 of 0.69; RAVLT TL, F-A-S total score, and RCFT copy score had an R^2 of 0.84. For mixed dementia, left hippocampal volume, right caudate volume and right paracentral volume had a R^2 of 0.77; Token test and RAVLT DR had a R^2 of 0.711. For SVD, right lateral ventricle and right posterocingulate volume had an R^2 of 0.78; TMT A had an R^2 of 0.51.

Receiver operating characteristic (ROC) analysis was then performed for each variable to calculate cut-off values based on the maximum of the sum of sensitivity and specificity. See Supplementary Table 1 for mean values and their corresponding area under the curve (AUC) and derived cut-off values. These cut-offs were used to calculate AUC, sensitivity/specificity and positive likelihood ratio where cut-offs from AD/control groups were applied to MCI-AD/MCI-MCI groups, mixed dementia/control groups to MCI-Mix/MCI-MCI groups etc. To provide complementary predictive information, all variables

were z-transformed and then entered into a logistical regression that also included age as a covariate. The results from the ROC analyses and logistical regressions are displayed in Table 3.

Additionally, we performed a direct comparison between the converting MCI groups using ANCOVA with age as a covariate (age and education for neuropsychological tests) to investigate if differences seen between the converting and stable MCI groups could also be detected between the converting MCI groups. Finally, we produced Kaplan-Meier curves to illustrate differences in survival between MCI patients with pathological or non-pathological values of selected markers based on the cut-off values derived from the dementia groups and control group (for A β ₄₂/T-tau ratio we used AD/controls, for WMC volume we used SVD/controls comparisons) The cut-off values from were then applied to the MCI-AD/MCI-MCI group etc. All analyses were performed using IBM SPSS software (version 19.0).

RESULTS

Demographic data of the study is presented in Table 1, where patients with dementia diagnoses (AD, mixed dementia, and SVD) were compared to controls and MCI patients converting to AD, mixed dementia or SVD (MCI-AD, MCI-Mix, and MCI-SVD) were compared with patients of stable MCI (MCI-MCI). Patients with AD and mixed dementia

Table 3
Predictive values

	MCI-AD versus MCI-MCI	MCI-mix versus MCI-MCI	MCI-SVD versus MCI-MCI
Aβ₄₂			
AUC (Sens, spec) %	69 (67, 70)	72 (64, 70)	62 (94, 29)
Pos LR (95% CI)	2.2 (1.6-3.2)	2.8 (2.0-3.9)	1.3 (1.1-1.6)
Odds ratio (95% CI)	4.2 (2.2-7.7)	5.7 (2.3-14.1)	1.8 (0.9-3.5)
T-tau			
AUC (Sens, spec) %	78 (81, 77)	75 (60, 78)	63 (92, 23)
Pos LR (95% CI)	3.6 (2.4-5.2)	2.7 (1.7-4.4)	1.4 (1.1-1.7)
Odds ratio (95% CI)	4.0 (2.1-7.6)	3.8 (1.9-7.4)	1.1 (0.4-2.7)
P-tau			
AUC (Sens, spec) %	79 (67, 84)	81 (45, 89)	55 (85, 40)
Pos LR (95% CI)	4.2 (2.5-6.9)	4.1 (2.0-8.5)	1.4 (1.1-1.9)
Odds ratio (95% CI)	6.1 (3.1-11.8)	2.6 (1.3-5.2)	2.1 (0.6-6.8)
Left HC			
AUC (Sens, spec) %	67 (47, 71)	69 (91, 64)	
Pos LR (95% CI)	1.7 (0.9-3.0)	2.6 (1.8-3.6)	
Odds ratio (95% CI)	1.5 (0.9-2.4)	3.0 (1.5-6.0)	
3rd ventricle			
AUC (Sens, spec) %	56 (53, 57)		
Pos LR (95% CI)	1.2 (0.7-2.1)		
Odds ratio (95% CI)	1.2 (0.7-2.0)		
Left ERC			
AUC (Sens, spec) %	68 (36, 75)		
Pos LR (95% CI)	1.45 (0.6-3.3)		
Odds ratio (95% CI)	1.1 (0.6-2.2)		
Right caudate volume			
AUC (Sens, spec) %		80 (27, 87)	
Pos LR (95% CI)		2.1 (0.7-6.3)	
Odds ratio (95% CI)		1.8 (0.98-3.4)	
Right paracentral volume			
AUC (Sens, spec) %		60 (80, 25)	
Pos LR (95% CI)		1.1 (0.8-1.5)	
Odds ratio (95% CI)		1.2 (0.7-1.8)	
RAVLT TL			
AUC (Sens, spec) %	75 (44, 87)		
Pos LR (95% CI)	3.3 (1.8-6.0)		
Odds ratio (95% CI)	4.1 (2.1-8.2)		
RAVLT DR			
AUC (Sens, spec) %		74 (72, 75)	
Pos LR (95% CI)		2.8 (1.9-4.2)	
Odds ratio (95% CI)		5.4 (2.3-12.4)	
F-A-S			
AUC (Sens, spec) %	56 (38, 63)		
Pos LR (95% CI)	1.0 (0.7-1.6)		
Odds ratio (95% CI)	1.4 (0.8-2.3)		
RCF copy			
AUC (Sens, spec) %	74 (38, 85)		
Pos LR (95% CI)	2.6 (1.4-4.9)		
Odds ratio (95% CI)	1.8 (1.2-2.9)		
Token test			
AUC (Sens, spec) %		73 (65, 75)	
Pos LR (95% CI)		2.6 (1.6-4.0)	
Odds ratio (95% CI)		3.0 (1.5-6.0)	
TMT A			
AUC (Sens, spec) %			70 (78, 69)
Pos LR (95% CI)			2.5 (1.5-4.2)
Odds ratio (95% CI)			6.9 (2.0-24.7)

AUC, area under the curve; Sens, sensitivity; Spec, specificity; Pos LR, positive likelihood ratio; CI, confidence interval; Left HC, left hippocampal volume; Left ERC, left entorhinal cortex volume.

at baseline or follow-up were more likely than the control and stable MCI groups to carry the *APOE* $\epsilon 4$ allele. Although small in terms of mean difference, MCI-Mix had lower MMSE scores compared to MCI-MCI. Women were generally overrepresented in all groups except for the SVD group. Sex and years of education had no impact on conversion to dementia but had an effect on some of the dementias compared to controls.

Table 2 displays the baseline data for CSF markers as well as FreeSurfer volumes and neuropsychological tests derived from variable selection. There was a clear difference between the converting MCI groups and the stable MCI group in many of the selected markers. Both MCI-AD and MCI-Mix were different from MCI-MCI for all the tested CSF biomarkers, while the MCI-SVD group did not differ from the MCI-MCI group. For the imaging data, the left hippocampus of the MCI-Mix group differed from the MCI-MCI group; otherwise there were no differences between groups. Most cognitive tests differed between converters and the stable MCI group.

Table 3 shows predictive values for the selected markers using the cut-off values derived from the dementia-controls comparisons. Again, CSF markers and neuropsychological tests performed better in the MCI-AD group and the MCI-Mix groups. For the MCI-AD and MCI-Mix, P-tau and $A\beta_{42}$ were the best predictors, while for the MCI-SVD group TMT A provided the best predictive value.

A direct comparison between the MCI subgroups that developed dementia is presented in Table 4. The

CSF markers separated between MCI-AD/MCI-Mix and MCI-SVD. MCI-AD and MCI-Mix were best differentiated using hippocampal volume, and the TMT A and F-A-S tests. The imaging markers were the least discriminating modality; the only between-group difference was the smaller left hippocampal volume in the MCI-Mix group compared to the other groups.

Figure 1 displays survival curves for the converting MCI groups individually compared to the MCI-MCI group. It shows that using a cut-off derived from AD/controls, MCI patients converting to mixed dementia are more likely to have pathological levels of $A\beta_{42}$ /T-tau ratio compared than converters to SVD, but less likely than converters to AD. It also shows that pathological levels of WMC is as common in MCI-Mix as in MCI-SVD.

DISCUSSION

Our main objective was to investigate if incipient mixed dementia could be distinguished from the incipient forms of AD and SVD using neuropsychological tests, brain volumetric and CSF markers. We found that incipient mixed dementia was characterized by an AD-like biomarker pattern combined with an SVD-like cognitive profile. Similar findings have been reported in studies of patients with manifest dementia, but this is the first comprehensive study to report such findings on a group of patients with incipient mixed dementia. Although $A\beta_{42}$ levels were lowest in the MCI-Mix group, intermediate levels of P-tau, T-tau and WMC suggest an interactive effect on cognitive functioning from AD pathology and SVD pathology in the MCI-Mix group.

The CSF markers did not differentiate between MCI-AD and MCI-Mix, but CSF P-tau and T-tau, unlike $A\beta_{42}$ in the MCI-AD group, effectively discriminated the former groups from both MCI-MCI and MCI-SVD. The low levels of $A\beta_{42}$ in the MCI-Mix group was unexpected but a recently published cross-sectional study has also found lower than expected levels of $A\beta_{42}$ in a group of memory clinic patients with both AD and cerebrovascular pathology [29]. Additionally, albeit CSF $A\beta_{42}$ is considered a marker for $A\beta$ deposition in the brain, P-tau seem to be a more AD specific marker which is in line with our findings [30]. WMC, the hallmark of SVD, have been linked to deviations in levels of several CSF $A\beta$ isoforms [31, 32], but as WMC are present in mixed dementia as well as in normal aging, these

Table 4
Direct comparison between the converting groups

	MCI-AD	MCI-Mix	MCI-SVD
N	43	26	16
$A\beta_{42}$	447 ± 132	408 ± 110 [†]	529 ± 172*
T-tau	744 ± 451 [†]	660 ± 304 [†]	365 ± 156*
P-tau	91 ± 41 [†]	76 ± 24 [†]	51 ± 19*
Left hippocampus	3.1 ± 1.0*	2.3 ± 0.8 [†]	3.3 ± 0.3*
3 rd ventricle	1.6 ± 0.7	1.8 ± 0.9	2.3 ± 0.6
L. ERC	1.7 ± 0.6	1.7 ± 0.7	1.8 ± 0.3
R. caudate volume	3.1 ± 1.0	3.0 ± 1.0	3.4 ± 0.6
R. paracentral volume	3.5 ± 0.9	3.2 ± 0.6	3.9 ± 0.2
RAVLT TL	30 ± 9	28 ± 9	29 ± 9
Token test	19 ± 2	17 ± 4	17 ± 4
F-A-S	36 ± 10* [†]	30 ± 12	28 ± 9
RCF copy	29 ± 8	30 ± 4	28 ± 4
RAVLT DR	2.7 ± 3 [†]	2.7 ± 3 [†]	4.9 ± 3*
TMT A	49 ± 15* [†]	57 ± 27	59 ± 17

CSF markers (ng/L), volumetric data (cm³) and neuropsychological test scores are presented as mean values ± SD. **p*-value < 0.05 versus MCI-mix, [†]*p*-value < 0.05 versus MCI-SVD.

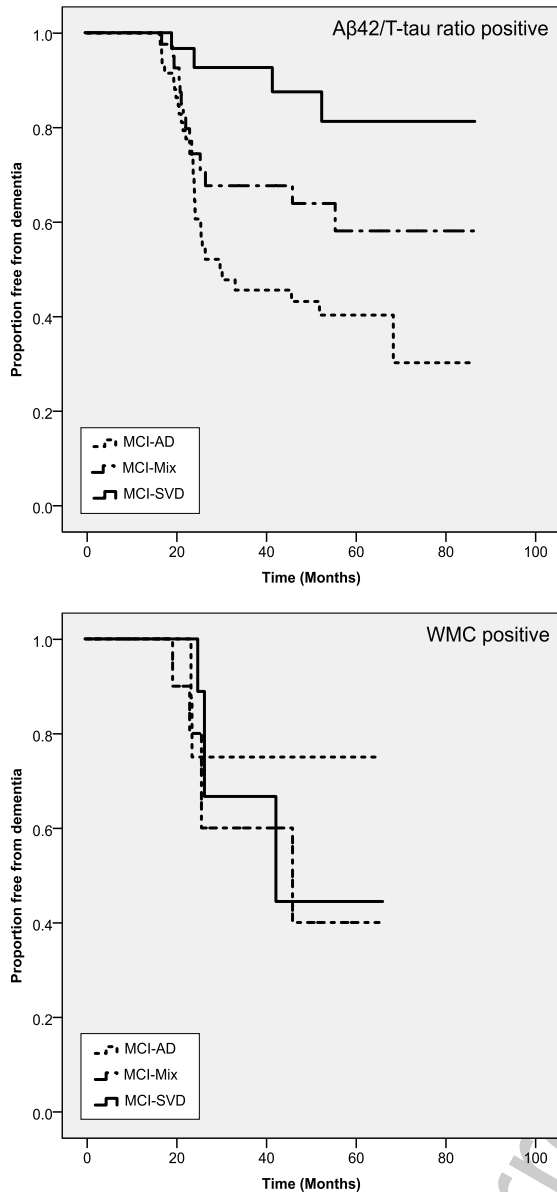


Fig. 1. Survival curves stratified by levels of $A\beta_{42}/T$ -tau ratio and WMC. Only patients with pathological values are displayed. Marker values were designated pathological or non-pathological based on the cut-off scores derived from dementia-control group comparisons. X axis shows proportion of non-demented patients at a given time (months) as shown on the y axis.

findings are difficult to interpret. CSF markers that more accurately reflect the pathological processes in SVD have been tested [33, 34], but are currently not widely available and need to be validated further.

The imaging markers analyzed provided the least value in terms of positive likelihood ratio and odds ratio for separating converting MCI from stable MCI.

These findings should be interpreted with caution, however, as MRI data was missing for some study participant which could have affected the results. For MCI-AD, the resulting markers from the variable selection (hippocampus, entorhinal cortex, and 3rd ventricle) directly or indirectly reflect central atrophy in areas affected by early accumulation of AD pathology. The markers in the MCI-Mix group were, however, less predictable, assigning hippocampal volume, caudate nucleus and right paracentral volume as the best markers. Surprisingly, the left hippocampus did in this study differentiate MCI-Mix from MCI-AD and MCI-SVD. While hippocampal atrophy is an established marker of AD, the small volumes in the MCI-Mix group could reflect an interactive effect from both AD pathology and WMC on hippocampal volume [35, 36]. The proposed mechanism is deafferentation of neuronal tracts by WMC, resulting in atrophy and reduced function of the affected region [37, 38].

The three derived neuropsychological tests from the AD group represent functions previously linked to AD, i.e., memory, verbal fluency, and visuo-spatial function. Although episodic memory impairment (RAVLT TL) best discriminated MCI-AD from MCI-MCI, MCI-AD outperformed MCI-Mix/MCI-SVD on phonemic verbal fluency (F-A-S). Previous studies have found that while both semantic and phonemic fluency are deteriorated in SVD, phonemic verbal fluency is relatively preserved in AD compared with SVD [39]. Impaired phonemic fluency in MCI has been described previously but has not been deemed a strong predictor for subsequent conversion to AD [40]. Our finding of impaired phonemic fluency in MCI patients with prodromal AD compared to stable MCI might be less important than the finding that the test seems to effectively discriminate AD from mixed dementia/SVD. Whereas the RAVLT TL suggested risk for general cognitive deterioration, the delayed recall subtest discriminated well between AD/mixed dementia and SVD. The most prominent cognitive feature of prodromal AD in our sample was impaired delayed recall, while prodromal SVD was predominantly characterized by reduced mental speed and verbal fluency. Prodromal mixed dementia had features resembling both AD and SVD, suggesting that both these pathological processes influenced the mixed dementia phenotype. Furthermore, our findings are similar to those of previous studies in manifest AD and mixed dementia [41]. In summary, impaired delayed recall is seen in both incipient mixed dementia and AD, but mixed dementia

504 also exhibits impaired verbal fluency and mental
505 speed which is not seen in AD.

506 There are discrepancies regarding the reported
507 prevalence and incidence of mixed dementia, and
508 especially so regarding its early phases. The current
509 view is that the proportion of patients with mixed
510 dementia increases with age, and a community-based
511 autopsy study observed that AD pathology and cere-
512 brovascular disease were common findings in very
513 old patients [4]. When examining the proportion of
514 mixed dementia as a part of AD, we found that 38%
515 of MCI patients progressing to dementia with AD
516 characteristics converted to mixed dementia, which is
517 slightly higher than that in a recent study by Claus et
518 al., in which mixed dementia patient made up a third
519 of all cases with AD characteristics [42]. The fact
520 that the prevalence in the present study was higher
521 despite the younger age of our patients might mir-
522 ror differences in classification of mixed dementia.
523 Both our study as well as the study by Claus et
524 al. classified AD patients with moderate to severe
525 WMC as mixed dementia with the difference that
526 we also classified AD patients with mild WMC and
527 a marked frontosubcortical-dysexecutive syndrome
528 as mixed dementia. The rationale for our approach
529 is based on the objective of the Gothenburg MCI
530 study as a naturalistic study and variation concern-
531 ing presentation of disease is consequently given a
532 high rank in the classification process. Additionally,
533 WMC may not be the best indicator of progressive
534 disease. Although severe WMC have been linked to
535 cognitive impairment [43], there are still uncertainties
536 regarding the link between mild-moderate WMC and
537 cognitive impairment and whether there are qualita-
538 tive differences between WMC in regard to cognitive
539 outcome [44, 45]. Further research will hopefully
540 not only elucidate the connection between WMC and
541 cognition, but also provide a foundation for a more
542 accurate classification of mixed dementia.

543 *Limitations*

544 Limitations of the present study include uneven
545 distribution of incipient dementia disorders, and
546 incomplete MRI and neuropsychological test data of
547 the patient cohort. The results, and especially the non-
548 significant results in the study should therefore be
549 interpreted with caution due to the low number of
550 patients in the MCI-mix and MCI-SVD group. Addi-
551 tionally, the study was performed on patients actively
552 seeking care at a memory clinic and thus may not nec-
553 essarily reflect the general population. Furthermore,

554 as a result of the low number of patients in the con-
555 verting MCI groups, we chose to perform the variable
556 selection on patients with dementia/controls instead
557 of a different set of MCI patients which may have
558 affected the results as, compared with neuropsycho-
559 logical and imaging markers [46], CSF markers reach
560 a threshold level relatively early in the disease process
561 [47]. Consequently, our approach may have provided
562 an advantage to CSF markers compared to cognitive
563 tests and volumetric markers.

564 *Conclusions*

565 Incipient mixed dementia can be separated from
566 incipient AD and SVD using CSF markers and cog-
567 nitive testing. Intermediate levels of pathological
568 markers suggest an interactive effect on cognitive
569 function in incipient mixed dementia.

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593 **SUPPLEMENTARY MATERIAL**

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