Inflammation, Amyloid and Atrophy in The Aging Brain:
Relationships with longitudinal changes in cognition

Roser Sala-Llonch PhD[a], Ane-Victoria Idland[a,b], Tom Borza MD[c], Leiv Otto Watne

MD, PhD [b,d], Torgeir Bruun Wyller MD, PhD [b,e], Anne Brækhus MD, PhD [e,f,g], Henrik

Zetterberg MD, PhD [h,i,j], Kaj Blennow MD, PhD [h,i], Kristine Beate Walhovd PhD [a],

Anders Martin Fjell PhD[a]

[a]Center for Lifespan Changes in Brain and Cognition, Department of Psychology, University
of Oslo, Oslo, Norway

[b]Oslo Delirium Research Group, Institute of Clinical Medicine, Department of Geriatric
Medicine, University of Oslo, Oslo, Norway

[c]Centre for Old Age Psychiatric Research, Innlandet Hospital Trust, Ottestad, Norway

[d]Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

[e]Department of Geriatric Medicine, Oslo University Hospital, Oslo, Norway

[f]Norwegian National Advisory Unit on Ageing and Health, Vestfold Hospital Trust, Tønsberg

[g]Department of Neurology, Oslo University Hospital, Oslo, Norway

[h]Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry,
The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

[i]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[j]Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London,
UK

Corresponding author:

1

ABSTRACT:

Amyloid deposition occurs in healthy aging even in individuals free from cognitive symptoms. In addition, newer CSF markers, such as YKL-40, can track neuropathological brain processes such as inflammation. Altered YKL-40 levels are described in Alzheimer's disease (AD), but little is known about their correlations with brain and cognition in healthy older adults. From a longitudinal sample, we selected 89 participants having CSF samples at baseline, and both MRI and cognitive assessments from two time-points separated by two years. Mean age at inclusion was 73.1 years (SD: 6.01). We tested how baseline CSF levels of Aβ42 and YKL-40 predicted changes in cortical thickness and in cognition. We found correlations between longitudinal cortical thinning and Aβ42 only in amyloid positive participants (< 600 pg/mL, n=27). Aβ42 did not predict change in cognitive scores, while increased YKL-40 was associated with less memory preservation in the total sample (r=-0.28, p=0.012) and less preservation of global cognition for amyloid positive participants (r=-.58, p=0.004). Our results suggest a role of inflammation in brain structure and cognitive changes in healthy aging, which may be partly dependent on amyloid status.

Keywords: cerebrospinal fluid, aging, biomarkers, cortical thickness, inflammation, YKL-40, memory, cognition

1. INTRODUCTION:

Low level of cerebrospinal fluid (CSF) Aβ42 represents a major risk factor for Alzheimer's disease (AD) (Blennow et al., 2010) but is also a commonly described feature within clinically normal aging populations (Mormino, 2014). We still do not understand why Aβ is related to the development of disease in some persons, while others seem able to cope with substantial amyloid burden. Therefore, many studies point to an inconsistent relationship between Aβ, brain atrophy and cognition in aging, which can be caused by other factors that interact with amyloid (Chételat et al., 2016). In this sense, neuroinflammation has been proposed as one critical factor. Similar to amyloid, neuroinflammation occurs in both AD and normal aging (Fleischman et al., 2010; Heneka et al., 2015). Although neuroinflammation is observed in pathologically vulnerable brain regions in AD, it remains unclear whether insoluble Aβ deposits or neurofibrillary tangles are causing inflammation (Akiyama et al., 2000) or whether inflammation may actually promote or accelerate deposition of Aβ (Lee et al., 2008). In addition, by means of neuropathological studies, we know that increasing age is associated with increased microglial activation accompanied by production of inflammatory cytokines and compromised neuronal function, including synaptic dysfunction (Costello et al., 2016). The chitinase-3-like protein 1 (YKL-40) is a CSF biomarker reflecting neuroinflammation (Craig-Schapiro et al., 2010). Some studies have reported increased YKL-40 in AD (Rosén et al., 2014), predicting conversion to dementia (Antonell et al., 2014; Craig-Schapiro et al., 2010; Janelidze et al., 2016). YKL-40 has also been related to brain atrophy in AD (Alcolea et al., 2015; Gispert et al., 2016). However, the capabilities of YKL-40 to distinguish AD from controls have so far been found to be moderate in comparison with other CSF-markers such as T-tau, P-tau and Aβ42 (Olsson et al., 2016), and some report no relationship with AD (Hellwig et al., 2015; Mattsson et al., 2011). This may suggest that YKL-40 levels correlate with brain and cognitive processes just as much in normal aging as in AD.

In the present study, we tested the role of neuroinflammation in cortical atrophy and cognitive reductions in older adults without cognitive symptoms with either abnormal or normal amyloid status. Baseline measures of Aβ42 and YKL-40 were used to

predict cortical thinning and change in cognitive test scores over time. Although there are reasons to expect a relationship between amyloid levels and neuroinflammation, no previous study has directly tested how these biomarkers interact in predicting brain and cognitive decline in aging. While the role of YKL-40 in normal aging is almost unexplored, previous research on amyloid-related changes in the aging brain are conflicting, with some reporting more atrophy (Fagan et al., 2009; Schott et al., 2010; Tosun et al., 2010) in subjects with lower CSF Aβ42 levels and others larger volumes (Chételat et al., 2010), or non-linear relationships (Fortea et al., 2011). It has also been suggested that the correlations between Aβ42 and atrophy can be different for Aβ positive vs. negative individuals. This has been described in the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (Becker et al., 2011; Fjell et al., 2010), but has not to our knowledge been tested in other independent samples. With regard to cognitive changes, studies indicate that amyloid levels correlate, albeit not strongly, with cognitive function in normal populations (Hedden et al., 2013). Because of these controversies, it is necessary to study other factors that may show either amyloid-independent or amyloid-dependent relationships to atrophy and cognitive changes.

2. METHODS:

2.1. Participants

The sample consisted of cognitively healthy older adults. Exclusion criteria were dementia, previous stroke with sequelae, Parkinson's disease or other neurological condition likely to affect cognition (see details in Idland et al., *in press*). From the full sample recruited, which included 172 participants, we selected those having available baseline CSF measures and both MRI and cognitive evaluation at the two timepoints (mean time between MRI scans: 2.18 years). A total of 89 participants were included in the current analyses. Demographics are summarized in Table I. The cognitive evaluation was identical at the two timepoints and included a range of tests from which we selected for the present analyses the Word List Memory Task from the Consortium to Establish a Registry for Alzheimer's Disease battery (CERAD)(ref) the Animal Naming Test (ANT)(ref) and the Trail Making Test B (TMTB) (ref), to represent different cognitive domains.

2.2. Rates of change and cognition factor

We computed the annual rate of change in the scores obtained from the cognitive tests using the *symmetrized annual percent change*. These values were obtained as the difference of the scores between timepoints divided by its mean and by the time between them. The obtained scores are referred to as: *CERAD-change*, *ANT-change*, and *TMTB-change*.

We also calculated a global cognition change factor using Principal Component Analysis (PCA) on the rates of change obtained from the three tests. We refer to this score as *COGfac-change*.

2.3. CSF measures

CSF samples were taken at the time of the induction of spinal anesthesia. CSF was collected in polypropylene tubes, centrifuged, the supernatant aliquoted in polypropylene vials, frozen as soon as possible, and stored at -80°C. CSF was thawed, aliquoted once more in polypropylene vials and frozen again before it was sent for analyses. Levels of Aβ42 were determined using INNOTEST enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium). YKL-40 concentrations were measured using a commercially available ELISA (R&D systems, Minneapolis, MN). All analyses were performed by board-certified laboratory technicians, who were masked to clinical data, using one batch of reagents with intra-assay coefficients of variation below 10 %.

2.2. Magnetic Resonance Imaging

In each scanning session, a T1-weighted MPRAGE 3D image was acquired in a 1.5T Siemens Avanto scanner using a 12-channel head coil (TR=2400 ms, TE=3.79ms, slice thickness=1.20mm, pixel spacing=1.25x1.25mm).

2.3. MRI processing

Structural MPRAGE scans were processed with FreeSurfer (version 5.3) and its longitudinal stream (https://surfer.nmr.mgh.harvard.edu). The standard FreeSurfer pipeline performs a set of automated procedures for the cortical reconstruction and volumetric segmentation of the individual scans (Dale et al., 1999; Fischl et al., 2002). In addition, the FreeSurfer longitudinal stream includes a set of methods designed to minimize the bias to any time point and which has been shown to lead to

increased statistical power, better separation of groups based on atrophy and higher reproducibility (Jovicich et al., 2013; Reuter and Fischl, 2011; Reuter et al., 2012). Using these tools, we obtained vertex-wise maps of symmetrized atrophy rate, defined as the normalized difference in thickness at each vertex per year.

FreeSurfer reconstructed brain surfaces were visually inspected and manually corrected when necessary. Manual interventions included the removal of non-brain areas and the use of control points to guide the automated reconstruction of white and pial surfaces.

2.4. Statistics.

The surface-based data representing thickness change for each subject was fit into a GLM model for group-statistics. We first calculated the mean rate of change between acquisitions for the whole sample (i.e., one-sample t-test). We then performed vertex-wise correlations and tested interactions to investigate relationships between thickness, CSF markers and cognition. Age and gender were introduced as covariates in all the surface-based analyses. Maps were corrected for family wise-error (FWE) using precomputed simulated data in FreeSurfer (Hagler et al., 2006). Significance threshold was set at a p-value of 0.05, both for the initial thresholding of the maps and for the identification of significant clusters.

In addition, we repeated the longitudinal analysis using a Linear Mixed Effects (LME) analysis implemented in Matlab and distributed as part of the FreeSurfer Pipeline (Supplementary Material).

Statistics on non-imaging data were performed with SPSS and MATLAB tools. These included partial correlation and linear multiple regression approaches.

3. RESULTS:

3. 1. Demographics and cognitive data

Participants were divided into two subgroups according to their levels of CSF Aβ42, Aβ+ (< 600 pg/mL) and Aβ- (≥ 600 pg/mL). Because several cutoff values of CSF Aβ42 levels are described in the literature, ranging from 500 to 650 pg/mL (Fagan et al., 2009; Mulder et al., 2010; Niemantsverdriet et al., 2016; Zwan et al., 2016), we used a rather conservative level of 600 pg/mL to accommodate possible variation or bias in the measurements and to ensure a considerable number of subjects within the

group of healthy older adults with amyloid positivity. A total of 27 subjects were included in the Aβ+ group and 62 in the Aβ- group. There were no group differences in the rates of change in any of the cognitive measures or in baseline CSF YKL-40 and Tau levels. The results of the TMTB at the second timepoint differed between groups (t=2.54, p=0.016), however, the rates of change within this measure were comparable (t=1.3, p=0.16).

	Full sample N = 89 Mean (SD) Range	Aβ42- N=62 Mean (SD) Range	Aβ42+ N=27 Mean (SD) Range
Sample & Demographics			
Age (years)	73.1(6.01) 64.74-89.79	73.2 (6.3) 64.74-89.79	72.91 (5.38) 64.95-82.52
Sex (men/women)	46/43	29/33	17/10
Time between (years)	2.17 (0.28) 1.55-2.88	2.18 (0.26) 1.55-2.87	2.17 (0.33) 1.71-2.88
MMSE baseline	29.13 (1.27) 25-30	29.09 (1.40) 25-30	29.22 (0.93) 27-30
MMSE 2years	29.05 (1.21) 24-30	29.14 (1.19) 24-30	28.85 (1.23) 26-30
Cognition			
TMTB - baseline [+]	118.55(60.28) 34-466	112.61 (44.33) 34-246	132.69 (64.13) 64-466
TMTB 2 years [+]	116.23(56.57) 31-306	106.64 (50.53) 31-267	137.89 (64.13) 72-306
CERAD baseline	6.78 (1.76) 3-10	6.73 (1.67) 4-10	6.89 (2) 3-10
CERAD 2 years	7.69 (1.72) 3-10	7.61 (1.55) 5-10	7.85 (2.1) 3-10
ANT baseline	20.67 (5.45) 5-32	20.69 (5.22) 10-32	20.63 (6.04) 5-30
ANT 2 years	23.27 (6.09) 7-38	23.85 (5.9) 7-38	21.93 (6.4) 10-38
CSF biomarkers			
CSF - Aβ-142 (pg/ml)	721.22 (204.65) 275-1175	831.25 (131.07) 605-1175	467.19 (72.45) 275-587
CSF – T-Tau (pg/ml)	373.4 (142.05) 114.7-785.9	361.28 (123.27) 181.8 – 767.9	401.23 (177.45) 114.7-785.95
CSF - Ptau (pg/ml)	60.42 (18.37) 26-110	59.76 (17.1) 32-110	61.93 (21.34) 26-103
YKL-40 (ng/ml) *	$220.02 (73.9)*10^3$ $92.11 - 441.5*10^3$	$219.80 (73.39) *10^3$ $97.19 – 441.5 *10^3$	$220.521 (76.47) *10^3$ $92.11-348.3 *10^3$

Table I: Sample summary. Demographics, cognititive and CSF data of all participants included in the analyses.
+ Missing cognitive data of N=1 subject
* excluded 1 subject below detection limit

3.3. Mean longitudinal changes in cortical thickness

We observed an average rate of cortical thinning of 0.53% (SD: 1%) per year in the full sample. General thinning was observable across large regions of the cortex (Figure 1). Reductions in cortical thickness were significant (FWE corrected level of p<0.05) in several clusters located bilaterally in superior parietal, supramarginal,

precuneus, isthmus cingulate posterior cingulate, postcentral and paracentral cortices, in the right hemisphere including the superior frontal, caudalmiddlefrontal, inferior parietal, precentral fusiform and parahippocampal cortices, as well as in left lingual cortex.
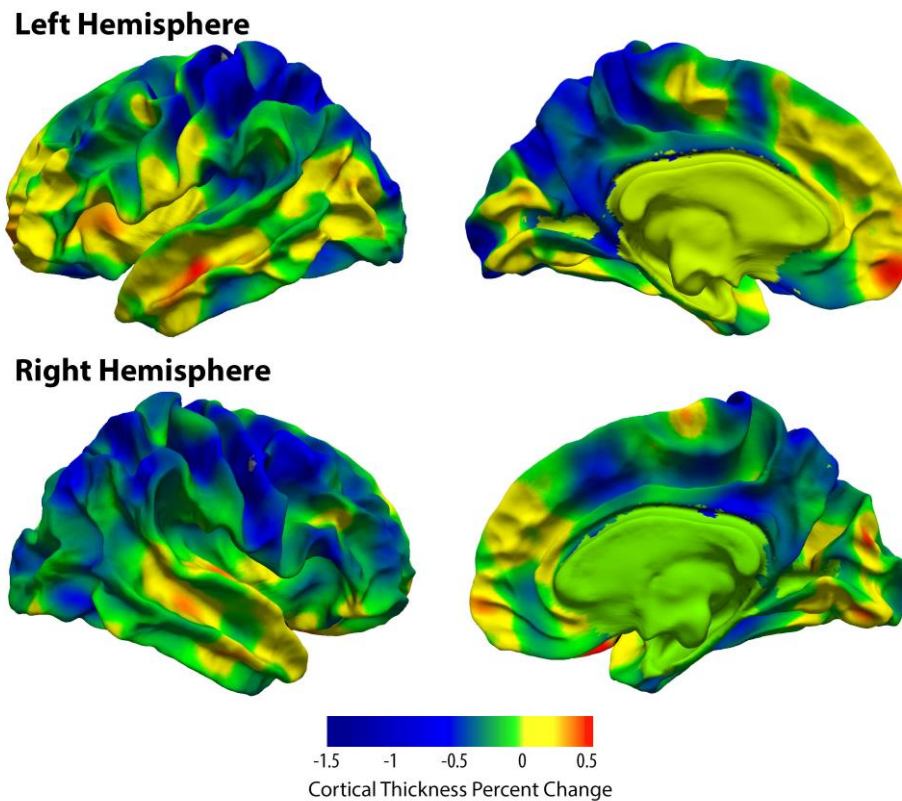

Figure 1. Mean effects of time on cortical thickness within the full sample. Percentage of thickness change measured between baseline and the 2-years scan. Green-blue areas indicate decreases over time and yellow-red indicate increases (see legend).

3.4. Aβ42 and cortical thinning

Overall change in cortical thickness did not differ between Aβ+ and Aβ- participants (-0.50% and -0.54 % respectively, t=0.11, p=0.91). We used a vertex-wise GLM on the surface to investigate the effect of CSF Aβ42 levels on regional thickness change. Using a between-group comparison, we found that regional change in thickness did not differ between groups. We then created a design with separate slopes for the Aβ+ and the Aβ- groups and tested it across the whole cortex. We found a cluster where this interaction was significant, located in the xxx (p<0.05, FWE corrected). In this

region there was a negative relationship between CSF Aβ42 levels and the reduction of cortical thickness in the Aβ+ group but not in the Aβ- participants (Figure 2).
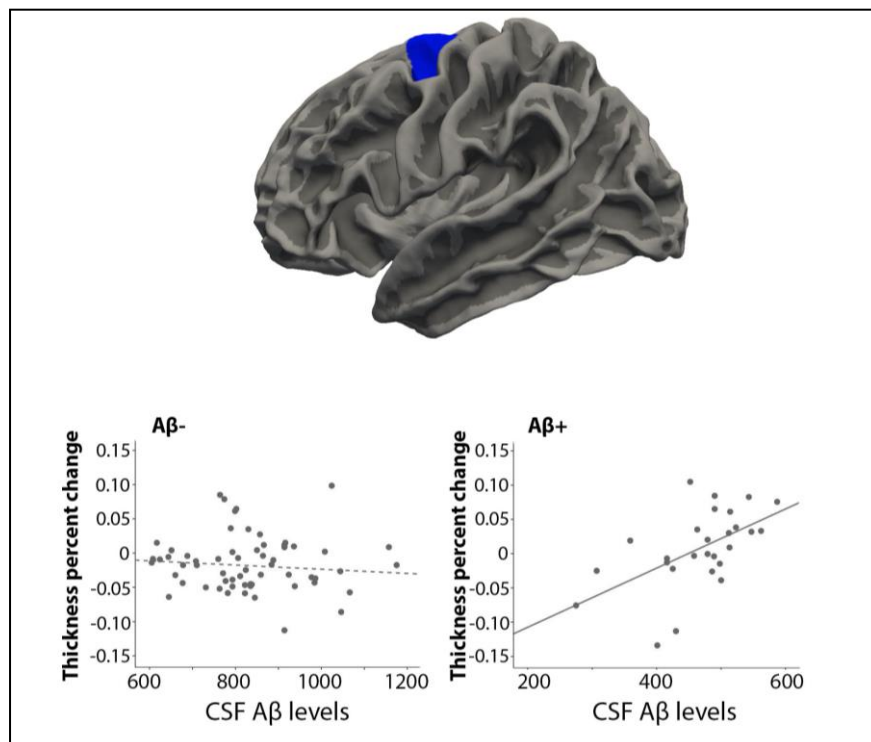

Figure 2. (A) Region with a group-interaction with Aβ42 levels and cortical thickness change. (B) Scatter plots showing the relationship between thickness change and Aβ42 levels for the two groups separately.

3.5. Aβ42 and cognition
We did not find any relationship between CSF levels of Aβ42 and the global cognitive change or change in any of the specific cognitive measures.

3.6. YKL and cortical thinning
We did not find any relationship between change in cortical thickness and YKL-40. We tested for direct relationships as well as for interactions with CSF Aβ42 levels.

3.5. YKL and cognition
Change in the animal naming test (*ANT-change*) correlated with CSF YKL-40 levels within the whole sample, in that higher YKL-40 levels were related to more negative change (r=-0.28, p=0.012) (Figure 3A). Note that we observed rates of change above zero due to practice effects (Galvin et al., 2005). No significant correlations between

YKL-40 and the other cognitive measures were found, but a trend was observed between YKL-40 and global cognition change (r=-0.185, p=0.096).

Finally, we explored correlations with the CSF YKL-40 levels and cognition in Aβ+ and Aβ- participants. In the Aβ+ group, we found a correlation between levels of YKL40 and the cognition change factor (r=-.58, p=0.004, see Figure 3B), indicating less cognitive maintenance. This correlation was not found in the *Aβ-* group (r=-0.008, p=0.955). These correlations were significantly different, as evidenced by use of the Fisher r-to-z transformation (z = 2.72, p = .0065, two-tailed).
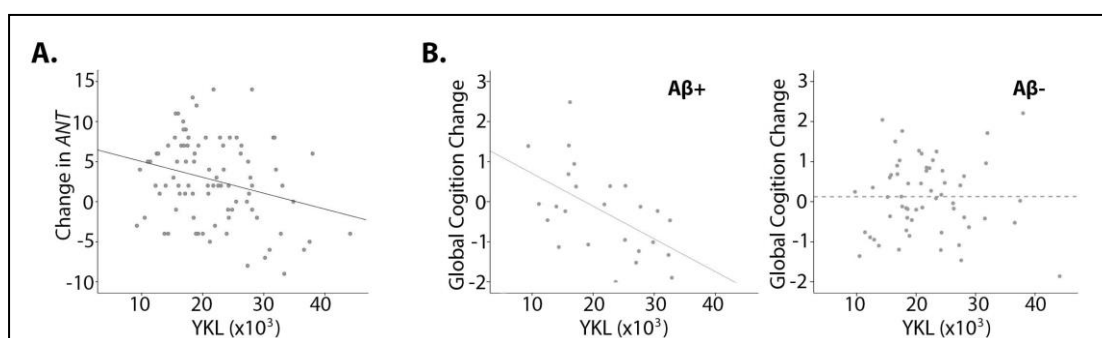

Figure 3. CSF YKL levels and cognition. (A) YKL-40 and changes in animal naming test in the full sample. (B) Correlation between YKL and global cognition in Aβ+ and Aβ- participants.

3.6. Cortical Thickness and cognition

Longitudinal change in cortical thickness correlated positively with change in the results of the animal naming test (p<0.05), meaning that more thinning was associated with reduction or less maintenance of cognitive scores. We found two significant clusters located in the right hemisphere, covering parts of the supramarginal, postcentral, and inferior parietal cortices (Figure 4).
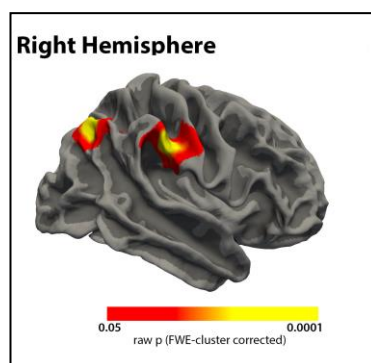

Figure 4. Correlation between change in cortical thickness and change in the results of the animal naming test. Red-yellow areas indicate that changes in thickness and changes in cognition have the same direction. Only results within significant clusters are shown

DISCUSSION:

In the present study we showed that neuroinflammation, as indexed by CSF levels of YKL-40, predicted reduced cognitive function two years later in cognitively normal older adults who were positive for Aβ. Intriguingly, no relationship was observed for Aβ negative participants. As CSF levels of Aβ42 and YKL40 were not related, this could indicate that these biomarkers are indicators of distinct processes in the brain that have additive detrimental effects on cognitive function in older adults at risk for AD. In addition, we found that CSF Aβ42 levels predicted cortical atrophy only in amyloid positive participants. Seen together, these results indicate that mechanisms for brain atrophy and cognitive decline might be different in older adults that are positive in comparison with those negative for amyloid, underscoring the need to understand how amyloid relates to brain atrophy in cognitively normal older adults. The implications of the results are discussed below.

Neuroinflammation and cortical thinning in Aβ positive older adults
Here we used CSF YKL-40 levels as a measure of neuroinflammation and we found that increased levels of this biomarker predicted global changes in cognition in the Aβ+ group, indicating that neuroinflammation might play an important role for cognitive changes in older adults with altered amyloid status. In addition, increasing age has been associated with increased microglial activation (Costello et al., 2016). Abnormal, increased levels of CSF YKL-40 are observed in AD patients (Craig-Schapiro et al., 2010; Janelidze et al., 2016; Olsson et al., 2016), but this is not invariably found (Hellwig et al., 2015; Mattsson et al., 2011). However, no studies have previously tested whether this biomarker is increased also in healthy older adults with altered amyloid levels. Intriguingly, our result suggests that even if the level of neuroinflammation as indexed by YKL-40 does not vary as a function of amyloid accumulation, the ability of the brain to cope with the inflammation is different depending on the subject amyloid status. Thus, inflammation predicts cognitive reduction over time in the presence of higher amyloid levels only, with the lower risk older adults probably being able to better cope with the burden of neuroinflammation. This is not an on-off mechanism, however, as we found that changes in verbal memory correlated with YKL-40 levels also within the full sample. Thus, the present results indicate that YKL-40 indexes neuroinflammatory processes that have mild but

nevertheless detrimental effects on cognitive function in older adults, and that these detrimental effects are significantly larger in participants with increased Aβ42 levels.

Cortical thinning at the different CSF Aβ42 levels
In addition to the Aβ-dependent effects of neuroinflammation on cognition, we found that decreased CSF Aβ42 correlated with cortical thinning in the Aβ+ participants. This further indicate that there might be degenerative processes occurring at this narrow range of Aβ42 levels even in individuals free from clinical symptoms. This pattern has previously been described within the ADNI cohort of normal older adults (Becker et al., 2011; Fjell et al., 2010). These two studies found that only under a certain threshold of Aβ42 levels in CSF, Aβ42 correlated with accelerated brain atrophy. The present study demonstrates this pattern in a different sample than the ADNI database. Taken together, these findings indicate that amyloid positive older adults are less able to cope with processes in the brain that might otherwise not be as harmful in older adults without evidence of brain amyloid accumulation.

Neuroinflammation, amyloid and changes in cognition
Previous literature has described null or relatively weak relationships between common CSF AD biomarkers such as Aβ42 and memory/cognition in healthy elderly participants (Aschenbrenner et al., 2015; Fjell et al., 2014; Hedden et al., 2013). This has encouraged the community to study the roles of alternative markers in addition to amyloid to better explain cognitive reductions and brain atrophy in aging. Our results, though being moderate in strength, indicate that YKL-40 is one such marker, primarily when seen in coexistence with altered levels of established risk markers such as Aβ42. This fits with theories of amyloid and neuroinflammation working together to cause decline in older adults with neurodegenerative conditions such as AD (Heneka et al., 2015), even if the evidence for these theories in humans is scarce and to a large extent based on genetic studies. Further, additive roles of amyloid accumulation and neuroinflammation have not been tested in cognitively normal older adults. The results of the present study suggest that effects of inflammation on cognitive decline in non-demented may partly depend on amyloid status. Adding to this, we found that the same cognitive test also correlated with longitudinal changes in cortical thickness. Participants with less capability to maintain or improve performance on the animal naming test showed more decreases in cortical thickness.

These results are in accordance with previous studies showing correlations between longitudinal changes in brain structure and changes in cognition (Fjell et al., 2013; Persson et al., 2016; Rodrigue and Raz, 2004).

Although being predictive of cognitive reductions, CSF YKL-40 levels did not correlate with cortical thinning. Some previous studies have reported correlations between thinner cortex and higher YKL-40 levels (Alcolea et al., 2015; Gispert et al., 2016) or other markers of inflammation (Fleischman et al., 2010), while others did not find relationships with measures of brain structures such as hippocampal volume (Melah et al., 2015). The studies mentioned above were all cross-sectional, in contrast to the present longitudinal design. Further, these former studies included patients with MCI or AD, thus not being fully comparable. Still, when the present results are seen in contrast to the identified relationship between amyloid levels and cortical thinning in the amyloid positive participants, it is evident that the relationship between amyloid levels, neuroinflammation and brain atrophy is complex. To get a deeper understanding of the mechanisms at play, we will likely also need to include additional biomarkers as well as genetic factors in the analyses.

Conclusion: One important theory holds that sustained inflammatory responses to amyloid accumulation increase brain atrophy in AD (Gouwens et al., 2016; Heneka et al., 2015), and the increased levels of neuroinflammation observed also in cognitively normal older adults make it important to investigate amyloid – inflammation relationships also in non-demented. Here we found that YKL-40, as a measure of neuroinflammation, correlated with cognitive reductions in amyloid positive older adults only. In contrast, CSF Aβ42 levels per se did not predict cognitive decline, but still were related to brain atrophy in amyloid positive participants. Put together, these findings highlight the need for considering how newer biomarkers interact with the established ones in predicting cognitive and brain changes in aging. This may provide better understanding of the processes that are crucial in the transitional phase between normal aging and neurodegeneration.

REFERENCES

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G.M., Cooper, N.R., Eikelenboom, P., Emmerling, M., Fiebich, B.L., Finch, C.E., Frautschy, S., Griffin, W.S., Hampel, H., Hull, M., Landreth, G., Lue, L., Mrak, R., Mackenzie, I.R., McGeer, P.L., O'Banion, M.K., Pachter, J., Pasinetti, G., Plata-Salaman, C., Rogers, J., Rydel, R., Shen, Y., Streit, W., Strohmeyer, R., Tooyoma, I., Van Muiswinkel, F.L., Veerhuis, R., Walker, D., Webster, S., Wegrzyniak, B., Wenk, G., Wyss-Coray, T., 2000. Inflammation and Alzheimer's disease. Neurobiol. Aging 21, 383–421.

Alcolea, D., Vilaplana, E., Pegueroles, J., Montal, V., Sánchez-Juan, P., González-Suárez, A., Pozueta, A., Rodríguez-Rodríguez, E., Bartrés-Faz, D., Vidal-Piñeiro, D., González-Ortiz, S., Medrano, S., Carmona-Iragui, M., Sánchez-Saudinós, M., Sala, I., Anton-Aguirre, S., Sampedro, F., Morenas-Rodríguez, E., Clarimón, J., Blesa, R., Lleó, A., Fortea, J., 2015. Relationship between cortical thickness and cerebrospinal fluid YKL-40 in predementia stages of Alzheimer's disease. Neurobiol. Aging 36, 2018–2023. doi:10.1016/j.neurobiolaging.2015.03.001

Antonell, A., Mansilla, A., Rami, L., Lladó, A., Iranzo, A., Olives, J., Balasa, M., Sánchez-Valle, R., Molinuevo, J.L., 2014. Cerebrospinal fluid level of YKL-40 protein in preclinical and prodromal Alzheimer's disease. J. Alzheimers. Dis. 42, 901–8. doi:10.3233/JAD-140624

Aschenbrenner, A.J., Balota, D.A., Fagan, A.M., Duchek, J.M., Benzinger, T.L.S., Morris, J.C., 2015. Alzheimer Disease Cerebrospinal Fluid Biomarkers Moderate Baseline Differences and Predict Longitudinal Change in Attentional Control and Episodic Memory Composites in the Adult Children Study. J. Int. Neuropsychol. Soc. 21, 573–83. doi:10.1017/S1355617715000776

Becker, J.A., Hedden, T., Carmasin, J., Maye, J., Rentz, D.M., Putcha, D., Fischl, B., Greve, D.N., Marshall, G.A., Salloway, S., Marks, D., Buckner, R.L., Sperling, R.A., Johnson, K.A., 2011. Amyloid-β associated cortical thinning in clinically normal elderly. Ann. Neurol. 69, 1032–42. doi:10.1002/ana.22333

Blennow, K., Hampel, H., Weiner, M., Zetterberg, H., 2010. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat. Rev. Neurol. 6, 131–44. doi:10.1038/nrneurol.2010.4

Chételat, G., Ossenkoppele, R., Villemagne, V.L., Perrotin, A., Landeau, B., Mézenge, F., Jagust, W.J., Dore, V., Miller, B.L., Egret, S., Seeley, W.W., van der Flier, W.M., La Joie, R., Ames, D., van Berckel, B.N.M., Scheltens, P., Barkhof, F., Rowe, C.C., Masters, C.L., de La Sayette, V., Bouwman, F., Rabinovici, G.D., 2016. Atrophy, hypometabolism and clinical trajectories in patients with amyloid-negative Alzheimer's disease. Brain. doi:10.1093/brain/aww159

Chételat, G., Villemagne, V.L., Pike, K.E., Baron, J.-C., Bourgeat, P., Jones, G., Faux, N.G., Ellis, K.A., Salvado, O., Szoeke, C., Martins, R.N., Ames, D., Masters, C.L., Rowe, C.C., 2010. Larger temporal volume in elderly with high

versus low beta-amyloid deposition. Brain 133, 3349–58. doi:10.1093/brain/awq187

Costello, D.A., Keenan, K., McManus, R.M., Falvey, A., Lynch, M.A., 2016. The age-related neuroinflammatory environment promotes macrophage activation, which negatively impacts synaptic function. Neurobiol. Aging 43, 140–148. doi:10.1016/j.neurobiolaging.2016.04.001

Craig-Schapiro, R., Perrin, R.J., Roe, C.M., Xiong, C., Carter, D., Cairns, N.J., Mintun, M.A., Peskind, E.R., Li, G., Galasko, D.R., Clark, C.M., Quinn, J.F., D'Angelo, G., Malone, J.P., Townsend, R.R., Morris, J.C., Fagan, A.M., Holtzman, D.M., 2010. YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. Biol. Psychiatry 68, 903–12. doi:10.1016/j.biopsych.2010.08.025

Dale, A.M., Fischl, B., Sereno, M.I., 1999. Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 9, 179–94. doi:10.1006/nimg.1998.0395

Fagan, A.M., Head, D., Shah, A.R., Marcus, D., Mintun, M., Morris, J.C., Holtzman, D.M., 2009. Decreased cerebrospinal fluid Abeta(42) correlates with brain atrophy in cognitively normal elderly. Ann. Neurol. 65, 176–83. doi:10.1002/ana.21559

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M., 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–55.

Fjell, A.M., McEvoy, L., Holland, D., Dale, A.M., Walhovd, K.B., 2013. Brain changes in older adults at very low risk for Alzheimer's disease. J. Neurosci. 33, 8237–42. doi:10.1523/JNEUROSCI.5506-12.2013

Fjell, A.M., McEvoy, L., Holland, D., Dale, A.M., Walhovd, K.B., Alzheimer's Disease Neuroimaging Initiative, 2014. What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. Prog. Neurobiol. 117, 20–40. doi:10.1016/j.pneurobio.2014.02.004

Fjell, A.M., Walhovd, K.B., Fennema-Notestine, C., McEvoy, L.K., Hagler, D.J., Holland, D., Blennow, K., Brewer, J.B., Dale, A.M., Alzheimer's Disease Neuroimaging Initiative, the A.D.N., 2010. Brain atrophy in healthy aging is related to CSF levels of Aβ1-42. Cereb. Cortex 20, 2069–79. doi:10.1093/cercor/bhp279

Fleischman, D.A., Arfanakis, K., Kelly, J.F., Rajendran, N., Buchman, A.S., Morris, M.C., Barnes, L.L., Bennett, D.A., 2010. Regional brain cortical thinning and systemic inflammation in older persons without dementia. J. Am. Geriatr. Soc. 58, 1823–5. doi:10.1111/j.1532-5415.2010.03049.x

Fortea, J., Sala-Llonch, R., Bartrés-Faz, D., Lladó, A., Solé-Padullés, C., Bosch, B., Antonell, A., Olives, J., Sanchez-Valle, R., Molinuevo, J.L., Rami, L., 2011. Cognitively preserved subjects with transitional cerebrospinal fluid ß-amyloid 1-42 values have thicker cortex in Alzheimer's disease vulnerable areas. Biol. Psychiatry 70, 183–90. doi:10.1016/j.biopsych.2011.02.017

Galvin, J.E., Powlishta, K.K., Wilkins, K., McKeel, D.W., Xiong, C., Grant, E., Storandt, M., Morris, J.C., 2005. Predictors of preclinical Alzheimer disease and dementia: a clinicopathologic study. Arch. Neurol. 62, 758–65. doi:10.1001/archneur.62.5.758

Gispert, J.D., Monté, G.C., Falcon, C., Tucholka, A., Rojas, S., Sánchez-Valle, R., Antonell, A., Lladó, A., Rami, L., Molinuevo, J.L., 2016. CSF YKL-40 and pTau181 are related to different cerebral morphometric patterns in early AD. Neurobiol. Aging 38, 47–55. doi:10.1016/j.neurobiolaging.2015.10.022

Gouwens, L.K., Makoni, N.J., Rogers, V.A., Nichols, M.R., 2016. Amyloid-β42 protofibrils are internalized by microglia more extensively than monomers. Brain Res. 1648, 485–495. doi:10.1016/j.brainres.2016.08.016

Hagler, D.J., Saygin, A.P., Sereno, M.I., 2006. Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. Neuroimage 33, 1093–1103. doi:10.1016/j.neuroimage.2006.07.036

Hedden, T., Oh, H., Younger, A.P., Patel, T.A., 2013. Meta-analysis of amyloid-cognition relations in cognitively normal older adults. Neurology 80, 1341–8. doi:10.1212/WNL.0b013e31828ab35d

Hellwig, K., Kvartsberg, H., Portelius, E., Andreasson, U., Oberstein, T.J., Lewczuk, P., Blennow, K., Kornhuber, J., Maler, J.M., Zetterberg, H., Spitzer, P., 2015. Neurogranin and YKL-40: independent markers of synaptic degeneration and neuroinflammation in Alzheimer's disease. Alzheimers. Res. Ther. 7, 74. doi:10.1186/s13195-015-0161-y

Heneka, M.T., Carson, M.J., El Khoury, J., Landreth, G.E., Brosseron, F., Feinstein, D.L., Jacobs, A.H., Wyss-Coray, T., Vitorica, J., Ransohoff, R.M., Herrup, K., Frautschy, S.A., Finsen, B., Brown, G.C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G.C., Town, T., Morgan, D., Shinohara, M.L., Perry, V.H., Holmes, C., Bazan, N.G., Brooks, D.J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C.A., Breitner, J.C., Cole, G.M., Golenbock, D.T., Kummer, M.P., 2015. Neuroinflammation in Alzheimer's disease. Lancet. Neurol. 14, 388–405. doi:10.1016/S1474-4422(15)70016-5

Janelidze, S., Hertze, J., Zetterberg, H., Landqvist Waldö, M., Santillo, A., Blennow, K., Hansson, O., 2016. Cerebrospinal fluid neurogranin and YKL-40 as biomarkers of Alzheimer's disease. Ann. Clin. Transl. Neurol. 3, 12–20. doi:10.1002/acn3.266

Jovicich, J., Marizzoni, M., Sala-Llonch, R., Bosch, B., Bartrés-Faz, D., Arnold, J., Benninghoff, J., Wiltfang, J., Roccatagliata, L., Nobili, F., Hensch, T., Tränkner, A., Schönknecht, P., Leroy, M., Lopes, R., Bordet, R., Chanoine, V., Ranjeva, J.-P., Didic, M., Gros-Dagnac, H., Payoux, P., Zoccatelli, G., Alessandrini, F., Beltramello, A., Bargalló, N., Blin, O., Frisoni, G.B., 2013. Brain morphometry reproducibility in multi-center 3T MRI studies: a comparison of cross-sectional and longitudinal segmentations. Neuroimage 83, 472–84. doi:10.1016/j.neuroimage.2013.05.007

Lee, J.W., Lee, Y.K., Yuk, D.Y., Choi, D.Y., Ban, S.B., Oh, K.W., Hong, J.T., 2008. Neuroinflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. J. Neuroinflammation 5, 37. doi:10.1186/1742-2094-5-37

Mattsson, N., Tabatabaei, S., Johansson, P., Hansson, O., Andreasson, U., Månsson, J.-E., Johansson, J.-O., Olsson, B., Wallin, A., Svensson, J., Blennow, K., Zetterberg, H., 2011. Cerebrospinal fluid microglial markers in Alzheimer's disease: elevated chitotriosidase activity but lack of diagnostic utility. Neuromolecular Med. 13, 151–9. doi:10.1007/s12017-011-8147-9

Melah, K.E., Lu, S.Y.-F., Hoscheidt, S.M., Alexander, A.L., Adluru, N., Destiche, D.J., Carlsson, C.M., Zetterberg, H., Blennow, K., Okonkwo, O.C., Gleason, C.E., Dowling, N.M., Bratzke, L.C., Rowley, H.A., Sager, M.A., Asthana, S., Johnson, S.C., Bendlin, B.B., 2015. Cerebrospinal Fluid Markers of Alzheimer's Disease Pathology and Microglial Activation are Associated with Altered White Matter Microstructure in Asymptomatic Adults at Risk for Alzheimer's Disease. J. Alzheimers. Dis. 50, 873–86. doi:10.3233/JAD-150897

Mormino, E.C., 2014. The relevance of beta-amyloid on markers of Alzheimer's disease in clinically normal individuals and factors that influence these associations. Neuropsychol. Rev. 24, 300–12. doi:10.1007/s11065-014-9267-4

Mulder, C., Verwey, N.A., van der Flier, W.M., Bouwman, F.H., Kok, A., van Elk, E.J., Scheltens, P., Blankenstein, M.A., 2010. Amyloid-beta(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. Clin. Chem. 56, 248–53. doi:10.1373/clinchem.2009.130518

Niemantsverdriet, E., Goossens, J., Struyfs, H., Martin, J.-J., Goeman, J., De Deyn, P.P., Vanderstichele, H., Engelborghs, S., 2016. Diagnostic Impact of Cerebrospinal Fluid Biomarker (Pre-)Analytical Variability in Alzheimer's Disease. J. Alzheimer's Dis. 51, 97–106. doi:10.3233/JAD-150953

Olsson, B., Lautner, R., Andreasson, U., Öhrfelt, A., Portelius, E., Bjerke, M., Hölttä, M., Rosén, C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., Zetterberg, H., 2016. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 15, 673–684. doi:10.1016/S1474-4422(16)00070-3

Persson, N., Ghisletta, P., Dahle, C.L., Bender, A.R., Yang, Y., Yuan, P., Daugherty, A.M., Raz, N., 2016. Regional brain shrinkage and change in cognitive

performance over two years: The bidirectional influences of the brain and cognitive reserve factors. Neuroimage 126, 15–26. doi:10.1016/j.neuroimage.2015.11.028

Reuter, M., Fischl, B., 2011. Avoiding asymmetry-induced bias in longitudinal image processing. Neuroimage. doi:10.1016/j.neuroimage.2011.02.076

Reuter, M., Schmansky, N.J., Rosas, H.D., Fischl, B., 2012. Within-subject template estimation for unbiased longitudinal image analysis. Neuroimage 61, 1402–18. doi:10.1016/j.neuroimage.2012.02.084

Rodrigue, K.M., Raz, N., 2004. Shrinkage of the entorhinal cortex over five years predicts memory performance in healthy adults. J. Neurosci. 24, 956–63. doi:10.1523/JNEUROSCI.4166-03.2004

Rosén, C., Andersson, C.-H., Andreasson, U., Molinuevo, J.L., Bjerke, M., Rami, L., Lladó, A., Blennow, K., Zetterberg, H., 2014. Increased Levels of Chitotriosidase and YKL-40 in Cerebrospinal Fluid from Patients with Alzheimer's Disease. Dement. Geriatr. Cogn. Dis. Extra 4, 297–304. doi:10.1159/000362164

Schott, J.M., Bartlett, J.W., Fox, N.C., Barnes, J., Alzheimer's Disease Neuroimaging Initiative Investigators, 2010. Increased brain atrophy rates in cognitively normal older adults with low cerebrospinal fluid Aβ1-42. Ann. Neurol. 68, 825–34. doi:10.1002/ana.22315

Tosun, D., Schuff, N., Truran-Sacrey, D., Shaw, L.M., Trojanowski, J.Q., Aisen, P., Peterson, R., Weiner, M.W., 2010. Relations between brain tissue loss, CSF biomarkers, and the ApoE genetic profile: a longitudinal MRI study. Neurobiol. Aging 31, 1340–1354. doi:10.1016/j.neurobiolaging.2010.04.030

Zwan, M.D., Rinne, J.O., Hasselbalch, S.G., Nordberg, A., Lleó, A., Herukka, S.-K., Soininen, H., Law, I., Bahl, J.M.C., Carter, S.F., Fortea, J., Blesa, R., Teunissen, C.E., Bouwman, F.H., van Berckel, B.N.M., Visser, P.J., 2016. Use of amyloid-PET to determine cutpoints for CSF markers: A multicenter study. Neurology 86, 50–8. doi:10.1212/WNL.0000000000002081