Chapter 1. General Introduction

Abstract

Mild cognitive impairment (MCI) and neuromyelitis Optica (NMO) are briefly described in this chapter as well as relevant questions related to brain perfusion in these diseases. A general introduction to brain perfusion SPECT, including a novel image analysis based on graph theory, is also provided. Finally, the aim of the thesis is established.
1. Introduction

Although there has been important progress in recent years, neurological diseases in humans, especially those affecting the central nervous system (CNS), are probably least understood disorders [1]. Thus, in several cases, the patient's diagnosis is only presumptive, which makes treatment and prognosis more difficult, particularly in the early stages of the disease.

The still limited knowledge we have of complex CNS disorders stimulates scientific research in various directions (e.g., brain structure and function, neurochemistry, neuroimmunology and genetic) and at different levels (from molecules to large brain networks) [2, 3] for which various technologies are employed. Among these technologies, nuclear medicine neuroimaging, such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT), play an important role [4]. The ultimate goal is to substantially improve the diagnostic accuracy and medical care of patients, especially in the early stages of the disease where there may be more possibilities to stop or slow down the pathological processes, based on a greater insight into complex CNS diseases.

Two examples that clearly show the complexity of CNS disorders are Alzheimer's disease (AD), particularly in the stage preceding dementia also termed as mild cognitive impairment (MCI) [5-7], and neuromyelitis Optica (NMO), known as Devic's disease as well [8-10]. These two clinical entities, which are the main subjects in this thesis, are briefly described below in sections 2 and 3, respectively.

Among the relevant questions without conclusive answers in MCI and NMO, alterations in brain perfusion occupy a special place. Brain perfusion SPECT, which is a low cost and accessible technology worldwide, can be used for this purpose and its general principles are presented in section 4.
In the case of patients with MCI, for example, it is unclear whether the cerebrovascular reactivity (CVR) is altered [11]. CVR is also known as the cerebrovascular reserve and describes the ability of cerebrovascular structures to increase cerebral blood flow (CBF) above a basal condition in response to a vasodilatory challenge. Although there is increasing evidence that patients with AD dementia have decreased CVR [11], this is less clear in patients during the MCI stage. Some studies show a decrease [12-15] while others do not [16-18]. This question is important in AD research because it could have implications for early diagnosis and treatment [19].

Considering that MCI is the transition from normal cognition to dementia [5-7], CVR abnormalities will be subtle which may partly explain the ambiguous findings, particularly in MCI patients with a low vascular burden. Furthermore, taking into account the complexity of the cerebral microvasculature network, the standard analysis of CVR might not reflect subtle network-related alterations since it relies on the analysis of individual regions (or the whole brain) rather than on the interaction between them.

Recently, graph theoretical analysis of neuroimaging data has shown its potential to reveal subtle pathological processes in MCI [20, 21]. In principle, this methodology can also be applied to brain perfusion SPECT data to investigate possible subtle network-related CVR abnormalities in MCI. In subsection 4.4, graph theoretical analysis is briefly presented and it is described how it works in the case of brain perfusion SPECT.

Regarding the other main topic of this thesis, previous neuroimaging studies have shown that structural brain abnormalities in NMO are more frequent than described earlier [22-32]. However, to better understand these structural abnormalities, more research considering multiple aspects of NMO is necessary. For example, a clinical feature of relapsing NMO (which is the most common form of the disease) is that the incremental disability is attack-related (optic neuritis and/or transverse myelitis attacks) [33, 34].
Therefore, an association between the attack-related process and neuroimaging need to be investigated. On the other hand, the immunopathological analysis of NMO lesions has suggested that CNS microvasculature could be an early disease target [35-37], which might be detectable by brain perfusion imaging. Thus, voxel-based analyses using multiple neuroimaging modalities (e.g., brain perfusion SPECT and structural MRI) could help to identify possible brain perfusion and structural changes behind the attack-related process in relapsing NMO. The study of these changes, if detected, could be relevant for the comprehension of the incremental disability and the development of brain structural abnormalities in this disease. Voxel-based analysis of neuroimaging data is also briefly presented in the subsection 4.3.

2. Mild cognitive impairment (MCI)

MCI is considered intermediary between normal cognitive aging and very early dementia [5-7]. The historical evolution of the MCI concept has recently been reviewed by Petersen [5], including the more recent formulation by the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5). In general, the MCI syndrome is characterized by a cognitive deficit greater than would be expected considering the individual’s age and educational level, but not enough to significantly interfere with functioning in daily life. The prevalence of MCI ranges from 15% to 20% in individuals older than 60 years [5].

MCI is defined as amnestic MCI when memory impairment is the predominant symptom [5, 38], especially episodic memory [7]. About 30% of all MCI individuals present amnestic MCI [39] and they tend to convert to probable AD dementia over time at a rate of approximately 10% to 15% per year [38]. These figures indicate the serious health problem that this clinical entity represents worldwide, and its prevalence is expected to grow in the coming years [40], mainly due to the increase in life expectancy.
Other MCI classifications include amnestic MCI plus impairment in other cognitive 
domains (multiple domains MCI) and non-amnestic MCI in a single cognitive domain 
(single domain non-amnestic MCI) or in multiple domains (multiple domain non-amnestic 
MCI) [5, 41]. Non-amnestic MCI individuals tend to evolve to another type of dementia 
[5]. In other situations, MCI subjects remain stable or even remit over time.

The diagnosis of MCI often needs clinical acuity [5-7]. Besides a complete clinical 
evaluation (including neurological and psychiatric examinations), also blood tests, 
neuropsychological tests and neuroimaging help to support the diagnosis. Intra-individual 
cognitive changes at follow-up are also important to substantiate the MCI diagnosis.

One of the most widely used cognitive tests in clinical and research settings is the Mini-
Mental State Examination (MMSE) because it is brief and easy to apply [42]. Albeit being 
useful to verify longitudinal intra-individual changes of global cognitive function, its use 
for AD research has been criticized mainly because of its low sensitivity to MCI in the 
early stage [43]. Thus, more sophisticated (neuropsychological) tests are also needed to 
better characterize the cognitive function in multiple and specific cognitive domains.

There are also two clinical scales to distinguish between normal aging and early dementia: 
the clinical dementia rating scale (CDR) [44] and the global deterioration scale for aging 
and dementia (GDS) [45]. However, the CDR is more suited to support amnestic MCI as it 
is strongly weighted towards memory evaluation [44].

Although the conclusive diagnosis of AD requires histological verification, the use of 
biomarkers increases the certainty of whether or not an MCI patient will convert to AD 
dementia over time. In 2011, the National Institute of Aging-Alzheimer's Association 
(NIA-AA) recommended two types of biomarkers for this purpose, though they were 
mainly intended for clinical research [7]. The first biomarker type reflects the cerebral 
amyloid burden as the initial event of AD (amyloidosis biomarker) and presumably present
many years before the first clinical symptoms appear [46-48]. Two biomarkers were recommended for this purpose: one based on measurements of soluble β amyloid (Aβ) 42 in the cerebro-spinal fluid (CSF) [46, 47] and the second based on PET amyloid imaging [48]. The second type of biomarker reflects neuronal injury that occurs later in the successive stages of AD, which include the transition stage of MCI. Three biomarkers of neuronal injury were recommended: one based on measuring CSF tau protein (CSF tau) [46, 47], another one on 18F-fluorodeoxyglucose (FDG) PET imaging (FDG-PET) [49], and the last one based on hippocampal atrophy measured with structural MRI [50]. The NIA-AA criteria of MCI due to AD also included brain perfusion SPECT as a neuroimaging biomarker of neuronal injury, similar to FDG-PET [7].

With regard to the disease progression in the MCI stage, neuronal injury biomarkers seem to have a more significant role than amyloid biomarkers [51]. The former are more associated with oncoming cognitive deterioration than the latter, which are already elevated in the initial asymptomatic stage of AD, and are close to a plateau at the MCI phase with few further changes. Other probable modifiers of MCI progression include APOE genotype, cognitive reserve (related to educational level), and co-morbidity of other cerebral diseases or lifestyles [51].

On the other hand, the etiology of AD remains unknown except for 1% to 5% of cases due to specific genetic profiles [52], which basically refer to monogenetic disorders with autosomal dominant transmission involving amyloid precursor protein and presenilin 1 and 2 mutations. Although there are several hypotheses, which attempt to explain AD’s etiology, the amyloid hypothesis prevails [53]. However, criticism of the amyloid hypothesis has been increasing in recent years [54, 55]. In particular, the amyloid model does not include early vascular dysfunction of AD, despite the body of evidence supporting its contribution [56-58]. The topic of the AD pathogenesis is probably one of the most
active research fields in neuroscience research today. The ability to identify more effective biomarkers in AD is directly related to the knowledge of its etiology.

3. Neuromyelitis Optica (NMO)

NMO, or Devic’s disease, is a severely disabling autoimmune inflammatory demyelinating disorder of the CNS [59-61]. The disease is clinically characterized by optic neuritis (ON) and longitudinally extensive transverse myelitis (LETM) attacks. In the past, it was considered a rare severe variant of multiple sclerosis (MS). The finding that most NMO patients have autoantibodies (NMO-IgG) against the water channel aquaporin-4 (AQP4) changed the comprehension of NMO pathogenesis [59-61]. AQP4 is the most abundant water channel in the CNS [62].

Wingerchuk et al. [63] formulated the revised diagnostic criteria for NMO in 2006. These guidelines include as absolute criteria the presence of ON and acute LETM; and at least two of the following three supportive criteria: (1) brain MRI negative or non-diagnostic for MS at onset, (2) spinal cord MRI with contiguous T2-weighted signal abnormality extending over three or more vertebral segments, and (3) a serological test positive for NMO-IgG. Using these criteria, the authors reported a sensitivity of 99% and a specificity of 90% for NMO [63].

More recently, the International Panel for NMO Diagnosis updated the diagnostic criteria for NMO [64]. The new criteria extend the concept of the disease to a broader disease spectrum and recommend the use of term ‘NMO spectrum disorders’. However, the main clinical criteria remain the same.

NMO is more frequent in women [65, 66] and the age of onset ranges from 35–45 years [66]. Although familial cases have been reported [62, 65], the disease is mainly sporadic.
The global prevalence of NMO is approximately 1 to 3 per 100,000 [65] and it is higher in non-Caucasian countries [62, 66].

In most of the cases, NMO follows a relapsing course with attacks of ON, LETM or both, that can be separated by weeks to years [33, 34]. Approximately only 10% of NMO patients follow a monophasic course [34, 67]. The typical symptoms of NMO include impaired vision, paraplegia or tetraplegia as well as bladder and bowel dysfunctions. About 60% of NMO patients show blindness in at least one eye after a disease duration of 7–8 years or severely impaired ambulation as measured by the expanded disability status scale (EDSS) [68]. Unlike MS, a secondary progression course is uncommon in relapsing NMO [33]. A distinctive clinical feature of relapsing NMO is that the incremental disability is attack-related [33, 34]. In general, NMO has a worse prognosis and response to treatment compared to MS [34, 60]. Most of the deaths occur due to respiratory failure as a result of cervical myelitis or brainstem involvement [60].

On the other hand, NMO has traditionally been regarded as a disease without brain involvement. However, recent neuroimaging studies have shown that the brain abnormalities are more frequent than earlier described [22-32]. The typical lesions by standard MRI are observed close to periaqueductal areas (peri-ependymal regions surrounding the third ventricle, cerebral aqueduct, and fourth ventricle), following the distribution of expression of AQP4 [69]. Nevertheless, more research considering multiple aspects of NMO is necessary for a better understanding of the pathogenic mechanisms that cause brain abnormalities in this disease.

Although the etiology of NMO is still not fully understood, recent studies have shown the pathogenic role of AQP4-IgG (NMO-IgG) [59-61]. The immunopathological analysis of NMO lesions has revealed a unique vasculocentric pattern of complement activation [35-37], which correlates with the regions of AQP4 expression in the peri-microvessel
astrocyte foot processes. Pathological changes occur primarily in the optic nerve and spinal cord, but do not exclude the brain [69]. The pathogenesis could be caused by complement-mediated astrocyte damage, cascading to leukocyte infiltration, oligodendrocyte death, and damage to neuronal cells [59-61].

4. Brain perfusion SPECT

Brain perfusion SPECT is a non-invasive nuclear medicine imaging technique that allows regional CBF evaluation. The technique includes three main components: the CBF radiotracers (radiopharmaceuticals), instrumentation plus image reconstruction, and image analysis.

4.1. CBF radiotracers

CBF radiotracers using Technetium-99m ($^{99m}$Tc) and Iodine-123 ($^{123}$I) labeled compounds are the standard in clinical SPECT. Given the relatively high cost of $^{123}$I as a cyclotron-produced radioisotope, the use of radiotracers using $^{123}$I is limited as compared to $^{99m}$Tc labeled compounds. $^{99m}$Tc-ethyl cysteine dimer ($^{99m}$Tc-ECD) and $^{99m}$Tc-hexamethyl propylene amine oxime ($^{99m}$Tc-HMPAO) are the two $^{99m}$Tc labeled compounds used as CBF radiotracers. The commercialization of ECD and HMPAO kits facilitate the labeling with $^{99m}$Tc, which is a low-cost radioisotope and available daily by extracting it from relatively long-lived $^{99}$Mo/$^{99m}$Tc generators delivered to hospitals on a weekly basis.

Both $^{99m}$Tc-ECD and $^{99m}$Tc-HMPAO are highly liposoluble agents, so that after administration to the patient intravenously, they cross the blood-brain barrier and remain retained in the perivascular space (at the capillary level) for several hours, enough for images acquisition [70, 71]. The regional distribution of both agents is nearly linear proportionally to regional CBF. To have a linear relationship, it is necessary to apply to the images the correction proposed by Lassen et al. [72]. The Lassen's correction is
particularly useful to avoid CBF underestimation in more perfused brain regions when vasodilatory challenges are used. Nevertheless, in other clinical situations the correction may not be necessary.

Since the regional CBF is in most circumstances tightly coupled to neuronal metabolism, it is assumed that the radiotracer distribution reflects levels of neuronal activity in different brain areas [70, 71]. Although both agents perform equally well in the classification of patients with AD, $^{99m}$Tc-ECD shows the highest image contrast [73].

Brain perfusion SPECT may also be used in combination with acetazolamide (ACZ). In healthy vascular conditions, ACZ increases regional CBF by increasing local pCO2 and, in turn, arteriolar dilatation [74], thus being a suitable vasodilatory challenge to evaluate CVR [11]. Therefore, it is particularly useful in patients with suspected cerebrovascular disease.

As another important point, it should be noted that brain perfusion SPECT is a safe technique since the doses used in adults, for example, are between 555 - 1110 MBq for $^{99m}$Tc-ECD and $^{99m}$Tc-HMPAO, which represent effective doses of 0.011 and 0.0093 mSv, respectively [75].

4.2. Instrumentation and image reconstruction

The SPECT technique is based on the gamma camera, which is a medical instrument that records a planar image of the three-dimensional distribution of a radiopharmaceutical administered to a patient by detecting the emitted gamma radiation [76]. The gamma camera consists of the detector, with one or multiple heads, and the acquisition and processing system, including algorithms for three-dimensional tomographic reconstruction from planar images (see below). In brain SPECT, multi-head detectors (two or more) or dedicated gamma cameras for brain imaging should be used as they offer superior results compared to single-head cameras. An important element of the detector head is the
A special type of converging collimator called fan-beam is preferred in brain perfusion SPECT since spatial resolution and the signal-to-noise ratio is better when compared with the most commonly used parallel holes collimator [76].

The tomographic image reconstruction calculates a representation of the three-dimensional distribution of the radiotracer using the set of planar images (projections) obtained by the gamma camera [77, 78]. In most systems, the projections from different angles are obtained by sequential rotation of the gamma camera. The planar data are then used for three-dimensional reconstruction using mathematical methods. The filtered back-projection (FBP) has been the most used reconstruction method in nuclear medicine [77]. More complex approaches, such as iterative reconstruction methods are gradually replacing FBP in nuclear medicine [77-80]. Iterative methods are better since they can include a model of the emission and detection of gamma radiation, thus producing better solutions for several physical problems affecting SPECT. The advantage of iterative methods is mainly when SPECT is used to quantify the concentration of radioactivity within a given volume of tissue in absolute units.

Physical problems affecting SPECT include the variation of spatial resolution with the distance between the radioactive source and the collimator, photon attenuation, photon scattering and partial volume effect (PVE) [81, 82]. Photon attenuation and PVE are perhaps the most important physical problems, especially in the differentiation of healthy control subjects from patients with probable early AD dementia. For instance, photon attenuation causes a substantial decrease of intensity in the reconstructed image towards the middle regions of the brain, including posterior cingulate and precuneus, which are frequently affected in MCI patients due to AD [7]. The correction of photon attenuation clarifies whether or not the intensity reduction is due to a pathological process (Figure 1). Among the approaches to correct for attenuation, the method proposed by Chang et al. has
been widely used [83]. Although this method simplifies the attenuation problem, which is much more complex [84], it usually offers a satisfactory solution in the case of brain perfusion SPECT.

![Uncorrected and corrected brain perfusion SPECT images](image)

**Figure 1.** Uncorrected and corrected brain perfusion SPECT images for photon attenuation for a 69-year-old cognitively normal male from our dataset. The figure shows a sagittal slice close to the midline of the brain. Note the apparent intensity reduction in the uncorrected image as compared to the corrected one, including posterior cingulate and precuneus.

On the other hand, PVE is directly related to the spatial resolution of SPECT [81, 82]. This problem becomes more noticeable for a region with dimensions close to the spatial resolution limit and causes the region to appear apparently less intense than it actually is. In recent years, several methods have been developed to correct PVE on PET images [85-87], some of which have been easily extended to SPECT [87]. These methods are based on co-registering the SPECT images with structural MRI images of higher spatial resolution. In structural MRI, PVE can be considered to be negligible, comparatively. Therefore, the spatial information provided by MRI can be used to correct this problem [87]. This correction is particularly useful in patients with cerebral atrophy, as is the case in MCI patients due to AD. Since brain perfusion SPECT images simultaneously reflect brain
volume losses (atrophy) and perfusion alterations, the PVE correction allows having more genuine perfusion measurement by removing the effect of perfusion underestimation due to brain atrophy (Figure 2). PVE correction also improves the sensitivity of voxel-based analysis [88].

![Figure 2. Structural MRI (T1-weighted), uncorrected and corrected brain perfusion SPECT images for partial volume effect. The images are from a 76-year-old male with amnestic MCI from our dataset. Note the typical AD-like pattern of bilateral hypoperfusion in the posterior parietal cortex (uncorrected image), although with a slight asymmetry toward the left side, which also corresponds to cortical atrophy in MRI images. The corrected image shows a similar hypoperfusion pattern but less pronounced.](image-url)

Lastly, while the quality control of the instrumentation and the use of rigorously validated standards for image acquisition and pre/post processing of both PET and SPECT is essential, it is even more crucial for SPECT as it is more subject to noise (less counts are acquired). Therefore, special attention should be paid to these technical and methodological aspects when brain perfusion SPECT is performed.
4.3. Image analysis

The analysis of clinical brain perfusion SPECT images is primarily visual. The visual analysis is based on a qualitative evaluation of the images, and even more importantly, it includes the reader's medical knowledge and the particular diagnostic question in each patient. The visual analysis also helps to prevent misinterpretation when there are technical artifacts present in the image. However, the visual analysis is susceptible to intra and inter-reader variability, especially in cases where CBF alterations may be subtle. This is one of the reasons why quantitative methods of image analysis are needed. Furthermore, quantitative methods are particularly useful in research, and make it easier, for example, to study associations with clinical variables of interest in a particular disease.

One of the quantitative approaches used for many years in brain perfusion SPECT has been the classical method based on regions of interest (ROI). The method has evolved from manual ROI tracing to automated procedures where the operator is less involved. Although the ROI method is useful in many situations, its main limitation is that the analysis is based on the average across all voxels in each ROI. Thus, a relevant effect may be overlooked if it is present in only part of a particular ROI or comprises small parts of several ROIs.

Among more recent quantitative methods of image analysis, Statistical Parametric Mapping (SPM) has gained interest [89-91]. Unlike the classical ROI method, SPM allows voxel by voxel analysis of the entire brain (i.e. voxel-based). SPM analysis is based on the general linear model and the random field theory [89-91]. The basic objective of the method is the construction of statistical maps that test scientific hypotheses using neuroimaging, including brain perfusion SPECT. The classic example is to compare 2 groups of images, the first belonging to a control group of healthy individuals and the second to a group of patients with a common disease. SPM also allows the identification of regions of the brain that correlate with a particular clinical variable within a group of
individuals. The method thus offers great flexibility to investigate a wide variety of problems. In the case of small samples of subjects, there is a version of this methodology based on a permutation test that is termed as Statistical Non-Parametric Mapping (SnPM) [92]. The detailed mathematical descriptions of these approaches can be found elsewhere [89-91, 92].

However, an important limitation of standard SPM analysis (or similar methods) is that it is not possible to investigate the relationship between brain regions (i.e. connectivity), thus ignoring important features of the complex network that is the brain. In recent years methods have been developed to study brain connectivity using neuroimaging [93, 94], one of which is based on graph theory (described below) that can be applied to brain perfusion SPECT data [95].

The quantitative methods explained so far, quantify the CBF (in one way or another) but in relative units, which implies that the regional CBF values are relative to the number of counts corresponding to a reference value. The reference value may be the mean value of a specific brain region (e.g., the cerebellum) or the whole brain. The reference value should not be affected by the disease under study, so that the regional relative CBF values are due to the effect of the disease in the patient and not to the reference used.

The reference region based CBF quantification (i.e. in relative units) is simple and works well in most clinical situations. Nevertheless, in cases where the reference value may be affected by the disease under study or by some intervention on the patient, it is not a good option. This is the case, for example, when studying the CVR using vasodilatory stimuli. A priori it is not possible to determine how the effect of the vasodilatory stimulus will be (e.g., in patients with a cerebrovascular disease it may be opposite to that observed in healthy controls). In these cases, the best option is to use CBF quantification in absolute units (mL/min/100 g).
Several methodologies have been developed to quantify CBF in absolute units using brain perfusion SPECT and $^{99m}$Tc labeled compounds ($^{99m}$Tc-ECD or $^{99m}$Tc-HMPAO) [96]. One of these methodologies is based on non-invasive radionuclide angiography of the CBF tracer and is relatively easy to implement in clinical practice as arterial blood sampling is not required. The method calculates a brain perfusion index (BPI) using spectral analysis of the time-activity curves for the aortic arch and brain hemispheres within the first 20-30 seconds after the bolus administration of the CBF tracer [97-99]. The BPI is then converted to global CBF (mL/min/100 g) using a linear regression equation between BPI and global CBF measured using $^{123}$I-N-isopropyl-p-iodoamphetamine ($^{123}$I-IMP) and dynamic SPECT with arterial blood sampling. $^{99m}$Tc-ECD (or $^{99m}$Tc-HMPAO) uptake images (in counts), obtained with the subsequent SPECT acquisition, are finally converted to regional CBF images using global CBF and mean SPECT counts (the Lassen's linearization algorithm is also used in the process). The procedure is reproducible [97] and shows a high correlation with $^{15}$O-PET [99], which is considered the gold standard for absolute CBF measurement.

4.4. Introduction to graph theoretical analysis. The case of brain perfusion SPECT

In the field of quantitative neuroimaging, graph theoretical analysis is one of the methods to study brain connectivity [93-95, 100, 101]. A key concept of this method is the notion of topology. This concept can be illustrated with a simple idea which is used when we travel in the subway of any large city. In figure 3 two maps of the London subway appear, the first map shows a precise spatial description of the railways (or lines) through which trains travel (i.e., the subway topography), whereas the second one is only concerned with the relative locations of subway stations and connecting lines (i.e., the subway topology). These two maps do not coincide with regards to the relative position of the stations, neither in the distances nor in the location of the lines. However, the topological map simplifies
the problem for the traveler. For example, two stations may be physically (topographically) distant but with a direct connection (topologically near) and vice versa. This information is relevant to the traveler and it is easy to extract from the topological map. Note also that the topological map is a graph (a representation of a network as well). A graph is composed of two main topological elements, the nodes (the stations in the subway example) and the connectors (connecting lines).

![Network – Graph](image)

**Figure 3.** The topographical (left) and the topological maps (right) of the London subway.

Now let's look at the problem of comparing the subways of two different cities. By simply inspecting the topological maps, it is not easy to know if one subway is better organized than the other (e.g., subway efficiency). One way to simplify this problem is to use metrics that quantify or analyze the subway network using graph theory [94, 101]. Hence the name of graph theoretical analysis.
A first aspect to measure could be how easy it is to travel between any two stations (e.g. the number of stations on average, between the start and end of the trip). This aspect is relevant, especially if the traveler wants to visit different parts of the city on the same day. This example illustrates the concept of global efficiency of a graph [94, 101]. The metric of global efficiency is a way to quantify the global connectivity (integration) of the network. In this example, it is assumed that the number of stations (nodes) and lines (connectors) are the same in the two subways that are compared.

Another feature of measuring could be how well a given station is connected to its topographical neighbor stations. Neighbor topographical stations are those that are relatively close physically, although not necessarily close in the topological sense, i.e. there is no direct connection between them (the connection is through intermediate stations); while stations may be relatively far away physically and yet be topological neighbors (with a direct connection). Then, for example, if the given station has a direct connection with three other topographical neighbor stations (i.e., they are also topological neighbors), which in turn have a direct connection to each other (topological neighbors as well), in that case, if the given station is out of service, it is relatively easy to reroute (reconnect) the passengers to the subway network through the topographical neighbor stations (e.g. by using other transportation means). In contrast, it would be more difficult in the case that the topographical neighbor stations are not also topological neighbors. In the first case, the local efficiency of the station is high, while in the second case is low. Thus, the metric of local efficiency is a way to quantify the local connectivity (segregation) of the network. Local connectivity can be also quantified using a similar metric termed as the clustering coefficient [94, 101].

A third aspect of measuring is how well connected a particular station (node) is with the rest of the subway network (i.e., station centrality). Nodes with high centrality are called
hubs and they are very important in network functioning. There are several metrics to quantify nodal centrality [94, 101], a simple metric is the degree of the node (the number of lines connected to a particular station in the subway example), which is equivalent to measuring the strength of association of the node with the rest of the network.

A fourth interesting feature to measure is to what extent the whole subway network can be divided into modules (groups of stations) with maximum within-module and minimum between-module connectivity (Figure 3). This metric is important since in the event that a group (module) of stations (with maximum within-module connectivity) were out of service (e.g., failure in power supply), the remaining subway would continue operating due to the relative independence of the other modules. This example shows the concept of network modularity [101], describing an important aspect of the network structure.

On the other hand, if the networks (graphs) were ranked, at one extreme would be a regular network, where each node is directly connected to its neighbor topographical nodes (i.e. they are also topological neighbors), but without direct connections to distant topographical nodes (i.e., a network with a high local but a low global efficiency) (Table 1). At the other extreme would be a random network, where direct connections between any two nodes are random. Thus, in a random network, the local efficiency is low and the global efficiency is high as compared to a regular network. In the middle of these two extremes would be what is known as a complex network, in which there is a balance between global and local efficiencies so that both are relatively high [94, 101] (Table 1). This kind of networks is said to have a 'small-world' topology. The concept of 'small world' comes from the social sciences, and reflects the fact that two persons (nodes) who do not know each other are nevertheless connected by a relatively short chain of persons known to each other. A complex network is also characterized by the presence of hubs and high modularity [94, 101].
Table 1. Basic topological features of three type of networks according to the degree of disorder.

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<th>Regular network</th>
<th>Complex network</th>
<th>Random network</th>
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<tr>
<td><strong>Global efficiency</strong></td>
<td>Low</td>
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<tr>
<td><strong>Local efficiency</strong></td>
<td>High</td>
<td>High</td>
<td>Low</td>
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<tr>
<td><strong>Hubs presence</strong></td>
<td>No</td>
<td>Yes</td>
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<td><strong>Modularity</strong></td>
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All of these relatively simple concepts can be extrapolated to neuroimaging. Actually, brain networks have a ‘small-world’ topology [94, 101]. Furthermore, this kind of topology is present from micro (networks of neurons) to macro scale level (networks of brain units).

Using the concepts explained so far, it is now easier to understand how graph theoretical analysis can be used to construct a CBF network (a topological map) from the brain perfusion SPECT data (the topographical map). Let’s, therefore, assume that there is a group of brain perfusion SPECT images corresponding to 30 healthy control subjects (or 30 MCI individuals). The images of each subject can then be segmented in different ROIs using some brain atlas (e.g., the AAL atlas). If we plot the mean voxel value of each ROI for the 30 subjects, we would have a measure of how each ROI varies across subjects (Figure 4). In this example, it can be seen that ROIs 1 and 60 (albeit with different amplitude) vary similarly. The same goes for ROIs 30 and 90. A simple way to measure how similar the variations are between any pair of ROIs across subjects is by calculating their Pearson’s correlation coefficient. If this process is performed for every pair of ROIs a matrix of Pearson’s correlation coefficients is obtained (Figure 4).
**Figure 4.** Construction of the CBF group-based correlation network. In the correlation matrix, the color bar indicates the value of the correlation coefficient coming from the CBF co-variations among 90 anatomical brain regions (AAL atlas) across 30 subjects. Prior to the correlation analysis, a linear regression is performed at every ROI to remove the effects of age, gender, age–gender interaction, and global values. Self-correlations are excluded, implying a diagonal of zeros in the symmetric correlation matrix.

This matrix represents a correlation matrix of the CBF that can be seen as a network (or graph). Here the nodes are the ROIs (equivalent to the subway stations) and the connectors are the Pearson’s correlation coefficients between any two ROIs. Thus the brain perfusion SPECT data of 30 individuals have been transformed from a topographical space to a
topological one (the CBF correlation network). We previously demonstrated the feasibility of this approach using brain perfusion SPECT data of healthy control subjects [95]. This approach has also been applied to FDG-PET and structural MRI (T1-weighted) [20, 21, 100]. Furthermore, this methodology has shown to be consistent in several aspects with diffusion tensor imaging and functional MRI, which are neuroimaging modalities that directly target brain connectivity [100].

A typical clinical example of CBF correlation (co-variation) is the diaschisis phenomenon present in patients with cerebral infarction in the middle cerebral artery territory. In these patients, there is a decrease of cerebellar perfusion due to infarction in distant (connected) contralateral cortex (Figure 5). In these cases, the connectivity phenomenon is visually detectable. In others, it is more subtle and therefore quantitative (like graph theoretical analysis) methods are needed.
Figure 5. The diaschisis phenomenon in a patient with a cerebral infarction in the middle cerebral artery territory. This example visually illustrates the CBF correlation (co-variation) between connected brain regions (the cerebellar hemisphere and contralateral cortex). This patient was examined in the Center for Neurological Restoration (CIREN), Havana, Cuba.

Another important question is related to how network metrics are calculated from the correlation matrix. In a first case, the network metrics are computed directly from the matrix of the Pearson's correlation coefficients, but excluding the negative correlations. Although currently there is a debate about whether or not to exclude negative correlations, many of the negative correlations are thought to be artificial [101]. In some cases, there are also metrics that can be calculated using the absolute values of the correlation coefficients. An example of these metrics is the strength of association (a metric of node centrality) defined as the mean of the absolute value of Pearson's correlation coefficients of a particular node with the rest of the nodes in the network. The interpretation of this metric is simple since it measures the correlation's mean (co-variation's mean) of a node with the rest of the network. In another methodology, the correlation matrix is first binarized by setting the correlation coefficient to one (connection) if it is above a threshold and zero (no
connection) otherwise. The mathematical details on how the different network metrics are calculated are beyond the scope of this introduction. The interested reader is referred to Chapters 3 and 4 (for network metrics used in this thesis) or to the literature for a broader review [101].

On the other hand, the CBF correlation network is based on a group of subjects (as FDG-PET and structural MRI), thus, it represents an average group network (i.e., only one network). However, using appropriate statistical procedures it is possible to compare the topological metrics of two group-based correlation networks. One of these procedures is described and used in Chapter 3 (based on the bootstrap methodology) and another one in Chapter 4 (based on permutation tests). The interested reader is also referred to the literature [102].

Finally, even though the correlation network is group-based (as the CBF correlation network), it is possible to extract the individual patient’s contribution to the topological metrics of the network [103], and therefore, for example, to investigate the possible association with the relevant clinical characteristics of MCI patients that might be used to support diagnosis and prognosis individually. One of the methods to extract the individual patient’s contribution stands out since it is relatively easy to implement it in clinical practice [103]. This method estimates an indirect measurement of a network metric for a single patient by extracting the patient contribution to that metric (Figure 6). The estimation is achieved by subtracting the metric of the network using control subjects only from the metric of the network using control subjects plus the patient.
Figure 6. Estimation of the individual contribution of a given patient (patient j) to a network metric.

5. Aim of the thesis

The aim of this thesis is to demonstrate that brain perfusion SPECT can help to clarify important questions regarding the prodromal MCI stage of AD and relapsing NMO. This is not only pertinent considering our limited understanding of neurological diseases but also because brain perfusion SPECT is a low cost and worldwide accessible technology.

6. Thesis outline

The present thesis proceeds with Chapter 2, which reviews the role of brain perfusion SPECT in the prediction of AD dementia in MCI patients as compared to FDG-PET. In the first part of this chapter, we also provide a state-of-the-art review of the role of FDG-PET in the prediction of AD dementia in subjects suffering MCI, with a particular focus on the predictive power of FDG-PET compared to structural MRI.

Next, Chapter 3 proposes a new approach to investigate CVR in amnestic MCI patients and healthy control subjects based on graph theoretical analysis of brain perfusion SPECT data at baseline and under the vasodilatory challenge of ACZ. CVR is also investigated by
the standard methodology in the same groups of subjects and compared with graph theoretical analysis findings.

As an extension of graph theoretical analysis applied to brain perfusion SPECT of amnestic MCI patients, Chapter 4 examines the possible association of episodic memory with metrics derived from the CBF correlation network at the individual level. This chapter also explored changes in the metrics corresponding to the MCI group network after one-year follow-up, including the association with the global cognitive function at the individual level.

On the other hand, Chapter 5 investigates in relapsing NMO patients the associations between regional brain white and grey matter volumes and/or perfusion on one side, and the number of ON attacks, LETM attacks and/or total attacks on the other side. Because disease duration and NMO-IgG status could be also related with brain structural and functional changes, possible associations of these two clinical variables with regional brain tissue volumes and perfusion are evaluated also. This study was carried out by voxel-based analysis using two neuroimaging modalities (brain perfusion SPECT and structural MRI).

Based on the results of Chapter 5, Chapter 6 investigates the potential confounding effect of the number of ON attacks when comparing patients with NMO vs. controls by voxel-based neuroimaging analysis.

Finally, Chapter 7 presents the thesis Summary, and Chapter 8 the concluding remarks and future research directions.
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