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# Combinatorial Markers of Mild Cognitive Impairment Conversion to Alzheimer's Disease - Cytokines and MRI Measures Together Predict Disease Progression

Simon J. Furney[a], Deborah Kronenberg[b], Andrew Simmons[a], Andreas Güntert[a], Richard J. Dobson[a], Petroula Proitsi[a], Lars Olof Wahlund[c], Iwona Kloszewska[d], Patrizia Mecocci[e], Hilkka Soininen[f], Magda Tsolaki[g], Bruno Vellas[h], Christian Spenger[i] and Simon Lovestone[a,*]

[a] *King's College London, Institute of Psychiatry and National Institute of Health Research (NIHR) Biomedical Research Centre for Mental Health, London, UK*
[b] *National Institute for Health Research (NIHR), Biomedical Research Centre Guy's and St. Thomas' NHS Foundation Trust and King's College London, London, UK*
[c] *Department of Neurobiology, Care Sciences and Society, Section of Clinical Geriatrics, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm, Sweden*
[d] *Department of Old Age Psychiatry & Psychotic Disorders, Medical University of Lodz, Lodz, Poland*
[e] *Section of Gerontology and Geriatrics, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy*
[f] *Department of Neurology, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland*
[g] *Third Department of Neurology, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[h] *Department of Internal and Geriatrics Medicine, Hôpitaux de Toulouse, Toulouse, France*
[i] *Department of Clinical Science, Intervention and Technology, Karolinska, Institutet, Stockholm, Sweden*

**Abstract**. Progression of people presenting with Mild Cognitive Impairment (MCI) to dementia is not certain and it is not possible for clinicians to predict which people are most likely to convert. The inability of clinicians to predict progression limits the use of MCI as a syndrome for treatment in prevention trials and, as more people present with this syndrome in memory clinics, and as earlier diagnosis is a major goal of health services, this presents an important clinical problem. Some data suggest that CSF biomarkers and functional imaging using PET might act as markers to facilitate prediction of conversion. However, both techniques are costly and not universally available. The objective of our study was to investigate the potential added benefit of combining biomarkers that are more easily obtained in routine clinical practice to predict conversion from MCI to Alzheimer's disease. To explore this we combined automated regional analysis of structural MRI with analysis of plasma cytokines and chemokines and compared these to measures of APOE genotype and clinical assessment to assess which best predict progression. In a total of 205 people with MCI, 77 of whom subsequently converted to Alzheimer's disease, we find

*Correspondence to: Simon Lovestone, King's College London, Institute of Psychiatry and National Institute of Health Research (NIHR) Biomedical, De, Crespigny Park, London SE5 8AF, UK. Tel.: +44 20 7848 0239/0550, Fax: +44 20 7848 0632; E-mail: simon.lovestone@kcl.ac.Uk.

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biochemical markers of inflammation to be better predictors of conversion than APOE genotype or clinical measures (Area under the curve (AUC) 0.65, 0.62, 0.59 respectively). In a subset of subjects who also had MRI scans the combination of serum markers of inflammation and MRI automated imaging analysis provided the best predictor of conversion (AUC 0.78). These results show that the combination of imaging and cytokine biomarkers provides an improvement in prediction of MCI to AD conversion compared to either datatype alone, APOE genotype or clinical data and an accuracy of prediction that would have clinical utility.

Keywords: Proteomics, MRI, mild cognitive impairment, Alzheimer's disease, biomarkers

## INTRODUCTION

The challenge of the neurodegenerative diseases is daunting; it is estimated that the global prevalence of dementia will rise from 24.3 million cases in 2005 to 81.1 million cases in 2040 [1]. Alzheimer's disease (AD) is the commonest form of dementia [2]. Rising to the challenge posed by this health burden, there are many potential disease modification therapies in development with approximately 10 compounds for AD in phase III and more than 50 in phase II [3]. There is a consensus that disease modification therapy is most likely to be efficacious very early in the disease process and because of this there is an increasing drive towards very early identification and diagnosis. Given the inherent difficulty of very early, and even prodromal, diagnosis, biomarkers are likely to play an important role. They are incorporated into the proposed revision of diagnostic criteria [4] for AD and have been increasingly examined for their potential to predict which people with mild cognitive impairment (MCI) are most likely to progress to full dementia [5]. This is important as only a minority with MCI progress to full dementia in the time frame of a typical clinical trial. A recent meta-analysis showed an annual conversion rate of 8.1% and a cumulative proportion of 33.6% for MCI conversion to AD [6] and in a previous systematic review we found study-related variables including recruitment strategy to be the most important factors predicting conversion [7].

The primary focus in the search for biomarkers for AD to date has been on neuroimaging, and on Aβ and tau proteins in cerebrospinal fluid (CSF) [8, 9]. Various studies using structural MRI have identified brain regions within the medial temporal lobe, particularly the hippocampus and entorhinal cortex, as potential biomarkers of conversion from MCI to AD [10–14]. In addition molecular imaging using amyloid PET ligands also report efficacy as markers predictive of conversion [15]. CSF is an excellent fluid for biomarker discovery in neurodegeneration as it is in direct contact with the extracellular space of the brain and is therefore supposed to reflect biochemical changes occurring in the brain [16]. Recently Shaw *et al.* showed in the US Alzheimer's Disease Neuroimaging Initiative (ADNI) study that the t-tau/$A\beta_{1-42}$ ratio discriminated between those who will remain MCI subjects and those who will convert to AD within one year follow-up [17]. In addition it has been reported that MCI subjects with abnormal results on both FDG-PET and episodic memory were more likely to convert to AD [18]. However, structural MRI is not sufficiently predictive of conversion [14, 19], PET imaging is a highly specialised approach available in relatively few centres, and lumbar puncture for CSF, although non-traumatic and without side effects in the majority of patients, necessitates a high level of skill and a hospital setting. A blood-based biomarker would be hugely advantageous especially in large-scale population and community based studies of elderly frail people.

Previously we and others have demonstrated using proteomics that blood-based biomarkers were feasible and reproducible in independent studies [20–22]. However, the most impressive evidence for a specific and sensitive marker of MCI conversion to date comes from a study reporting that 18 signalling proteins in blood plasma could be used to predict conversion from MCI to AD 2–6 years later with an accuracy of 91% [23]. However these results have not yet been independently replicated. Recently, O'Bryant *et al.* developed a serum protein-based classifier for the prediction of AD patients and controls [24].

For the most part, these studies have concentrated on single modality biomarkers although there are theoretical reasons and increasing data from combinatorial studies to think that combining biomarkers might have added benefits [25–28]. Some studies have combined imaging with clinical variables to try to increase predictive power, with mixed results. One such study found no added benefit of combining structural MRI data with clinical measures [12] whereas another developed a predictor including three clinical predictors

(SRT immediate recall, FAQ and UPSIT) as well as two imaging markers (hippocampal and entorhinal cortex volume) [29]. Davatzikos *et al*. recently integrated MRI and CSF biomarkers and noted improved predictive accuracy compared to either individual data type [9] and Ewers *et al*. investigated the accuracy of MRI and CSF biomarkers and neuropsychological tests for predicting the conversion from MCI to Alzheimer's disease [30].

However no study, to our knowledge, has investigated the potential added benefit of combining biomarkers that are readily obtained in routine clinical practice. To explore this we combined automated regional analysis of structural MRI with analysis of serum cytokines and APOE genotype to assess if the combination of data types improved prediction of progression in a small cohort of participants from the AddNeuroMed study, a European ADNI-like biomarker study [31]. In addition, we have examined the predictive accuracy of a cytokine panel in a larger dataset, which includes the AddNeuroMed cohort.

## MATERIALS AND METHODS

### *Subjects*

The study population used in this report was derived from the AddNeuroMed study, a European multi-centre study, aiming to identify biomarkers for AD [31] and the Alzheimer Research Trust–funded cohort at King's College London (KCL-ART) [32]. The participating AddNeuroMed clinical centres were in Kuopio, Perugia, Lodz, Thessaloniki, Toulouse and London. Subjects were patients who attended local memory clinics and received a diagnosis of MCI. Diagnosis of dementia was made according to NINCDS-ADRDA criteria and DSM IV, amnestic MCI diagnosis was based on CDR (CDR = 0.5), MMSE (MMSE $\geq$ 24) and amnestic cognitive impariment according to word list learning recall task of the CERAD (<1.5 SD of population mean adjusted for gender, age and level of education).The follow-up period was one year. At baseline and follow-up information was obtained on demographic characteristics, medical history, current health status, medication use and family history. In addition to the clinical data, blood and urine samples were obtained and participants underwent a neuropsychological assessment.

In the KCL-ART cohort, per MCI converter case we randomly sampled two MCI non-converters matched on gender and age. In the AddNeuroMed cohort, per MCI converter case we randomly sampled one MCI non-converter matched on gender and year of baseline assessment, sampled in five-year age categories. For the integration of cytokine levels with imaging measures, data from AddNeuroMed subjects who had undergone successful baseline structural MRI imaging, and whose APOE status and cytokine levels were determined were used (cytokine-imaging cohort; $n = 48$).

Assessment of the patient samples was conducted in a randomized fashion. Demographics can be found in Table 1.

### *Samples*

At baseline and follow-up blood samples were drawn by veni-puncture and collected into EDTA glass tubes, after a minimum of 2 h fasting prior to draw. After coagulation for 30 minutes serum was obtained by centrifugation for 8 min at 3,000 g at 4°C. Samples were aliquoted and frozen at −80°C until further use.

### *Cytokine multiplex analysis*

Serum samples were analyzed for 36 cytokines and chemokines (supplementary table 1) using a commercially available Cytokine Human 30-plex panel and a customized 6-plex (Biosource International). Samples were measured at baseline and follow-up in duplicate and according to the manufacturer's recommendations. The samples were measured in a randomized and blinded fashion using the antibody bead mix in duplicate with a biotinylated detection antibody followed by streptavidin-phycoerythrin. The plate was read using the Luminex platform (BioRad), and data were collected for 100 beads per cytokine from each well. Cytokine concentrations were calculated using Bio-Plex Manager 5.0 software with a five parameter curve-fitting algorithm applied for standard curve calculations.

### *Neuroimaging*

#### *Data acquisition*

Data acquisition took place using six different 1.5T MR systems (four General Electric, one Siemens and one Picker). At each site a quadrature birdcage coil was used for RF transmission and reception. Data acquisition was designed to be compatible with the Alzheimer Disease Neuroimaging Initiative (ADNI) [33]. The imaging protocol included a high resolution sagittal 3D T1-weighted MPRAGE volume (voxel size

Table 1
Demographics of MCI converter and non-converter cytokine cohort and cytokine and imaging cohort, including mean APOE ε4 dosage and p-values of difference between Non-Converters (MCI-N) and Converters (MCI-C). (MMSE, Mini Mental State Exam, Maximum score = 30)

| | Cytokine MCI-N ($n = 128$) | Cytokine MCI-C ($n = 77$) | *p*-value | Cytokine/imaging MCI-N ($n = 26$) | Cytokine/imaging MCI-C ($n = 22$) | *p*-value |
|---|---|---|---|---|---|---|
| Age | 80.4 | 77.5 | 0.003 | 74.1 | 73.4 | 0.68 |
| Female % | 55 | 59 | 0.77 | 58 | 41 | 0.38 |
| MMSE | 27.1 | 26.2 | 0.006 | 27.1 | 26.6 | 0.39 |
| APOE ε4 dosage | 0.31 | 0.54 | 0.009 | 0.35 | 0.77 | 0.02 |

$1.1 \times 1.1 \times 1.2\,mm^3$) and axial proton density / T2-weighted fast spin echo images. Full brain and skull coverage was required for both of the latter datasets and detailed quality control carried out on all MR images [34, 35]. All MR images received a clinical read by an on-site radiologist in order to exclude any subjects with non-AD related pathologies.

*Image analysis*

A highly automated structural MRI image processing pipeline developed by Fischl et al and producing both regional cortical thickness measures and regional volume measures was utilized for data analysis [36–38]. Cortical reconstruction and volumetric segmentation included removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. Surface inflation was followed by registration to a spherical atlas which utilized individual cortical folding patterns to match cortical geometry across subjects and parcellation of the cerebral cortex into units based on gyral and sulcal structure. All volumes were normalized by the subjects' intracranial volume.

The regional cortical thickness was measured from 34 areas and the regional cortical volume was measured bilaterally from 24 areas (supplementary Table 2).

*ApoE genotyping*

The APOE haplotype was determined using two allelic discrimination assays (rs7412 and rs429358) based on fluorogenic 5' nuclease activity, the Taq polymerase single nucleotide polymorphism genotyping assay (TaqMan, Applied Biosystems Inc., www.appliedbiosystems.com).

*Statistical analysis*

As an initial step unsuccessful cytokine readings with less than 50 bead counts were excluded from the analysis.

Observations with more than 50% missing values and/or outliers were consequently excluded from further analysis. Outliers were any data values which lay more than 1.5 times the interquartile range (IQR) below the first Quartile ($Q_{25}$) or above the third Quartile ($Q_{75}$).

*Machine-learning approach*

*Datasets*

The total sample size with cytokine data (AddNeuroMed + KCL-ART] was 205 (MCI-N = 128, MCI-C = 77). This cohort was divided into an approximate two thirds -one third stratified training set – test set where the training set sample size was 136 (MCI-N = 85, MCI-C = 51) and the test set size was 69 (MCI-N = 43, MCI-C = 26). Using the training set only we created 5 different datasets containing: (1) APOE ε4 dosage, (2) Age, Gender and MMSE score (Clinical data), (3) Cytokine data, (4) A subset of 7 cytokines in common with the panel of 18 identified by Ray et al. [23] (EGF, G-CSF, GDNF, IL-1α, IL-3, MCP-3 AND TNF-α; see supplementary table 1 for details), and (5) Cytokine, APOE ε4 dosage and Clinical data. Missing values were replaced using global class means.

The number of subjects with both imaging and cytokine data was 48 (MCI-N = 26, MCI-C = 22). This cohort was divided into an approximate two-thirds-one third stratified training set – test set where the training set sample size was 31 (MCI-N = 17, MCI-C = 14) and the test set size was 17 (MCI-N = 9, MCI-C = 8). Using the training set only we created 8 different datasets containing: (1) APOE ε4 dosage, (2) Age, Gender and

Table 2
Classification area under the ROC curve for the training and test datasets for entire cohort (best training set classifier in bold)

| | Training SVM (all data) | Training SVM (10 attributes) | Training SVM (5 attributes) | Test |
|---|---|---|---|---|
| Cytokine, clinical + APOEε4 | **0.69** | 0.67 | 0.67 | 0.61 |
| Cytokine | **0.64** | 0.63 | 0.62 | 0.65 |
| Cytokine subset | **0.66** | N/A | N/A | 0.60 |
| Clinical | **0.72** | N/A | N/A | 0.59 |
| APOEε4 dosage | **0.59** | N/A | N/A | 0.62 |

MMSE score (Clinical data), (3) Cytokine data, (4) Imaging data, (5) Cytokine and Clinical data, (6) Imaging and Clinical data, (7) Imaging and Cytokine, and (8) Imaging, Cytokine, APOE ε4 dosage, and Clinical data.

*Classification*

Feature selection and class prediction by machine-learning was conducted using Weka [39]. To address the class imbalance between the two classes with cytokine data (MCI-N = 128, MCI-C = 77), a cost-sensitive approach was employed (weka.classifiers.meta.CostSensitiveClassifier) using a cost matrix of the ratios of the two classes. The class imbalance in the dataset with both cytokine and imaging data was relatively minor (MCI-N = 26, MCI-C = 22) and therefore a cost-sensitive classifier was not used. Three different approaches were assessed using a ten-fold cross validation on the training data with 100 iterations : (1) A Support Vector Machine (SVM - the SMO algorithm in Weka) using default settings (Polykernel kernel) and all the data variables, (2) a within-loop feature selection using the best 10 attributes for classification with an SVM (weka.classifiers.meta.AttributeSelectedClassifier). The feature selection phase was conducted using the SVMAttributeEval (weka.attributeSelection.SVMAttributeEval) and Ranker (weka.attributeSelection.Ranker) algorithms, and (3) a within-loop feature selection using the best 5 attributes for classification with an SVM. Approaches (2) and (3), which incorporate feature selection, were not conducted for the datasets comprising APOE ε4 dosage only or the Clinical data only.

The effect of the SVM complexity parameter (C) was investigated by setting C = 0.01, 0.1, 1 (the default value) and 10 and the C value resulting in the highest AUC in the training set was used in evaluating the test set.

For each dataset, the machine learning approach resulting in the highest area under the ROC curve (AUC) after the ten-fold cross validation was applied to the relevant test dataset. To obtain proper probability estimates, the option that fits logistic regression models (-M) to the outputs of the support vector machine was used. An accuracy (ACC), sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and area under the curve were calculated for each test set.

## RESULTS

*Cytokine dataset*

We successfully measured 35 cytokines for analysis in a total of 205 subjects. Only one cytokine - RANTES – showed evidence of technical failure and was excluded from all analyses. In addition IL-17 and IL-1B were excluded from the multivariate analysis because of the high proportion of missing values (>50%). Baseline cytokine levels of three MCI non-converter subjects, were excluded from the analysis, because of overall highly elevated cytokine levels (i.e. more than 60% of all cytokines measured showed apparently arbitrarily high levels) or because more than 50% of all cytokine measures were missing.

*Multivariate analysis*

A machine-learning approach (Support Vector Machines) to class prediction was used to identify a set of combined analytes that might discriminate between converters and non-converters. Support Vector Machines (SVMs) are used extensively in computational biology as they have been shown to predict binary outcomes with high accuracy and possess the ability to model diverse and high-dimensional data [40].

The total number of subjects with cytokine data was 205 (MCI-N = 128, MCI-C = 77). The feature selection stage did not improve the accuracies of any of the classifiers and therefore the model built with all of the data

for each of the training datasets was applied to the relevant test dataset (Table 2). The AUCs (Figure 1) from the test datasets were 0.62 (APOE ε4 dosage), 0.60 (Cytokine subset), 0.61 (Cytokine data, Clinical data and APOE ε4 dosage), 0.59 (Clinical data) and 0.65 (Cytokine data).

*Cytokine and imaging dataset*

We then sought to assess whether combining structural MRI data with the cytokine data would improve classification accuracy. MRI brain scans of a subset of the subjects were processed and the regional cortical thickness was measured from 34 areas and the regional cortical volume was measured bilaterally from 24 areas (see Methods for details).

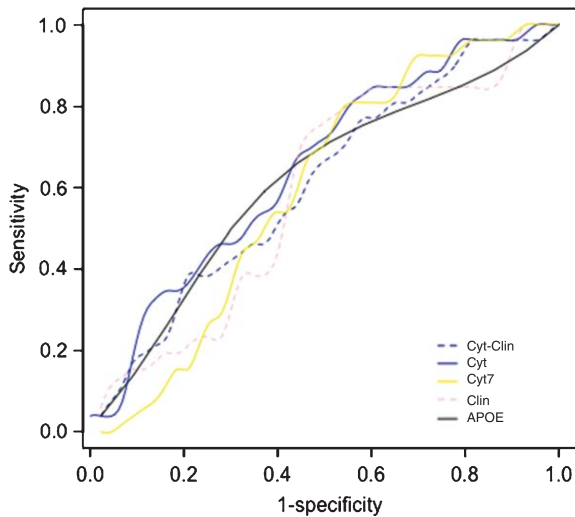


Fig. 1. Entire cytokine cohort receiver operating characteristic (ROC) curves of most accurate training classifiers applied to the test datasets. Cyt-Clin = Cytokine and Clinical data, Cyt = Cytokine data, Clin = Clinical data, Cyt7 = subset of 7 cytokines in common with the panel of 18 identified by Ray et al. , and APOE = APOE ε4 dosage.

The number of subjects with both imaging and cytokine data was 48 (MCI-N = 26, MCI-C = 22). Feature selection improved the training set prediction accuracy for the Cytokine dataset and the combined Cytokine and Clinical dataset. The other datasets showed greatest accuracy when including all data (Table 3). The most accurate model for each training dataset was then applied to the relevant test dataset.

In this smaller dataset, the cytokine data by themselves do not classify the test subjects particularly well (AUC = 0.60). In fact, in this cohort APOE ε4 dosage alone (AUC0.74) is a better predictor of conversion than the cytokine or imaging data (Table 3). The combination of the cytokine and imaging data is the most accurate classifier (AUC = 0.78) showing a modest improvement over APOE ε4 dosage.

The imaging or cytokine data alone predict the test subjects with AUCs = 0.68 and 0.60, respectively. The sensitivity, specificity, positive and negative predictive values of each classifier is shown in supplementary table 3.

## DISCUSSION

This study focused, for the first time, on combinatorial biomarkers using readily available techniques in order to identify a marker set predictive of conversion from MCI to dementia within the time frame of a typical disease modification trial. Alone, cytokine levels showed some predictive value for MCI conversion while imaging data showed a modest predictive accuracy. However, the predictive model using combined cytokine levels and imaging measures outperformed either individual classifier (Table 3 and Figure 2). The cytokine classifier exhibits low sensitivity and a higher specificity (supplementary table 3) whereas the converse is true of the imaging classifier. When the two data-types are combined the classification accuracy is improved (Table 3 and supplementary table 3).

Table 3
Classification area under the ROC curve for the training and test datasets for cohort with cytokine and imaging data ($n = 48$; best training set classifier in bold)

| | Training SVM (all data) | Training SVM (10 attributes) | Training SVM (5 attributes) | Test |
|---|---|---|---|---|
| Cytokine + imaging + clinical + APOEε4 dosage | **0.60** | 0.50 | 0.50 | 0.74 |
| Cytokine + imaging | **0.56** | 0.51 | 0.54 | 0.78 |
| Cytokine + clinical | 0.49 | 0.50 | **0.50** | 0.53 |
| Imaging + clinical | **0.63** | 0.54 | 0.52 | 0.67 |
| Cytokine | 0.48 | 0.50 | **0.50** | 0.60 |
| Imaging | **0.62** | 0.52 | 0.54 | 0.68 |
| Clinical | **0.50** | N/A | N/A | 0.50 |
| APOEε4 dosage | **0.59** | N/A | N/A | 0.74 |

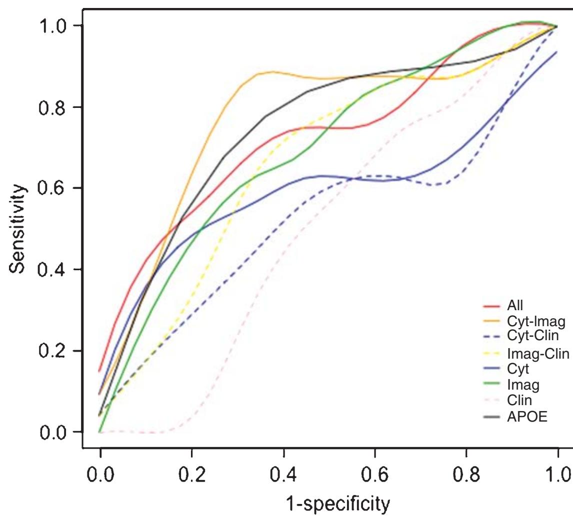


Fig. 2. Cytokine and imaging cohort receiver operating characteristic (ROC) curves of most accurate training classifiers applied to the test datasets. All = Cytokine, Imaging, Clinical data and APOE ε4 dosage, Cyt-Imag = Cytokine and Imaging data, Cyt-Clin = Cytokine and Clinical data, Imag-Clin = Imaging and Clinical data, Cyt = Cytokine data, Imag = Imaging data, Clin = Clinical data, and APOE = APOE ε4 dosage.

Considering that the potential disease modifying therapies are non-hazardous and aim to focus on an early stage of the disease the ideal predictive model would have a high negative predictive value and a high specificity. From a clinical point of view our here introduced model combining imaging and cytokine measures is favorable above combined imaging measures alone, which have been a point of primary interest in the literature so far.

Previous studies have looked at a number of brain regions (e.g. volumes of hippocampus, entorhinal cortex, ventricles, and whole brain) as potential biomarkers of conversion from MCI to AD [10, 29, 41]. Devanand *et al.* integrated the baseline predictors cognitive test performance, informant report of functional impairment, APOE genotype, olfactory identification deficit, and magnetic resonance imaging (MRI) hippocampal and entorhinal cortex volumes [29]. Ultimately, they used five variables in a predictor: Pfeffer Functional Activities Questionnaire (FAQ; informant report of functioning), University of Pennsylvania Smell Identification Test (UPSIT; olfactory identification), Selective Reminding Test (SRT) immediate recall (verbal memory), MRI hippocampal volume, and MRI entorhinal cortex volume. A recent imaging study, also based upon the AddNeuroMed patient cohort, has analyzed regional MRI volumes and thicknesses as predictors of conversion from mild cognitive impairment to AD [14]. Analysis of the expanded patient cohort of 103 subjects (22 converters at year one follow up) showed that the bilateral hippocampus and amygdala, and right caudate baseline volumes were significantly smaller in MCI to AD converters compared to stable MCI subjects.

We analyzed a subset of 7 of the 18 signaling proteins for MCI conversion identified by an earlier study [23] (see Materials & Methods for details). We trained an SVM using these 7 cytokines and applied the model to a test set resulting in an AUC of 0.60. Our findings suggest that the inflammatory markers identified by Ray *et al.* may show some differential expression in people with established AD, and we are able to show some prognostic value of these markers for the more demanding, but more clinically important task of predicting MCI conversion.

Some of the cytokines we have examined have been previously implicated in AD as potential markers. Reports describing IL-1β levels in serum of AD patients have been conflicting with some groups describing an increase in IL-1β serum levels in AD patients compared to controls [42, 43], but others finding no change [44]. A meta analysis also found a genetic association of a IL-1β polymorphism and AD [45-47]. It has been hypothesized that proinflammatory cytokines, such as IL-1β are activating a cascade of neurotoxic changes in the brain, that are related with the development of neuritic plaques and neurofibrillary tangles characteristics in AD [48]. VEGF is associated with neuroprotection and regeneration in the brain. It co-localizes with plaques in AD brain [49] and some studies report an increase in VEGF levels in serum and CSF [50, 51], whereas others find a decrease in serum [52] and no change in CSF [53]. In the cytokine/imaging cohort the classifier using APOE ε4 dosage alone performs better than most other classifiers. It is not surprising that APOE performs well as it is clear that the ε4 allele is associated with AD and has previously been associated with the time to progression from MCI to AD [54]. In addition, in this smaller dataset the mean APOEε4 dosage per converting patient is much greater than in converters in the entire cohort (0.77 compared to 0.54 – Table 1).

There are limitations to our study. Most obviously, the sample size of the cytokine and imaging cohort is relatively small ($n = 48$), which meant constructing a predictive model with a training set of 31 subjects. When applying our predictors to the set of 17 subjects, although the combination of cytokines outperforms APOEε4 dosage, the increase in performance is mod-

est. It is noteworthy that adding clinical information (including basic cognitive tests, diagnosis and medical history but not very detailed neuropsychometry) adds nothing to the predictive power. This emphasises that the clinical assessment of MCI alone does not predict conversion.

As such, our predictor would need to be applied to a larger dataset to independently assess its accuracy. Nonetheless, we show preliminary evidence that a combined set of imaging and cytokine measures provides a small improvement in prediction of MCI to AD conversion than either cytokine or imaging data alone. It remains to be seen whether the accuracy reported here may be improved still further by the addition of other biomarkers or by alternative MRI analytical routines. As the prediction in this case was over only one year after test these data hold out the promise of a combinatorial biomarker for use in both clinical practice and, perhaps more pressingly, for patient stratification and enrichment in trials of disease modification agents.

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Supplementary Table 1
Proteins measured with luminex 30- and 6-plex (HGNC, HUGO Gene Nomenclature Committee)

| Cytokine | Official full name (HGCN) | Gene symbol (HGNC) |
|---|---|---|
| BDNF | Brain-derived neurotrophic factor | BDNF |
| DR5 | Tumor necrosis factor receptor superfamily, member 10b | TNFRSF10B |
| EGF | Epidermal growth factor | EGF |
| Eotaxin | Chemokine (C-C motif) ligand 11 | CCL11 |
| FGF | Fibroblast growth factor 2 (basic) | FGF2 |
| G-CSF | Colony stimulating factor 3 (granulocyte) | CSF3 |
| GDNF | Glial cell derived neurotrophic factor | GDNF |
| GM-CSF | Colony stimulating factor 2 (granulocyte-macrophage) | CSF2 |
| HGF | Hepatocyte growth factor | |
| IFN-α | Interferon, alpha 1 | IFNA1 |
| IFN-γ | Interferon, gamma | IFNG |
| IL-1α | Interleukin 1, alpha | IL1A |
| IL-1β | Interleukin 1, beta | IL1B |
| IL-1RA | Interleukin 1 receptor, type I | IL1R1 |
| IL-2 | Interleukin 2 | IL2 |
| IL-2R | Interleukin 2 receptor, alpha | IL2RA |
| IL-3 | Interleukin 3 | IL3 |
| IL-4 | Interleukin 4 | IL4 |
| IL-5 | Interleukin 5 | IL5 |
| IL-6 | Interleukin 6 | IL6 |
| IL-7 | Interleukin 7 | IL7 |
| IL-8 | Interleukin 8 | IL8 |
| IL-10 | Interleukin 10 | IL10 |
| IL-12 | Interleukin 12 | IL12 |
| IL-13 | Interleukin 13 | IL13 |
| IL-15 | Interleukin 15 | IL15 |
| IL-17 | Interleukin 17 | IL17 |
| IP-10 | Chemokine (C-X-C motif) ligand 10 | CXCL10 |
| MCP-1 | Chemokine (C-C motif) ligand 2 | CCL2 |
| MCP-3 | Chemokine (C-C motif) ligand 7 | CCL7 |
| MIG | Chemokine (C-X-C motif) ligand 9 | CXCL9 |
| MIP-1α | Chemokine (C-C motif) ligand 3 | CCL3 |
| MIP-1β | Chemokine (C-C motif) ligand 4 | CCL4 |
| RANTES | Chemokine (C-C motif) ligand 5 | CCL5 |
| TNF-α | Tumor necrosis factor | TNF |
| VEGF | Vascular growth factor A | VEGFA |

Supplementary Table 2
MRI regional cortical thickness from 34 areas and the regional cortical volume measured bilaterally from 24 areas

| Volumes | Cortical thicknesses |
|---|---|
| 3rd Ventricle | Banks of STS |
| 4th Ventricle | caudal anterior cingulate |
| 5th Ventricle | caudal middle frontal |
| Brain Seg Vol | corpus callosum |
| Brain Stem | cuneus |
| CC_Anterior | entorhinal |
| CC_Central | frontal pole |
| CC_Mid_Anterior | fusiform |
| CC_Mid_Posterior | inferior parietal |
| CC_Posterior | inferior temporal |
| CSF | isthmus cingulate |
| Left Accumbensarea | lateral occipital |
| Left Amygdala | lateral orbito frontal |
| Left Caudate | lingual |
| Left Cerebellum Cortex | medial orbitofrontal |
| Left Cerebellum White Matter | middle temporal |
| Left Cerebral Cortex | para central |
| Volumes | Cortical thicknesses |
| Left Cerebral White Matter | para hippocampal |
| Left choroid plexus | pars opercularis |
| Left Hippocampus | pars orbitalis |
| Left Inf Lat Vent | pars triangularis |
| Left Lateral Ventricle | pericalcarine |
| Left non WM hypointensities | post central |
| Left Pallidum | posterior cingulate |
| Left Putamen | precentral |
| Left Thalamus Proper | precuneus |
| Left Ventral DC | rostral anterior cingulate |
| Left vessel | rostral middle frontal |
| Left WM hypointensities | superior frontal |
| Optic Chiasm | superior parietal |
| Right Accumbensarea | superior temporal |
| Right Amygdala | supra marginal |
| Right Caudate | temporal pole |
| Right Cerebellum Cortex | transverse temporal |
| Right Cerebellum White Matter | |
| Volumes | Cortical thicknesses |
| Right Cerebral Cortex | |
| Right Cerebral White Matter | |
| Right Hippocampus | |
| Right Inf Lat Vent | |
| Right Lateral Ventricle | |
| Right non WM hypointensities | |
| Right non WM hypointensities | |
| Right Pallidum | |
| Right Putamen | |
| Right Thalamus Proper | |
| Right Ventral DC | |
| Right vessel | |
| Right WMhypointensities | |

Supplementary Table 3
Sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and Accuracy (ACC) for the cytokine and imaging cohort classifiers

| | SN | SP | PPV | NPV | ACC |
|---|---|---|---|---|---|
| Cytokine + imaging + clinical + APOEε4 dosage | 0.75 | 0.67 | 0.67 | 0.75 | 0.71 |
| Cytokine + imaging | 0.75 | 0.78 | 0.75 | 0.78 | 0.76 |
| Cytokine + clinical | 0.38 | 0.78 | 0.60 | 0.59 | 0.59 |
| Imaging + clinical | 0.63 | 0.67 | 0.63 | 0.67 | 0.65 |
| Cytokine | 0.50 | 0.78 | 0.67 | 0.64 | 0.65 |
| Imaging | 0.88 | 0.44 | 0.58 | 0.80 | 0.65 |
| Clinical | 0.25 | 0.67 | 0.40 | 0.50 | 0.47 |
| APOEε4 dosage | 0.75 | 0.67 | 0.67 | 0.75 | 0.71 |

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