Draft Manuscript From Review

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Journal:	*Journal of Neurology*
Manuscript ID:	jneuro-08-07-0567.R1
Manuscript Type:	Original Communication
Keyword:	Alzheimer\x27s disease, SPECT, Dementia and neuroimaging

The final publication is available at link.springer.com
http://link.springer.com/article/10.1007%2Fs00415-008-0897-4

BRAIN SPECT IN SUBTYPES OF MILD COGNITIVE IMPAIRMENT: FINDINGS FROM THE 'DESCRIPA' MULTICENTER STUDY.

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Running head: SPECT in MCI subtypes
Words count: 4,449 (main text)

ABSTRACT

The Descripa multicentre study enrolled patients with MCI or subjective cognitive complaints (SUBJ), a part of whom underwent optional brain perfusion SPECT. These patients were classified as SUBJ (n=23), non-amnestic MCI (naMCI; n=17) and amnestic MCI (aMCI; n=40) based on neuropsychology. Twenty healthy subjects formed the Control (CTR) group. Volumetric Regions of Interest (VROI) analysis was performed in 6 associative cortical areas in each hemisphere. ANOVA for repeated measures, corrected for age and centre, showed significant differences among groups ($p=0.01$) and VROI ($p<0.0001$) with a significant group-region interaction ($p=0.029$). At post-hoc comparison, SUBJ did not differ from CTR. aMCI disclosed reduced uptake in left hippocampus and bilateral temporal cortex (comparison with CTR) or left hippocampus and bilateral parietal cortex (comparison with SUBJ). In naMCI group, reduced VROI values were found in bilateral temporal cortex and right frontal cortex. In the comparison between aMCI and naMCI, the former had lower values in left parietal cortex and precuneus. Discriminant analysis between SUBJ/CTR versus all MCI patients allowed correct allocations in the 73% of cases. Mean VROI values were highly correlated ($p<0.0001$) with the learning measure of a verbal memory test, especially in the bilateral precunei and parietal cortex and in the left hippocampus. In a subset of 70 patients, mean VROI values show a significant correlation ($p<0.05$) with the white matter hyperintensities score on MRI. In conclusion, MCI subtypes have different perfusion patterns. The aMCI group exhibited a pattern that is typical of early Alzheimer's disease, while naMCI group showed a more anterior pattern of hypoperfusion. Instead, a homogeneous group effect was lacking in SUBJ.

Key words: subjective cognitive impairment, amnestic MCI, non-amnestic MCI, brain SPECT, verbal memory, MRI.

INTRODUCTION

There is an increasing interest to understand the cerebral pathophysiology underlying Mild Cognitive Impairment (MCI) in subjects who substantially maintain their daily living functional autonomy, including patients already affected by either neurodegenerative or cerebrovascular diseases but still in a pre-dementia phase (35) as well as subjects in the extreme tail of the norm who will remain stable over time or even improve (25).

In view of the development of drugs that could modify the natural history of neurodegenerative diseases causing dementia, it is of paramount importance to identify these subjects in the pre-dementia phase. In order to reduce the heterogeneity of MCI, subtypes have been proposed. Amnestic MCI (aMCI) refers to subjects with a memory deficit and may prelude Alzheimer's type dementia (2). Non-amnestic MCI (naMCI) refers to deficits in other cognitive domains and may be the presenting symptom of other types of dementia, such as Vascular Dementia, Fronto-Temporal Dementia, Lewy-body dementia (45) or of non-dementing conditions, such as a depressive trait (7).

However, whether MCI subtypes have a different underlying pathophysiology remains unclear. In aMCI, brain perfusion-metabolic failure has been found by SPECT (3, 12, 14, 16-18, 20, 21) and PET (1, 6, 8, 9, 30, 31) studies especially in the posterior associative cortex (1, 3, 6, 8, 12, 14, 17), medial temporal lobe (1, 9, 15, 20, 30) and posterior cingulate/precuneus (1, 3, 8, 14, 16, 20, 21), thus reproducing the typical functional deficit of early Alzheimer's disease (AD). However, data on brain perfusion-metabolic failure in naMCI is still lacking. Moreover, another category of patients is receiving increasing attention. These patients present subjective cognitive complaints (SUBJ) but neuropsychological and neurological evaluations fail to disclose significant abnormalities. This appears as a potentially relevant, although heterogeneous, group because may contain some subjects who will develop an objective cognitive deficit (28) and thus have an underlying cerebral pathology. Moreover, these patients have potentially practical implications, because they represent the group that should be distinguished by MCI patients in the clinical setting. As for naMCI, SPECT data is lacking in this group as well.

In order to provide further support for the idea that subclassification of MCI may differentiate between various underlying aetiologies, we investigated brain function with perfusion SPECT in aMCI, naMCI and SUBJ patients, in comparison with a control group of healthy subjects. Moreover, since this classification lies on the neuropsychological profile, we investigated whether SPECT perfusion correlated with cognitive test performances. Finally, the correlation between perfusion values and the severity of white matter hyperintensities (WMH) at the Magnetic Resonance Imaging (MRI) was evaluated in a subset of patients, because WMH are frequently reported in MCI and are suspected to play a role in brain dysfunction (37).

METHODS

Study design. The patients took part in the DESCRIPA multi-center study (http://www.descripa.eu/), of the European Alzheimer's Disease Consortium (http://eadc.alzheimer-europe.org/), aiming to the development of clinical criteria and screening guidelines for AD in the predementia stage. The inclusion criteria basically included outpatients aged 55 years or older, newly referred for cognitive complaints to an European center dedicated to the evaluation of cognitive disorders. All kinds of referrals were considered, including self- or relative-referral, referral from general practitioner and from first-level neurological or geriatric clinics. Cognitive complaints mainly included memory complaints but they could also include difficulties in other cognitive domains, such as attention and orientation. Exclusion criteria were: dementia; any somatic, metabolic (e.g., vitamin deficiency, endocrine untreated disorders, kidney, liver or heart failure), psychiatric, or neurological disorder that may cause cognitive impairment, such as cerebrovascular accidents; neurodegenerative diseases, such as Parkinson's disease; severe head trauma; brain tumor; history of alcohol abuse; severe depression. In more detail, dementia was excluded by the clinical interviews with patients and caregivers and by means of formal questionnaires assessing the basic and instrumental activities of daily living.

Six DESCRIPA centres regularly used perfusion SPECT with ^{99m}TC-ECD or ^{99m}TC-HMPAO in clinical practice. SPECT was performed after patient enrolment in the DESCRIPA study in these 6 centres taking part in the optional SPECT sub-study. SPECT was not a mandatory tool for subjects to be enrolled in

the DESCRIPA, nor SPECT results were used to classify patients. Because significant perfusion distribution differences between the two radiopharmaceuticals have been shown both in healthy (34) and in pathological (40) conditions, including AD (23), we only selected subjects from the 3 centres in which ^{99m}TC-ECD had been performed, as this resulted in the largest sample.

The three centres enrolled 174 patients, of which 80 had underwent SPECT. Reasons for no SPECT included patient refusal, poor quality of SPECT scan, and avoidance of waiting lists. Subjects with and without SPECT did not differ with respect to demographic characteristics, Mini-Mental State Examination (MMSE) score and prevalence of vascular risk factors.

As the DESCRIPA study did not enroll normal subjects, a group of 20 healthy controls (CTR) was identified from the database of normal subjects of two of the three centers (controls unavailable in one centre). These subjects had been accepted as volunteers taking part in recent SPECT studies and were selected with the only criteria to be in the same age range of patients. They were judged to be in good health by general medical and neurological examinations, routine blood and urine assays. Mild and well controlled medical conditions, such as hypertension and diabetes, and a mild depressive trait were not considered as exclusion criteria. Only subjects not taking neuropsychoactive drugs or drugs known to interfere with cerebral perfusion were accepted in the CTR group. An extended neuropsychological test battery, including the tests performed on patients, ruled out any cognitive deficit.

The study was approved by the local Medical Ethics Committee in each centre.

Baseline clinical assessment. All subjects underwent a standard battery of examinations, including clinical history, medical and neurological examinations, laboratory tests, functional evaluation using the Clinical Dementia Rating scale (CDR), rating scales for depression and neuropsychiatric symptoms, a neuropsychological test-battery (see below), and structural neuroimaging. The same neuropsychological battery was employed for DESCRIPA patients and for CTR, because they were studied in the same time span as DESCRIPA patients. Digital MRI was available in 52 patients and 18 CTR subjects and was centrally scored for the presence of WMH on the axial FLAIR images in Amsterdam, using the Age-Related White Matter Changes scale (ARWMC) (46). Briefly, this scale evaluates five brain regions in each hemisphere, including the frontal, parieto-occipital, temporal areas, the infratentorial area, and the basal

ganglia. A 0-3 score is provided for each region, ranging from no WML (0) to diffuse involvement of the entire region, with or without involvement of U fibers (3). A sum score (range 0-30) can be computed expressing the severity of WMLs in all the regions taken together.

General cognition was assessed using the MMSE (patients with a score lower than 24 were excluded). Depression was assessed by the Hamilton depression scale (20 subjects), the 15-item Geriatric Depression Scale (26 subjects) or the Center for Epidemiologic Symptoms of Depression (CES-D) (36) scale (54 subjects). A depressive trait was defined according to the standard cut-off of each scale.

Hypertension (blood pressure >140/90 mmHg or use of antihypertensive medication), diabetes mellitus, hypercholesterolemia, ischemic heart disease, the presence of depressive symptoms, a history of thyroid disease, and current smoking (Table 1) were included in the analysis. All these conditions were adequately treated and well controlled by therapy during neuropsychological and SPECT examinations.

Neuropsychological examination and definition of MCI subtypes. In each center, a battery of neuropsychological tests was performed to assess cognitive performance in the domains of memory, language, executive function and attention, and visuoconstruction. Raw scores were converted to age, education, and gender corrected Z-scores according to locally collected or published normative data and these Z-scores were used for further analysis.

Patients were classified into 3 groups on the basis of test performances in these cognitive domains. Impairment was defined as a Z-score of -1.5 or lower. Subjects without impairment in any domain were classified as SUBJ. Subjects with impairment in the memory domain only or with impairment in the memory domain plus impairment in non-memory domains were defined as aMCI. Subjects with impairment in one or more non-memory domains were defined as naMCI. The aMCI and naMCI subgroups were not subdivided in single or multiple domain subgroups in order to avoid groups with too few patients to be analysed.

Due to variability among the neuropsychological protocols, the tests used to define MCI subtypes varied between centres. The tests for memory were the learning measure (1 centre, 39 patients and 15 control subjects) and delayed recall (1 centre, 39 patients) measure of the Rey Auditory Verbal Learning test (39), Selective Reminding test (27) (1 centre, 21 patients and 5 control subjects), and Grober-Buschke test (13) (1 centre, 20 patients). The tests for language were 1-minute verbal fluency for animals (1 centre, 21 patients

and 5 control subjects), 2-minute verbal fluency for animals (1 centre, 20 subjects), and 1-minute verbal fluency for fruits, animals or car trades (1 centre, 39 patients and 15 control subjects). Executive function and attention were assessed with the Trail Making Test part A and B (TMT A and B) in all centres (80 patients and 20 control subjects). The tests for visuoconstruction were the copy subtest of the Rey-Osterrieth complex figure (2 centres, 59 patients and 15 control subjects) and the copy of figures from the Mental Deterioration Battery (4) (1 centre, 21 patients and 5 control subjects). Mean neuropsychological data are shown in Table 2.

SPECT acquisition and reconstruction. Subjects underwent ^{99m}Tc-ECD SPECT within 0.1 ± 0.2 (mean ± SD) years of the baseline clinical assessment, according to the guidelines of the European Association of Nuclear Medicine (43). About 1000 MBq of ^{99m}Tc-ECD were injected i.v. through a catheter while the subject was lying on a reclining chair, in a silent and dimly-lit room, eyes closed and ears unplugged, being unaware of injection. After 30 to 90 min after injection, image acquisition started for a time ranging from 20 to 35 min. Radius of rotation was <15 cm. A 2-head camera, equipped with low-energy, high-resolution, parallel beam collimators, was employed in Brescia (Helix, Elscint) and in Genoa (Millenium VG, General Electric), and a 3-head camera, equipped with low-energy, ultra high-resolution, fan beam collimators, in Montpellier (Prism 3 000, Picker). A 1-mm thick glass capillary was acquired in each centre and transmitted together with the patients' scans to the elaboration centre in Genoa, where data reconstruction was performed. On the basis of the analysis on the capillary data, the conclusion was drawn that the camera plus collimator performance was nicely comparable among the three centers in terms of spatial resolution. The number of acquired angular views was 120 in Brescia and Genoa and 90 in Montpellier. In principle, the 3-head gamma camera could generate data of better quality in comparison with the other 2 cameras, but the choice of acquiring only 90 projections in Montpellier generated images of quite comparable quality. The projections were reconstructed with the Ordered Subsets Expectation Maximization algorithm (8 iterations, 10 subsets) followed by 3D Gaussian postfiltering (FWHM = 9 mm). The projector-backprojector pair embedded in the iterative algorithm compensated for the camera spatial resolution and for attenuation **(44, 47). Attenuation was modelled to be uniform (linear attenuation coefficient = 0.10 cm^{-1}) inside the skull volume, which was delimited in an automatic fashion of the contour of skin uptake. In this way, the**

peculiar anatomical features of each patient were accounted for. No scatter subtraction was performed. After inspecting some sample images, the same value for the linear attenuation coefficient was judged to be applicable to the reconstruction of data from the three centres. Independently of the original pixel size on the detector, all images were reconstructed with an isotropic voxel side of 2.35 mm.

The issue of different equipments in multicentric SPECT/PET studies has been analysed in ad-hoc investigations. In a methodological study, dealing with three different cameras, it was shown that the major source of variablity was the physiological/pathological condition, accounting for about 17% of between-subject variability. The between-camera variability was slightly higher (about 8%) than the within-camera (about 5%) variability, when some compensation was applied during the reconstruction step, even by using different reconstruction protocols (either filtered back-projection or iterative) (22). Moreover, between-camera inhomogeneities seemed ininfluent when using a ROI-based approach in a FDG-PET study (15).

Volumetric Regions of Interest (VROI). SPECT images were normalized in the Montreal Neurological Institute space using Statistical Parametric Mapping (SPM2). A study-specific ^{99m}Tc-ECD SPECT template, generated using both SPECT and MRI scans of a MCI group (5), was used. This allows for a better normalisation procedure, since both the deep and cortical hypoperfusion effects are taken into account. The following VROI were chosen from the Pick atlas by a sub-routine implemented on SPM2 (26). Hippocampus and para-hippocampal gyrus, posterior cingulate, precuneus, medium frontal gyrus, inferior parietal lobule and superior temporal gyrus were selected in each emisphere (Fig. 1 a-f). The choice of these regions was based on previous SPECT and PET studies in patients with MCI (1, 3, 6, 8, 9, 12, 16-18, 20, 21, 30). The whole cerebellum was chosen for normalization of VROI counts. **Voxel-based analysis was not performed because we judged more prudent to utilize VROI when managing data coming from three different cameras. Despite the efforts done in SPECT reconstruction phase, some residual inhomogeneity at voxel level might have been more likely persisted applying voxel-based instead than VROI analysis. In fact, the latter approach more likely minimizes such inhomogeneities by operating an average across all voxels within the VROI. Furthermore, an *a-priori* hypothesis was set on likely affected areas, based on the current knowledge, thus specifying the statistical test and reducing the problem of multiple comparisons.**

Statistics. General data, clinical and neuropsychological outcomes (mean values and standard deviations) were summarized by descriptive statistics; nominal significances of between-group differences were evaluated by multiple application of ANOVA or by the chi-square test, when appropriate.

Statistical analysis of perfusion SPECT data was performed using the statistical package of SAS software (SAS/STAT, v.8.1, 1999 SAS Institute Inc., Cary, NC, USA) throughout the following steps.

1. Stepwise regression analysis dealt with the identification of those variables influencing SPECT. A number of heterogeneous factors related to anagraphic data, physiological conditions, risk factors and severity of cognitive impairment, were included in a single analysis in order to separate the various sources of perfusion variability accounting for possible between-factor correlations and sampling inhomogeneity. The dependent variable was the mean values of the 12 VROIs in each subject, while the regressors were: recording centre, age, sex, years of education, presence of hypertension, ischemic heart disease, hypercholesterolemia, thyroid disease, depression, current smoking, MMSE score and Z scores of neuropsychological tests, which were considered as quantitative indexes of the pathological process. According to stepwise selection method, the regressors were included in the model one at a time by selecting the one whose contribution to F-statistics of the model was maximum, as long as this contribution was significant. At each step, the contribution of each variable was checked again and non-significant regressors were removed.
2. Repeated measures ANOVA tested perfusion SPECT differences among the four groups and among VROIs. The dependent variable was each of the 12 VROI value, while the group (among-subjects factor), the VROI (within-subjects factor) and their interaction were considered as sources of variation. Post-hoc group-mean comparison was performed by Ducan's multiple range test.
3. Discriminant analysis was performed in order to evaluate the contribution of SPECT values to the diagnostic process. VROI values and age were checked as predictive variables for group membership.
4. WMH score on MRI scans were examined apart, as a preliminary exploration, due to the incomplete data set. Stepwise regression analysis was repeated adding the WMH score to the other regressors.

Non-parametric analysis of variance (Kruskal-Wallis test) was performed considering the WMH score as the dependent variable and the group as the factor.

RESULTS

Table 1 shows the baseline characteristics for the whole of patients and controls and for the three patient groups. There was no significant difference for age and years of education among groups. A prevalence of females was found in naMCI group. The MMSE score was lower in aMCI group whereas the CDR-SB was lower in CTR. Patients with naMCI more frequently had hypertension and depression, but the differences with the other groups was not significant. There were no differences in the prevalence of the other vascular risk factors across the three groups. Finally, WMH was lower in CTR than in patients, especially those in the naMCI group.

Stepwise regression analysis showed a strong dependence ($p<0.0001$) of mean perfusion SPECT values on the verbal learning test score; moreover, both the recording centre ($p=0.03$) and age ($p=0.02$) more weakly influenced perfusion SPECT. Therefore, data were corrected for the influence of age and centre. Repeated measures analysis of variance showed significant differences among groups ($p=0.012$) and regions ($p< 0.0001$) and a significant group-region interaction ($p=0.029$). The regional distribution of SPECT values is shown in Fig. 2.

Post-hoc comparison of group means showed no significant difference between CTR and SUBJ. In the comparison between aMCI and CTR, the group effect was significant for the left hippocampus and temporal VROI ($p=0.01$) and the right temporal VROI ($p=0.003$). In the comparison between aMCI and SUBJ, the group effect was significant in the left ($p<0.05$) and right ($p=0.02$) parietal VROI and again in the left hippocampus ($p=0.01$).

In the comparison between naMCI and CTR, significant reduced uptake was found in the bilateral temporal VROI, while in comparison between naMCI and SUBJ, naMCI group disclosed reduced uptake in the right frontal VROI ($p=0.05$). Finally, aMCI group had lower uptake values than naMCI group in the precuneus ($p=0.03$) and parietal ($p=0.02$) VROI of the left hemisphere.

Given the similar position of CTR and SUBJ resulting from ANOVA, discriminant analysis was performed in all MCI patients versus the whole of SUBJ and CTR subjects, and lead to correct identification of subjects in the 73% of cases.

Mean VROI SPECT values were highly correlated (partial correlation r= 0.45; p<0.0001) with the score on verbal learning test (Fig. 3) and, more weakly, with categorical verbal fluency (partial correlation r= 0.18; p=0.05). There was no significant correlation between perfusion values and either the other neuropsychological test scores or the risk factors considered. Post-hoc ANOVA for repeated measures was then applied to the relationship between the 12 VROI and verbal learning score, corrected for age and centre. Eight of the 12 VROIs were significantly correlated with verbal memory score; highest correlation indexes were reached in the right cingulate, left hippocampus, bilateral parietal VROI and bilateral precunei.

Stepwise regression analysis in 70 subjects showed a significant inverse correlation between WMH score and meanVROI values (partial correlation r = -0.23; p<0.05).

DISCUSSION

Brain perfusion is already impaired in the group of patients with aMCI in some of the cortical areas typically affected in early AD. This finding confirms previous SPECT studies (3, 12, 14, 16-18, 20, 21), parallels the glucose hypometabolism found by PET (1, 6, 8, 9, 30) and point to aMCI as a group containing a substantial part of patients already affected by AD, but not demented yet.

The finding of hippocampal hypoperfusion raises the question on why hippocampal dysfunction has not been reported as an early marker of aMCI by a part of SPECT/PET literature (3, 6, 12, 14, 17, 18, 21), despite it is largely expected on the basis of current knowledge on the pathophysiology of AD. This issue has been discussed by a recent review (31), pointing to the problems of low spatial resolution of first-generation SPECT/PET equipments and to the smoothing procedure needed by the softwares performing voxel-based analysis (VBA). As a matter of fact, hippocampal dysfunction has been reported also by a VBA approach by more recent reports, employing both SPECT (16, 20) and PET (1, 8) last-generation equipments. aMCI group also showed significant hypoperfusion in left parietal and precuneus in comparison to naMCI group,

thus further qualifying these areas as typical of MCI in a pre-AD stage. In fact, it has recently been shown that the 90% of MCI patients with memory complaints developing dementia receives a diagnosis of AD (45). On the other hand, perfusion reduction in the posterior cingulate did not reach the statistical significance. Posterior cingulate hypoperfusion has been frequently reported in series of MCI patients (18, 20, 21), but often hypoperfusion has been rather reported in the posterior parietal cortex and the precuneus (3, 12, 16, 17). **To note that among SPECT studies, only those performed by means of ^{99m}Tc-HMPAO (18, 21) or ^{123} I-Iodoamphetamine (20) disclosed posterior cingulate hypoperfusion which on the contrary has not been reported by ^{99m}Tc-ECD studies (3,12). This may be correlated with the different distribution of these tracers within the brain in healthy condition and in AD as well (23). Moreover, other phenomena such as clinical differences among groups studied in various centres may play a role in finding hypoperfusion in precunei rather than in posterior cingulate, considering that these are actually contiguous structures.** Recent evidence suggests that hypoperfusion in the precuneus, beyond temporal-parietal cortex, may be the functional pattern occurring in very early AD, distinguishing prodromal AD with accuracy greater than 80% (3, 10, 19).

In naMCI group, perfusion values often fell between aMCI and Subj/CTR groups and reached the statistical significance at bilateral temporal cortex in comparison to CTR. Moreover, perfusion levels were the lowest of all the other groups at right frontal cortex, reaching the statistical significance in comparison to SUBJ. In the present study, naMCI patients had a high prevalence of female gender, and tended to have more hypertension, depression and WMH. These data may suggest that either a vascular aetiology or depression may underlie cognitive impairment in this group. In fact, WMH have been reported to be associated with executive dysfunction in MCI (37), which are driven especially by the role of dorsolateral prefrontal cortex. Moreover, frontal hypoperfusion has been reported in depression, independently of the presence of WMH (33). Hypertension is the most frequent underlying mechanism for chronic hypoperfusion and WMH, and it is characterized by reduced cerebral blood flow, especially in frontal and temporal areas (41). While the prevalence of females seemed due to chance, the present data points to a SPECT picture of fronto-temporal impairment rather than parietal-precuneus impairment as it happens in aMCI, although bilateral temporal hypoperfusion was common to both aMCI and naMCI.

The outcome of patients in the Subj group still remains unclear (38) and, to date, no SPECT/PET study has investigated such a group of patients. It has been suggested that some of these patients may actually have brain impairment and that this group has a slightly increased risk to develop dementia (28). However, no significant group effect was found for SPECT values in this series. As a matter of fact, analysis of individual values showed hypoperfusion in at least two VROI in 4 patients (especially in the temporal lobes), who also exhibited a high WMH score (individual data not shown). Thus, it is likely that the patients with an underlying brain dysfunction are too sparse to produce a detectable group effect; this is in keeping with recent prospective investigations (29). On the other hand, we confirm an increase of WMH in comparison to controls, a finding already reported by other authors (28).

Discriminant analysis yielded a 73% correct identification of all MCI patients versus subjects in the CTR and Subj groups. This figure is very close to some PET studies comparing aMCI to healthy controls by a VROI approach (6, 30), and makes of SPECT a reasonably sensitive tool to investigate MCI patients on a pathophysiological basis, a suggestion agreed also by other authors (3, 19).

Among the neuropsychological test scores, the mean perfusion values of the 12 VROI was highly dependent on verbal learning, mostly in the bilateral precunei, parietal cortex and left hippocampal structures. This finding further underscores the specificity of verbal learning in a clinical context of patients with MCI. It should be remarked that some of the aMCI patients had a Z score higher than –1.5 on the learning measure, but a score lower than –1.5 on delayed recall.

Verbal learning is a complex task, with both a linguistic and a memory aspect. The wide areas of correlation, more significant in the left hemisphere, likely reflect mainly the linguistic component. The extension of correlation to the bilateral precuneus and posterior cingulate cortex points to the involvement of the wider memory network component of the word learning test, as recently shown in a sample of mild AD patients (32). The right-sided correlation in posterior associative areas between brain perfusion and a word learning task has been recently underlined in a small group of patients with very mild AD by a VROI approach (11).

Mean VROI values showed a weaker but significant correlation with the severity of WMH lesions in a subset of patients. Widespread damage to the white matter may affect thalamo-cortical projections and thus reduce cortical function, with a ‘deafferentation’ phenomenon. In fact, glucose consumption and blood flow

are maximum at synaptic levels within the cortical layers. This mechanism could account for a lower cortical perfusion in patients with more severe white matter damage, independently of the underlying aetiology. The pathogenesis of cortical hypoperfusion in patients with WMH is still debated, because neuropsychological performance in patients in cerebral microangiopathy was found to be correlated with cortical perfusion and atrophy rather than with white matter lesions (42). Notwithstanding, cortical perfusion has been proposed as a surrogate marker in vascular cognitive impairment (24).

The cross-sectional design of our study limits interpretation about causal mechanisms underlying cerebral hypoperfusion and MCI subtypes. The DESCRIPA study includes a 2-year follow-up and thus it will be feasible to assess the predictive value of basal SPECT toward the onset of dementia. Another limitation may be the fact that both the neuropsychological test batteries and the gamma camera differed across centres, although these differences should have been accounted for by correcting for the recording centre. A debatable issue may be the criteria used to classify the three groups of patients, i.e., on the basis of their neuropsychological test scores. This choice was made after discussion within the Steering Committee of the DESCRIPA study and was aimed to reduce the higher variability that is likely to come from local differences in clinical diagnosis of MCI and MCI subtypes.

In conclusion, these data provide evidence for differences in pathophysiological substrates among clinical subtypes of MCI. SPECT data in naMCI and Subj groups has never been reported to date. Further longitudinal analysis will reveal the clinical outcome of MCI subtypes in relation to SPECT measures, which is important with respect to preventive and possible early therapeutic interventions in MCI.

ACKNOWLEDGMENTS

This research has been partly funded by the European Commission within the 5th Framework, programme 'Quality of Life and management of living resources', key action 6: The aging population, and priority 6.1: Age-related illnesses and health problems.

Study title: 'Development of Screening Guidelines and Clinical Criteria of Predementia Alzheimer's Disease (DESCRIPA)'.

Concerted action QLRT-2001-02455/ Contract number QLK6-CT-2002-02455.

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FIGURE 1. An example of Volumetric Regions of Interest (VROIs) selected according to the Pick atlas, a sub-routine implemented in Statistical Parametric Mapping (SPM), superimposed to transaxial sections of a ^{99m}Tc-ECD SPECT MCI-specific template. Example images of medium frontal gyrus, superior temporal gyrus, inferior parietal lobule, hippocampus and the para-hippocampal gyrus, precuneus and posterior cingulate are shown in the left hemisphere.

FIGURE 2. Graphs of the mean (and SDs) relative perfusion values (normalized on mean cerebellar counts) are shown for the 12 VROIs in the four groups of patients: Controls (CTR; black lines), patients with subjective cognitive complaints (SUBJ; gray lines), amnestic MCI (aMCI; red lines) and non-amnestic MCI (naMCI; blue lines). For each VROI, the vertical lines represent the extension of one SD above and the below the mean value. *: $p<0.05$ and **: $p\leq0.01$ at post-hoc Ducan's multiple range test.

FIGURE 3. Scatterplot and regression line of mean VROI values (normalized on mean cerebellar counts) versus the Z score at the verbal memory test in the whole sample of 100 subjects. Mean VROI value was highly dependent on verbal learning score ($p<0.0001$). Symbols and colors for the 4 groups are reported within the figure.

Table 1. Baseline characteristics in 20 control subjects, and in the total sample of 80 patients according to groups.

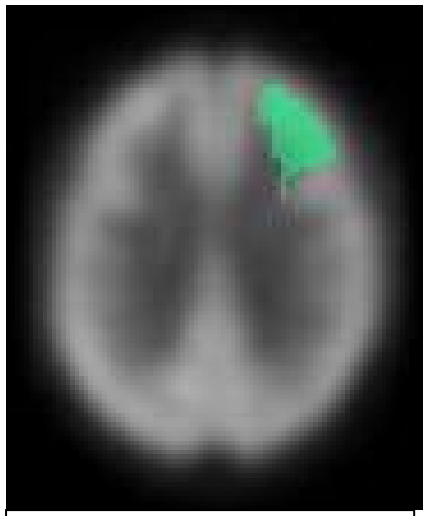
	1. Controls	All patients	2. Subjective cognitive complaints	3. Non-amnestic MCI	4. Amnestic MCI	Statistics	
	(n=20)	(n=80)	(n=23)	(n=17)	(n=40)	Overall p-value	Group comparisons
Demographics							
Age, years (SD)	71.3 (3.5)	69.8 (7.1)	67.2 (6.4)	70.8 (6.0)	70.9 (7.7)	n.s.	
Sex, n. female (%)	12 (60.0)	43 (53.7)	10 (43.5)	15 (88.2)	18 (45.0)	P<0.02	1,2,4 < 3
Education, years (SD)	9.3 (4.5)	9.7 (4.4)	10.3 (4.1)	8.2 (4.8)	10.1 (4.3)	n.s.	
MMSE (SD)	28.5 (1.2)	28.2 (1.5)	28.9 (1.1)	28.5 (1.4)	27.6 (1.5)	P<0.01	4 < 1,2,3
CDR-SB (SD)	0 (0)	1.24 (0.87)	0.71 (0.76)	1.33 (1.37)	1.44 (0.63)	P<0.01	1 < 2,3,4
Risk factors/other factors							
Hypertension, n (%)	7 (35.0)	42 (52.5)	11 (47.8)	12 (70.6)	19 (47.5)	P=0.056	1 <2,3,4
Diabetes Mellitus, n (%)	2 (10.0)	1 (1.25)	0	0	1 (6)	n.s.	
Hypercholesterolemia, n (%)	8 (40.0)	38 (47.5)	12 (52.2)	9 (52.9)	17 (42.5)	n.s.	
Ischemic heart dis., n (%)	3 (15.0)	10 (12.5)	4 (17.4)	3 (17.6)	3 (7.5)	n.s.	
Thyroid disease, n (%)	1 (5.0)	10 (12.5)	2 (8.7)	4 (23.5)	4 (10)	n.s.	
Current smoking, n (%)	1 (5.0)	9 (11.25)	3 (13)	2 (11.8)	4 (10)	n.s.	
Depression, n (%)	4 (20.0)	12 (15)	2 (8.7)	5 (29.4)	5 (12.5)	n.s.	
White matter hyperintensities	**(n=18)**	**(n=52)**	**(n=15)**	**(n=14)**	**(n=23)**		
ARWMC score (SD)	1.8 (2.0)	5.5 (4.6)	5.5 (4.1)	7.2 (5.1)	4.5 (4.6)	P<0.01	1 < 2,3,4

ANOVA with age and center of origin as covariates or chi-square test for categorial variables were performed. Abbreviations: MMSE: Mini-Mental State Examination; CDR-SB: Clinical Dementia Rating scale- Sum of Boxes; ARWMC: Age-Related White Matter Changes scale. The last column reports separation among groups when descriptive statistic indicates a significant difference.

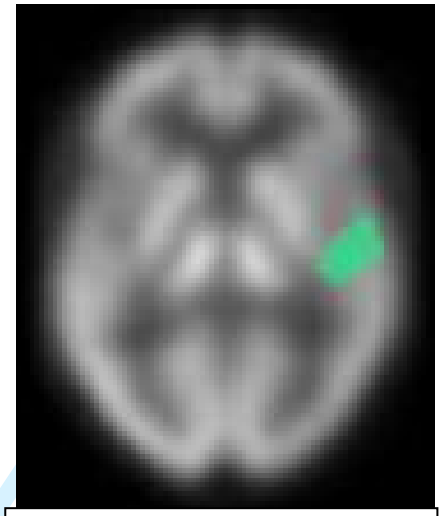
Table 2. Baseline neuropsychological Z scores in the Control group, in the total sample of 80 patients as a whole and according to the 3 groups.

	Controls (n=20)	All patients (n=80)	1. Subjective cognitive complaints (n=23)	2. Non-amnestic MCI (n=17)	3. Amnestic MCI (n=40)
Verbal learning mean (SD)	0.14 (0.89)	-0.90 (1.17)	-0.11 (0.82)	0.11 (0.78)	-1.79 (0.71)#
Delayed recall mean (SD)	n.a.	-1.10 (1.46)	0.0 (0.84)	0.17 (0.80)	-2.26 (0.94) #
TMT-A mean (SD)	0.17 (0.92)	-0.85 (1.58)	-0.02 (0.76)	-2.12 (1.68)#	-0.79 (1.57)*
TMT-B mean (SD)	0.17 (1.09)	-0.79 (1.76)	0.15 (0.80)	-1.78 (1.64)**	-0.94 (1.97)*
Categorial verbal fluency mean (SD)	2.05 (1.56)	-0.82 (1.14)	0.09 (0.90)#	-1.00 (0.80)#	-1.26 (1.09)#
Copying figures mean (SD)	0.17 (1.16)	0.08 (1.19)	0.67 (0.57)	-0.36 (1.25)	-0.07 (1.33)

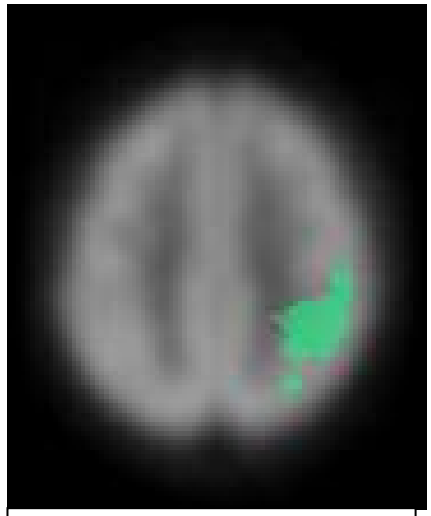
Abbreviations: TMT-A and TMT-B: Trail-making test A and B. n.a.: not available
Analysis of variance of the neuropsychological score differences among groups and post-hoc comparisons between each patient group and controls (or versus SUBJ for delayed recall).
*p<0.05; ** p<0.01; #p<0.001.



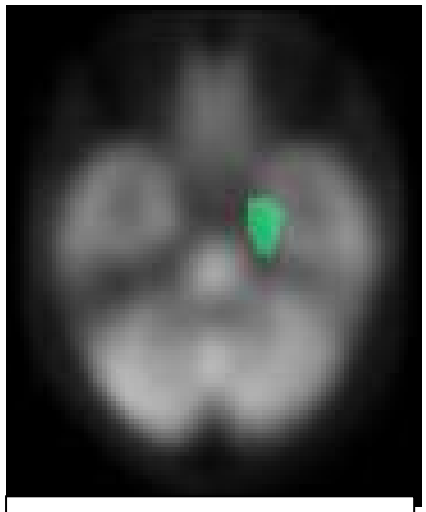
medium frontal gyrus



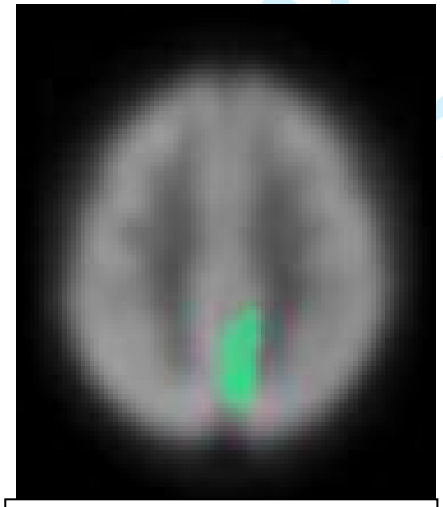
superior temporal gyrus



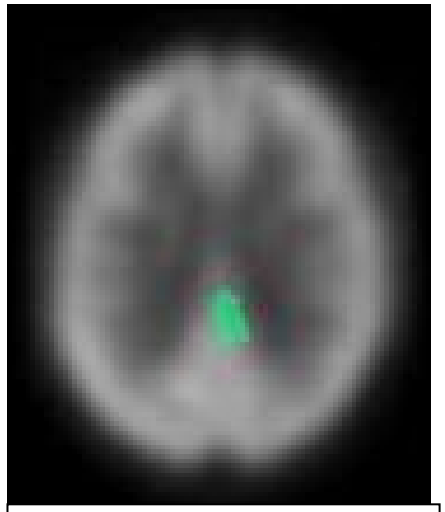
inferior parietal lobule



Hippocampus-parahippocampus



precuneus



Posterior cingulate

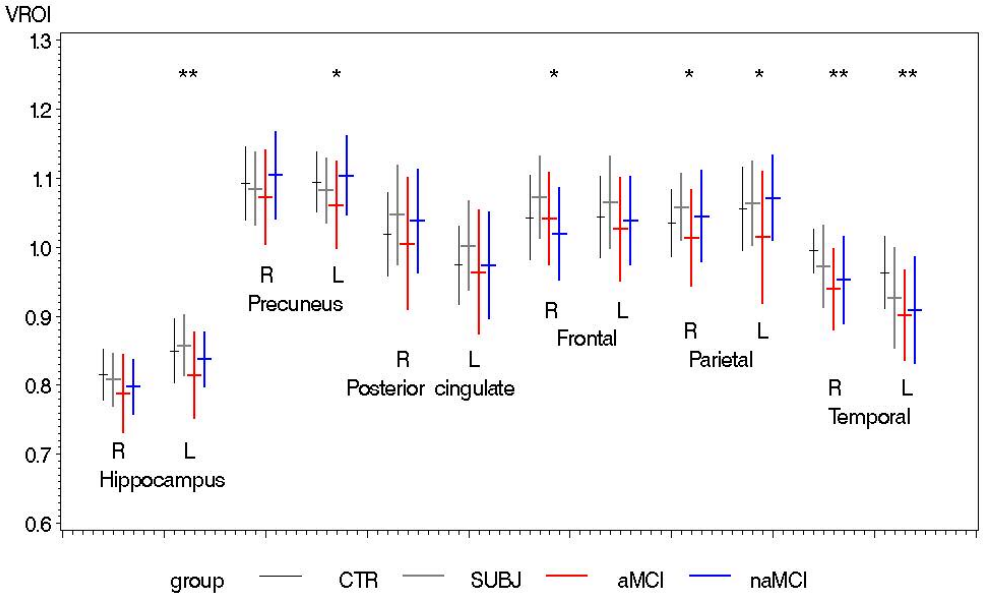


Figure 2
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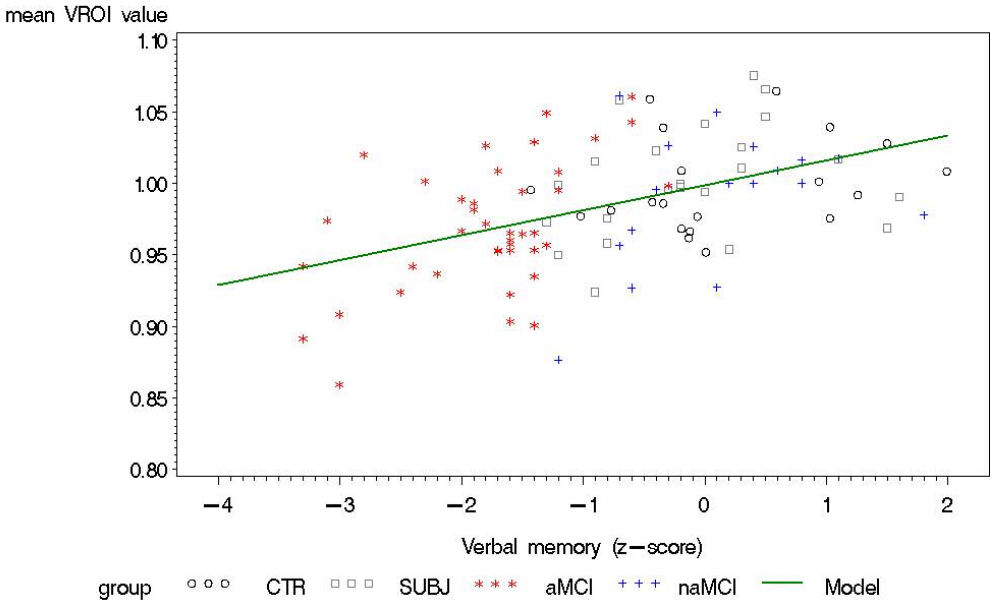

Figure 3
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