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Quantification of cerebral cholinergic activity with [11C]-MP4B from positron emission tomography images

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Chapter 1 Introduction

Alzheimer's dementia is a chronic degenerative disease characterized by a slow and progressive neuronal degeneration that damages and eventually destroys brain cells, leading to memory loss and changes in thinking and other brain functions. It usually develops slowly and gradually gets worse as more brain cells wither and die. Eventually, Alzheimer's is fatal, and currently, there is no cure [1]. It is the most common type of dementia and, due to the aging of the population, continuously increasing. Because of this, it is one of the most significant "emergencies" that the health and social systems are facing, also because of the number of years in which this disease burden on the welfare services and families. The data regarding the prevalence of dementia reported in studies conducted in several industrialized countries, indicate comparable rates, hovering around 5% in subjects older than 65 years old.

There isn't a well-demarcated border between normality and disease, rather a kind of transition. The main difficulty is to identify the onset, that it could happen several years before first symptoms appear. Therefore, one of the main challenges today is to be able to diagnose in the very early stage the Alzheimer's disease, even before the emergence of significant memory problems. This provides an opportunity for possible new therapeutic approaches that may alter the underlying disease process and have the potential to slow down or stop the progression of the disease.

Degeneration of the cortical cholinergic system is one of the most consistent neurochemical changes in degenerative diseases like Alzheimer disease (AD) [2]. The cholinergic system and its components, above all Acetylcholine, a brain neurotransmitter involved in cholinergic transmission, and the 2 enzymes that degrade it, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) become, more often, object of study, in order to investigate any deficit which might be symptomatic of an eventual dementia.

During the last two decades, N-methylpiperidinyl esters have been extensively characterized as synthetic substrates for AChE [3] while, until recently, the role of BuChE has been largely ignored. However, increasing evidence supports the role of BuChE in the pathophysiology of AD. In severe AD, in fact, the level of AChE is decreased by as much as 90% compared with normal values, whereas the level of BuChE increases by approximately 30%. In such cases, BuChE may be a more appropriate therapeutic target [4], [5]. 4

1-11C-Methyl-4-piperidinyl n-butyrate (11C-MP4B), is a new radiopharmaceutical for the in vivo assessment of BuChE activity using PET [6]. It is the first radioligand validated for the study of BuChE activity in human brain. It is the specific substrate for BuChE and is inert for AChE. PET can be applied to detect cholinergic deficits at an early stage of a dementing disease and also to study the effect of cholinesterase (ChE) inhibitors or other cholinergic drugs in vivo [7].

PET quantification consists of an estimate of the kinetics coefficients using the relationship between the data of the tissue measured through the scanner PET and the input function, typically extracted from arterial sampling corrected for metabolites [8], [9]. This method is based on the assumption that there is a known and validated model for the tracer from which it is possible to start the estimation process. One of the purpose of this work is to develop a compartmental model for the 11C-MP4B since , to our knowledge, no model for this tracer has been developed yet.

Quantification can be done either by averaging time-activity curves (TAC) on functionally defined regions of interest (ROIs) and/or at voxel level using a tracer kinetic model structure. At ROI level, TACs are characterized by a good signal-to-noise ratio (SNR). In general, the number of regions to be analyzed is limited, so the state of art would expect using non linear estimators. However, the ROI analysis leads to a loss of the original spatial resolution. Voxel level analysis solves the model for each element of the image producing parametric maps having the same spatial resolution as the original PET image. Due to the low SNR of the voxel kinetics and very high number of voxels to be analyzed, nonlinear estimators cannot be used and faster algorithms are needed.

The other purpose of this work is then to quantify cholinergic activity, previously through a ROI-level analysis, where images were partitioned through a functional clustering based on parametrization of Time Activity Curves, and then through voxel-level analysis. In order to lighten the burden of the method and obtained precise estimates we used Basis Functions Method estimation (BFMs) (Gunn et al., 1997; Hong and Fryer, 2010; Hong et al., 2011) and Bayesian estimation (Zhou et al., 2002; Alpert and Yuan, 2009; Rizzo et al., 2012).

Chapter 2

Alzheimer's disease and Cholinergic System implications

Alzheimer's disease is a pathology that, to be identified, diagnosed, treated and managed, it must be known. In this first chapter we try to define what are the characteristics of this disease, to indicate who and what affects, to describe symptoms and course and to give an overview of what are the current methods of therapies.

2.1 Alzheimer's disease

Alzheimer's disease (AD), also known in medical literature as Alzheimer disease, is the most common disabling degenerative senile dementia, it was first described by German psychiatrist and neuro-pathologist Alois Alzheimer in 1906 and was named after him. Current therapies for the treatment or the incisive slowing of the disease are not effective enough and the progression of symptoms leads inevitably to death within ten years. The problem of the limited and not conclusive effectiveness of available therapies , combined with its wide and growing spread in the population and the enormous resources required for its management (social, emotional, organizational and economic), which fall largely on family members of patients, make of the Alzheimer's Disease one of the most serious diseases with a social impact in the world.

2.1.1 Epidemiology and economics

Economically speaking, the Alzheimer's disease is undoubtedly an interesting disease. Statistics say that about 5% of people over age 65 suffer from Alzheimer's and, except for a reduced percentage of individuals affected in young age, in over 95% of cases, people are affected by this neuro-degenerative disorder in elderly age. The same statistics tell us that the average age of the population is rapidly increasing thus expanding the number of potential patients and, as a result, potential users of medication to treat the disease. It is useless to deny that scientific research and economics are intertwined issues.

The determination of the social cost of Alzheimer's disease involves the analysis of all the costs incurred for the care and assistance of the sick ,that may come not only from the sick and his family, but also from health service, and the analysis of the impact of the disease in the community in general. Care, assistance, supervision to a person with Alzheimer's require, in fact, a consisting series of expenses, some costs can be directly quantifiable for the purchase of goods and services (direct costs), some not, and they represent the indirect costs related to the disease. These, which do not provide a real expenditure, are evaluated using the concept of opportunity cost, considering the alternative use of time that the person could have done and applying to this time an economic value. Indirect costs are therefore the result of a loss of resources for the community associated with the disease, especially in terms of time taken to any production activity that affects both the patient and the caregiver involved in assistance. In addition, in the calculation of indirect costs, we can insert also that of informal care given by family members, calculated by estimating the costs that would have been needed to support, in case of employment of paid staff.

From the reports of the association of Alzheimer's Disease International, 2009, it was estimated at 36 million the number of people worldwide suffering from dementia. This number is expected to double every 20 years, reaching 66 million in 2030 and 115 in 2050. Worldwide spending on dementia (604 billion U.S. dollars in 2010) amounts to more than 1% of global GDP, according to the 2010 Report, if spending on dementia was a national budget, would be 18th among the richest countries in the world.

In particular, in the European Community, based on UN's demographic forecast combined with Eurocode's new prevalence figures for Europe, it has been estimated that the number of demented will increase considerably from about 10 million today to about 14 million demented persons in 2030 (Figure 2.1). The relative cost prognosis is seen in Figure 2.1. The demographic forecast of costs will result in an increase in the whole Europe by about 43% between 2008 and 2030 to over 250 billions euros [1].

In addition to large economic interests at stake, the possibility of defeating a disease that can affect all, highly debilitating and extremely high social costs, pushes many research groups around the world to study how this disease born, progresses, attacks the cells of our brain and destroys them.

2.1.2 Pathophysiology

Alzheimer's disease is a disease of the central nervous system characterized by the progressive and relentless cell death of neurons belonging to the association areas of the cerebral cortex.

The onset of Alzheimer's disease is sneaky and insidious, often hardly recognizable even to those who are closest to the patient. It is characterized by the appearance of non-cognitive symptoms: depression, social withdrawal, affective flattening, low interest rates, neglect of the person, often delusional ideas of persecution or reference, anxiety. Usual associated memory disorders are deficits in learning new information, difficulty in remembering taken commitments and first occurred events.

The short-term memory, working tool needed to learn new information and retrieve memory traces, is the first to undergo to a deficit, causing dis-



Figure 2.1: Prognosis for the number of demented and future costs of dementia in Europe until 2030.

comfort to the individual and his family. The Episodic memory, which contains information about episodes or events and their space-time relations, is the first to fall, crumbling gradually the many episodes that have established the identity of the person, leading to insecurity and hesitancy. As the disease progresses sicks loose also the memory semantics, wealth of general knowledge: words, symbols, rules, formulas.

The 99% of cases of Alzheimer's disease is "sporadic", that means it occurs in people who do not have a clear familiarity and only 1% of cases of Alzheimer's disease is caused by an altered gene that determines the transmission from one generation to another. The cause of both sporadic cases and those family seems to lie in an alteration in the metabolism of a protein called APP (amyloid beta protein precursor) and for unknown reasons at a certain time of life it begins to be altered metabolized, leading to the formation of a neurotoxic substance (precisely the beta amyloid) that slowly accumulates in the brain leading to progressive neuronal death [10].

The accumulation of beta amyloid seems to start decades before the typical disorders of memory, from the appearance of which, within a few years, more and more disabling difficulties occur in the management of activities of daily living, until the total loss of self. In patients with Alzheimer's disease there is a loss of nerve cells in areas of the brain vital to memory and other cognitive functions (Figure 2.2). Today, the plaques formed by amyloid proteins and tangles are considered the effects on nerve tissue of a disease of which, despite the considerable efforts made in the field, we still do not know the causes.

In the mid-70s it was discovered that in people with Alzheimer's disease the levels of the neurotransmitter acetylcholine were much lower than normal. The discovery was interesting for several reasons, first of all it finally connected Alzheimer's disease with the biochemical changes in the brain. Acetylcholine is a neurotransmitter of crucial importance in the process of



Figure 2.2: Brain areas involved in Alzheimer's disease degeneration.

memory formation, in addition to being the one commonly used by neurons in the hippocampus and cerebral cortex, areas most damaged by the disease. After this discovery, acetylcholine has been the subject of hundreds of studies. The levels of acetylcholine are reduced slightly in all older people, but in people with Alzheimer's, they are reduced since 90%. People with more severe memory disorders show the major deficiency of acetylcholine. These discoveries have led to the hope that compensate the lack of acetylcholine with the use of drugs is a possible treatment for dementia [11].

2.2 Cholinergic System

The cholinergic system is a system of nerve cells that uses acetylcholine in transmitting nerve impulse. Impairment of cholinergic neuro-transmission in the central nervous system leads to severe cognitive disorders. The brain contains a network of cholinergic neurons, those with cell bodies in the basal forebrain project to areas of the brain linked with cognitive function, memory and learning (Figure 2.3).



Figure 2.3: The medial septal nuclei and basal nucleus of Meynert project widely upon the cerebral cortex, including the hippocampus. The pontomesencephalotegmental complex projects to the thalamus and parts of the forebrain

Degeneration of neurons in the cholinergic system occurs in Alzheimer's

disease and this may contribute to impaired cognitive function and learning, characteristics of the disease. Acetylcholine is an important neurotransmitter in both the peripheral nervous system (PNS: skeletal and smooth muscle, autonomic sympathetic and parasympathetic ganglia), and in the central nervous system (CNS) and, as recently discovered, has a fundamental role in the evolution of the disease. In the next paragraphs we will describe what is Acetylcholine and what is its role in the cholinergic transmission of impulses, what are the enzymes involved in the activity of cholinergic neurons (Acetylcholinesterase and Butyrylcholinesterase) and eventually what are the therapies for the treatment of AD that work on the cholinergic system.

2.2.1 Acetylcholine

The chemical compound known as acetylcholine (ACh), was the first neurotransmitter to be identified. Acetylcholine acts as a chemical mediator on the transmission of nerve impulses (called cholinergic transmission in this case), in multiple points of the central (CNS) and the peripheral nervous system (PNS) [12]. Many of the cholinergic neurons of the CNS form a large ascending system; while the bodies of these neurons are in the reticular formation, the axons radiate to all parts of the forebrain, including the hypothalamus, thalamus, the optic tract, the basal ganglia, the hippocampus and neocortex (Figure 2.3).

ACh has been linked directly or indirectly with various brain functions. More in detail, acetylcholine is secreted by the following neurons:

CNS neurons are involved in three specific regions:

- o neurons in the dorsolateral pons, which have numerous targets within the CNS and are involved in REM sleep;
- o neurons of the basal forebrain, the major source of cholinergic innervation of cortical origin, involved in learning;
- o neurons of the medial region of the pellucid septum, which projects its axons mainly to the limbic system;
- o many interneurons of the basal ganglia;

 $\mathbf{PNS}\,$'s involved neurons are:

- o somatic motor neurons, causing the contraction of skeletal muscle;
- o or the neurons of the autonomic nervous system:
 - neurons pre-and post-ganglionic of the parasympathetic nervous system;
 - pre-ganglionic neurons of the sympathetic nervous system;

Neurochemistry of Acetylcholine relates to its biosynthesis, deposit, release and metabolism. These processes are summarize in Figure 2.4.



Figure 2.4: Acetylcholine is made from choline and acetyl CoA, then is rapidly broken down by the enzyme acetylcholinesterase in the synaptic cleft. Choline is transported back into the axon terminal and is used to make more ACh.

Biosynthesis

Acetylcholine (often abbreviated ACh) is an organic, polyatomic cation; it is an ester of acetic acid and choline, with chemical formula $CH3COO(CH2)2N^+(CH3)$. Acetylcholine is synthesized from its two immediate precursors, choline (Ch) and acetyl coenzyme A (ACoA). The synthesis reaction is a single step catalyzed by choline acetyltransferase (ChAT), an enzyme synthesized in the cholinergic neuron.

 $\label{eq:Choline} {\rm Choline} \, + \, {\rm Acetyl} \ {\rm coenzyme} \ {\rm A} \, \stackrel{ChAT}{\rightleftharpoons} \, {\rm Acetylcholine} \, + \, {\rm Coenzyme} \ {\rm A}$

Part of the choline is synthesized from the amino acid Serine but the major part comes from the recycle subsequently the enzymatic hydrolysis in the synaptic space. Extracellular choline is actively transferred in the presynaptic nervous terminal from high and low affinity reuptake system. High affinity sites are responsible of the most part of the choline recycled by the synapsis and used for the biosynthesis of the neurotransmitter[13] [12]. The reuptake choline process is considered the passage that determines the speed of the whole biosynthetic process.

Deposit

Most of the Acetylcholine just synthesized is actively transferred through the use of cytosolic depositary vesicle located in the presynaptic nervous terminal, here the ACh is preserved until its release. Part of this Acetylcholine remains in the cytosol and it is eventually hydrolyzed in choline and acetate. Only the Acetylcholine in the vesicles is used as a neurotransmitter.

Release

The Acetylcholine release process from the depositary vesicles is initialized from an action potential transmitted towards the axon up to the nervous presynaptic membrane. This potential action induces the opening of the voltage dependent calcium channels , and leads to the release of Acetylcholine in the synapsis.

Metabolism

In the synapsis the Ach can ligate, to make a reaction, with the receptors located on the pre-synaptic or post-synaptic membrane. Acetylcholine must be rapidly removed from the synapsis to allow repolarization so free Ach, not ligated with receptors, is hydrolyzed in acetate and choline. This reaction is catalyzed from the Cholinesterase and this mechanism represents the physiological way to interrupt its action. This enzyme is also called specific cholinesterase and it has his maximum affinity with acetylcholine but also hydrolyzes other choline esters.

2.2.2 Acetylcholine receptors

The ACh acts using two different types of cholinergic receptors (AChR) that are widely distributed in the whole brain. These receptors are classified as muscarinic and nicotinic receptors. Some regions of the brain are characterized by the presence of the only muscarinic subtype (midbrain, medulla, and pons), while other regions (substantia nigra, locus caeruleus and septum) by the only nicotinic one. Both subtypes are localized in: striatum, cerebral cortex, hippocampus, thalamus, hypothalamus and in the cerebellum. Both muscarinic and nicotinic receptors have as endogenous ligand acetylcholine but, historically, the differentiation of the two receptor classes was made using non-endogenous ligands: muscarine and nicotine. The muscarinic receptors, in fact, are activated (as well as by acetylcholine) also by muscarine, but not by nicotine; nicotinic receptors instead, are activated by nicotine, but not by muscarine, and this explains the denomination [14].

Nicotinic Receptors

The nicotinic receptors are ionotropic receptors permeable to sodium, potassium, and chloride ions; they are divided into three main classes: muscle, ganglion and the central nervous system. Ion channels are activated by ligand.

The muscle receptors are localized at the level of the neuromuscular junction of skeletal muscle, the ganglionic receptors are responsible for transmitting at sympathetic and parasympathetic ganglia level and the receptors in the CNS are widespread in the brain.

The nicotinic receptors, with a molecular mass of about 280 kDa, are constituted by 5 sub-units, that are arranged symmetrically so as to circumscribe a pore through which occurs the flow of cations (Na and Ca incoming, outgoing K). Each subunit is composed of four transmembrane domains (Figure 2.5), with the N-terminal and C-terminal both on the extracellular side [15].



Figure 2.5: Structure of a neuronal nicotinic acetylcholine receptor (nAChR)

Currently we have identified several isoforms of the sub-units according to the different anatomical localization of the receptors. Multiple combinations of different types of sub-units generate different receptor subtypes. Among various receptors, the two most important and best characterized receptor types since today are the receptors of the neuromuscular junction and the receptors in the CNS. Alzheimer's disease is characterized by a reduction in the number of nicotinic receptors, without particular differences between various subtypes.

Muscarinic Receptors

Muscarinic receptors bind both acetylcholine and muscarine, an alkaloid present in certain poisonous mushrooms (it was first isolated in Amanita muscaria). Cholinergic transmission (acetylcholine-mediated) that activates muscarinic receptors occurs mainly at autonomic ganglia, organs innervated by the parasympathetic division of the autonomic nervous system and in the central nervous system.

All muscarinic receptors are G-protein coupled receptors. These receptors are formed by a single sub-unit that hasn't a directly associated channel. The receptor comprises a polypeptide that traverses the membrane seven times, the N-terminal is out and the C terminal is inside the cell. Inside, it contains a binding site for the G protein, which is activated when the ACh binds to the receptor (Figure 2.6).

Binding studies have identified five subclasses of muscarinic receptors each one with its own locations: M1,M4 and M5 are receptors of the CNS, M2 are the receptors of the heart and M3 are the receptors of smooth muscle. While the nicotinic type are ligand-gated ion channels that mediate a fast synaptic transmission of the neurotransmitter, the activation of muscarinic receptor triggers a cascade of intracellular reactions mediated by the release



Figure 2.6: Structure of a muscarinic nicotinic acetylcholine receptor (mAChR)

of a second messenger that mediate a slow metabolic response. Symptoms of AD regarding muscarinic receptor, show a decrease of M2 presynaptic receptor but not a decrease of M1 postsynaptic receptor [16].

2.2.3 Cholinesterase

In biochemistry, cholinesterase is a family of enzymes that catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return its resting state after activation. This family consists of two types of cholinesterase: Acetylcholinesterase and Butyrylcholinesterase (also known as Pseudocholinesterase), vertebrates possess both enzymes that probably come from the duplication of a single gene.

Acetylcholinesterase (AChE), also known as RBC cholinesterase, erythrocyte cholinesterase, or (most formally) acetylcholine acetylhydrolase, is a serine protease that hydrolyzes the neurotransmitter acetylcholine. AChE has a very high catalytic activity - each molecule of AChE degrades about 25000 molecules of acetylcholine (ACh) per second, approaching the limit allowed by diffusion of the substrate. During neurotransmission, ACh is released from the nerve into the synaptic cleft and binds to ACh receptors on the post-synaptic membrane, relaying the signal from the nerve. AChE, also located on the post-synaptic membrane, terminates the signal transmission by hydrolyzing ACh. The liberated choline is taken up again by the pre-synaptic nerve and ACh is synthesized by combining with acetyl-CoA through the action of choline acetyltransferase. For a cholinergic neuron to receive another impulse, ACh must be released from the ACh receptor. This occurs only when the concentration of ACh in the synaptic cleft is very low. Inhibition of AChE leads to accumulation of ACh in the synaptic cleft and results in impeded neurotransmission. AChE is found in many types of conducting tissue: nerve and muscle, central and peripheral tissues, motor and sensory fibers, and cholinergic and non-cholinergic fibers. Acetylcholinesterase is also found on the red blood cell membranes. The activity of AChE is higher in motor neurons than in sensory neurons.

Acetylcholinesterase exists in multiple molecular forms, which possess similar catalytic properties, but differ in their oligomeric assembly and mode of attachment to the cell surface.

Butyrylcholinesterase (BuChE), also known as plasma cholinesterase, pseudocholinesterase, or (most formally) acylcholine acylhydrolase, is a non-specific cholinesterase enzyme that hydrolyses many different choline esters. It is located in the liver, where it is synthesized and secreted in plasma, gut, heart, kidneys and lungs. Despite the high homology with AChE,(in 539 residues h AChE and h BuChE have 52.8% identity and 69.8% homology [4]), BuChE differs in many aspect. These two enzymes are distinguished essentially by the specificity of substrate: the AChE hydrolyzes the acetyl-choline neurotransmitter more faster than the choline esters having an acyl chain more bulky ,as the butyrylcholine. The BuChE, however, shows the same activity toward both substrates and is, therefore, less selective. Furthermore, it doesn't show a sort of substrate inhibition but it is active only at high substrate concentrations.

The main role of the AChE has long been known but the role of BuChE still remains an open question, it seems that this cholinesterase has an important role in cellular differentiation and development and it was also recently hypothesized that BuChE is not essential for survival but may play an important role, for such as "reserves" at the Ache, if it is in a condition of less or no activity [17].

The biochemical deficits of AD are reduced levels of acetylcholine because of substantial reduction in the activity of AChE, and by contrast, increased activity of BuChE. Both AChE and BuChE, that have differentiated kinetic and molecular properties than normal neuronal forms found in the brain, accumulate BuChE and AChE in brain can cleave >10 000 molecules of acetylcholine per second. It is shown that AChE knockout mouse survives for several weeks, since BuChE compensates the absence of AChE and serves as a backup to AChE in supporting and regulating cholinergic transmission. In a similar way, cytochemical studies have revealed that cholinergic neurons contain BuChE instead of AChE, suggesting that specific cholinergic pathways are regulated by BuChE in the brain of patients with AD [17].

2.2.4 Therapies

Alzheimer disease (AD) is a chronic and progressive neurodegenerative disease that is characterized by degeneration of cholinergic neurons in the areas of the brain particularly associated with memory, higher intellectual functions and consciousness. Although other neurotransmitter systems are affected, the most profound loss is that of cholinergic transmission. The possible effectiveness of an intervention on the cholinergic system in symptomatic treatment of AD, brought the research to move in different directions. A first way is intended to increase the functionality of precursors of cholinergic synapses by AChE precursors or by modulators of secretion and storage of the neurotransmitter (Amino pyridine, already used in the treatment of Multiple Sclerosis) or use of trophic factors. Another way is the one that provides the use of direct cholinergic agents which include agonists (both muscarinic nicotinic) that directly stimulate receptors. Finally you can use cholinergic agents that increase concentration of acetylcholine by inhibiting its enzymatic hydrolysis and thereby extending the physiological function [18].

AChE inhibitors: focus on Donepezil and Rivastigmine

Among the above pharmacological agents, AChE inhibitors seem to be the most effective method to improve cholinergic deficit thus reducing the symptoms of the disease [60]. Different types of AChE inhibitors have been studied for the treatment of AD and they differ in their mechanism of action, metabolism and brain selectivity. There are four CHE-I: tacrine, galantamine, donepezil and rivastigmine but only the last two are involved in our study and we will focus on it. Cholinesterase inhibitors can be reversible, pseudo-irreversible, or irreversible. In reversible inhibition, the inhibitor molecule is bound to the enzyme molecule for a short period of time, after which the inhibitor and enzyme molecules dissociate and enzyme activity is restored. In pseudo-irreversible inhibition, the inhibitor molecule binds to the enzyme molecule, but the bond is more slowly broken down, delaying the return of enzyme activity to normal. Irreversible inhibitors bind permanently to the enzyme, and thus the enzyme does not become available again. Donepezil is a reversible cholinesterase inhibitors. Although rivastigmine is considered a reversible cholinesterase inhibitor, its extended interactions with cholinesterase have been referred to as pseudo-irreversible. Cholinesterase inhibitors can also be characterized as selective for acetylcholine-depleted areas of the central nervous system. Animal studies show that donepezil and rivastigmine are brain selective, but there is evidence to suggest that rivastigmine is also brain-region selective, i.e. selective for the cerebral cortex and hippocampus, areas mainly involved in cognitive function. There are two types of cholinesterase that can be affected by CHE-I - acetylcholinesterase and butyrylcholinesterase. Both enzymes are present in the CNS, with butyrylcholinesterase also found in smooth and cardiac muscle, skin and serum. Because of its presence in the periphery, a high degree of inhibition of butyrylcholinesterase may be associated with a greater degree of systemic cholinergic adverse effects, such as nausea and vomiting [18]. The four CHE-I differ in their selectivity for acetylcholinesterase vs. butyrylcholinesterase.

Donepezil selectively inhibits acetylcholinesterase. Its elimination halflife is approximately 70 hours. It is well absorbed and reaches peak plasma concentrations in 3 to 4 hours. Donepezil is extensively metabolized to four major metabolites, two of which are known to be active, and a number of minor metabolites. A small portion of donepezil is excreted in the urine intact.

Rivastigmine inhibits both butyrylcholinesterase and acetylcholinesterase. It is rapidly absorbed after oral administration (time to maximal plasma concentration ranges from 0.8 to 1.2 hours). The elimination half-life of rivastigmine is approximately 1 hour. Renal elimination of the drug's metabolite is rapid and essentially complete after 24 hours. No significant drug-drug interactions have been observed with 22 drug classes. Because rivastigmine demonstrates low protein binding (approximately 40%), no displacement of other drugs is expected.

Neither food nor time of administration (morning vs evening dose) influences the rate or extent of absorption of donepezil. Rivastigmine is administered with food to reduce gastrointestinal side effects and improve tolerability by delaying absorption, prolonging time to maximal plasma concentration. In clinical trials, rivastigmine was associated with a high incidence of GI adverse effects [18].

Chapter 3

PET neuroimaging for AD's diagnostic investigation

The recognition and diagnosis of the Alzheimer's disease are still an open question. Thus symptoms are undeniable just only at an advance stage, the difficulties lie in being able to diagnose the disease at its onset, so you can treat it right away and be able to, if not stop, at least slow its progression. Current diagnosis of Alzheimer's disease is made by clinical, neuropsychological, and neuroimaging assessments. Routine structural neuroimaging evaluation is based on nonspecific features such as atrophy, which is a late feature in the progression of the disease. Therefore, developing new approaches for early and specific recognition of Alzheimer disease at the prodromal stages is of crucial importance. In this section we will describe how a PET facility works and what are its main features.

3.1 Positron Emission Tomography technique

Positron Emission Tomography (PET) is a nuclear medical imaging technique that employs the electronic detection of short-lived positron-emitting radiopharmaceuticals (substances containing a carrier molecule, such as glucose, and a positron-emitting radioactive isotope that labels or tags the carrier molecule) to study and visualize human physiology [8]. The PET scanner or camera generates three-dimensional images of the distribution of a radiopharmaceutical administered within the body. During the scanning procedure, doctors and researchers are able to measure in detail the functioning of the human brain and other organs, while patients are comfortably aware and vigilant. The generated images allow to monitor and evaluate bodily processes such as glucose metabolism, oxygen metabolism, and cerebral blood flow. New developments have shown that the study of metabolic activity of brain areas is of crucial importance in the early diagnosis of degenerative diseases or brain tumors. The monitoring and evaluation of the metabolic processes of the body through the precious instrument of PET have added a new dimension to the diagnosis and treatment of diseases, while also allowing proper care and follow-up more targeted and timely.

PET uses radiopharmaceuticals that contain positron emitting radioisotopes, unlike other diagnostic technique imaging that uses radiopharmaceuticals that directly emit gamma-rays. This emission occurs when a positron emerge from the decay of nuclei of radioactive isotopes which are created in cyclotrons specifically dedicated to the synthesis of radiopharmaceuticals. Positrons are subatomic particles that have all the features of electrons (ie, mass, size, size of office), except the polarity of charge. When the electrons collides with their corresponding positrons, electrons and positrons are destroyed and their mass is transformed into a pair of high-energy gamma rays that run away from the collision point towards opposite directions.

It's on the basis of this principle that positrons emitted from radiopharmaceuticals during PET scanning procedures are transformed into gamma rays, that are then detected by a gamma camera or a PET scanner and prospectively reconstructed into images.

The procedure of a PET exam starts with the intravenously injection of the radiopharmaceuticals into a patient's bloodstream. The tracer is distributed throughout the patient's body and accumulates in the organ or body system being examined where positrons are emitted and travel in the surrounding tissue dispersing kinetic energy until they encounter and collide with one of many nearby electrons. During the collision, the two particles combine and destroy each other in a process that physicists refer to as annihilation.

The annihilation of two particles produces a burst of energy that leads to the generation of two 511-keV gamma rays (according to Einstein's famous equation: E = mc2), which radiate in opposite directions from each other, thus forming a line of coincidence (Figure 3.1). The coincidence line is the indicator that annihilation has occurred somewhere along its trajectory and it serves as a vital component in the detection scheme by which PET images are created.



Figure 3.1: After annihilation (A), the two gamma rays are detected by the detectors in the scanner that encircles the patient (B). PET scanner sorts all the coincidences in a sinogram (C) and links to a computer that reconstructs coincidence events to produce two- or three-dimensional images (D).

The two gamma rays, speeding away in opposite directions (180 degrees apart) from each other, are detected on opposite sides of the patient's body by a PET scanner or a gamma camera that surrounds the patient as he or she moves slowly through the scanner. The detection of this coincidence is possible because the detectors (thousands of them) are arranged in a ring configuration around the interior of the scanner (Figure 3.1), with each detector having an associate partner detector on the opposite side of the ring. In some instances, each detector consists of a scintillating crystal and a photomultiplier tube but more recent and common configuration, the block detector, consists of a rectangular bundle of crystals optically coupled to several photomultipliers. When a gamma ray is sensed by a detector, the gamma ray activates the scintillation crystal, which converts it into a burst of light photons. The light photons are detected and amplified by a photomultiplier, and that event is registered by the electronics of the scanner. The scanner electronics record the electronic signals and determine which ones are coincident. The coincidence is determined considering a time frame (coincidence window), based on which if two coincident gamma rays are detected on opposite sides of the patient's body within nanoseconds of each other, the computer pairs and records them into coincident events, forming a coincidence line.

The PET scanner collects all coincident events (usually about 500,000 events) and sorts them in the form of lines into a sinogram, which stores information in a way that is favorable for image reconstruction. The sinogram is then reconstructed with corrections by a computer linked to the scanner (Figure 3.1) to produce a two-dimensional image, that portray the activity of the radiopharmaceutical in the patient's . All commercially available PET scanners simultaneously acquire data for three-dimensional images, either by imaging the entire volume as a unit or by stacking adjacent two-dimensional slices. Before the advent of the PET scanner, the study of physiologic phenomena was not possible.

3.2 PET tracers

PET imaging process is based on the use of radiopharmaceuticals [19]. Most radiopharmaceuticals contain two components: a carrier molecule that contains a natural substrate, such as glucose, and a positron-emitting radioisotope that labels the carrier molecule. Bound to each other, the carrier molecule and the positron-emitting radioisotope make it possible for the PET scanner to detect, record, and image the results of the collision and interaction of emitted positrons with electrons inside the body in the form of gamma radiation detected outside the body. It is important to point out that the radioisotopes used in PET radiopharmaceuticals mimic substances, such as sugars, water, proteins, and oxygen-substances that occur naturally in the human body, and it is for this reason the radioisotopes can be used to label natural substrates without altering the substrates' biological and chemical properties.

Most of the radioisotopes used in PET radiopharmaceuticals are manufactured in a cyclotron. The cyclotron is a machine that accelerates protons by moving them along a circular orbit inside a chamber controlled by powerful electromagnetic fields until the protons' speed is near the speed of light and smashes them into the nuclei of stable or nonradioactive elements (e.g., fluorine, nitrogen, oxygen, and carbon), transforming the nonradioactive elements into positron-emitting radioactive isotopes. The excess protons acquired by the newly created radioisotopes during the particle acceleration process that takes place in the cyclotron, cause the radioisotopes to be unstable, such that they decay by positron emission, in keeping with their innate propensity to revert back to their stable state. Because they instinctively try to return to their original stable state, PET radioisotopes have short half-lives. While it is true that these short half-lives complicate the PET procedure because of the time constraints they impose, the manufacture of these radioisotopes and their transformation into PET radiopharmaceuticals must take place inside or close to the PET imaging site where they are to be used, this feature contributes to the low-risk profiles of PET radiopharmaceuticals. Together the short half-lives of PET radiopharmaceuticals along with their low levels of radiation make them ideal choices in terms of safe levels of radiation for the patient.PET radiopharmaceuticals are usually synthesized a single dose at a time and administered to the patient within minutes after their synthesis.

Based on the body function that they measure, PET radioisotopes can be divided into three categories. The first category includes radioisotopes, such as Fluorine-18, that are used to measure such general metabolic data as glucose uptake and protein synthesis. First category radioisotopes leave the bloodstream and enter cells. Quite contrarily, the second category of radioisotopes, which includes Oxygen-15, remain in the bloodstream throughout the study duration, making them appropriate candidates for measuring blood flow. The third category, that we will deepen in the next section, is the one of radioisotopes, such as Carbon-11, that quantify and delineate cellular receptors.

3.3 11C-MP4B : a radioligand for BuChE activity investigation

Because of their diagnostic potential and therapeutic values, investigators have synthesized various radiolabeled acetylcholine and butyrylcholine analogs as positron emission tomography (PET) tracers. These tracers are used to quantify levels of ChE in human's brains, to detect its activity and to verify the efficiency of cholinesterase inhibitors. As said in the previous chapter, more than AChE, BuChE may be an appropriate receptor to investigate and diagnose Alzheimer's Disease at an early stage of its onset because of both a quantitative and a qualitative reason. First of all, researchers and post-mortem studies demonstrated that the disfunctions of the cholinergic system associated with AD are reduced levels of AChE but, for what concern BuChE, its levels increase as much as 90% [6] [5]. In addition,to divert the attention towards this new tracer, there are also technical and qualitative considerations, which deal with the fact that, at present, there are no kinetic model globally suitable for quantification of AChE activity using acetylcholine radiolabeled analogs.

N-methylpiperidinyl esters are a group of synthetic substrates for ChE; of

them, 1-11C-methyl-4-piperidinyl acetate (11C-MP4A) and 1-11C-methyl-4piperidinyl propionate (11C-MP4P) have already been used in the clinic as PET tracers for in vivo assessment of AChE activity associated with AD [3] [9]. Both are classified as irreversible radiotracers. These lipophilic acetylcholine analogs enter the brain by diffusion and are hydrolyzed by AChE to a common hydrophilic metabolite, which is trapped in the brain according to the distribution of AChE activity. The standard method to estimate regional hydrolysis rate of [11C]MP4A by AChE (k3; an index of AChE activity) is to fit the regional time-activity curve (TAC) obtained by PET to the theoretical function derived from the kinetic model and the arterial input function using nonlinear least squares (NLS) optimization [7]. In NLS analysis, however, the procedure involves measurement of the arterial input function that is invasive to patients and requires considerable technical expertise, which may restrict widespread use of this method in daily clinical practice. In consequence of this, several alternative methods have been proposed.

A first alternative analysis method without the use of an arterial input function is the shape analysis, that has been proposed for estimation of regional cerebral k3 of [11C]-PMP (Koeppe et al., 1999) [20] and [11C]-MP4A (Tanaka et al., 2001) [21]. Shape analysis demonstrated to be very sensitive to bias effect of scan duration and showed larger coefficient of variation (CV) of k3 estimates as compared with NLS analysis, particularly in regions with high AChE activity. In the case of [11C]-MP4A, moreover, shape analysis allowed quantitative measurement of AChE activity only in neocortical regions.

Nagatsuka et al. (2001) proposed, then, the Multi-linear Reference Tissue Model for [11C]-MP4A (RLS), that is a method that uses a reference with very high AChE activity, in contrast to reference methods for receptor tracers which use a reference devoid of specific binding [22]. Findings in different publications indicate that cerebellum yields more stable results than striatum, most likely due to the higher impact of motion on the signal from the small striatum than the large cerebellum. The sensitivity of RLS analysis for detecting cortical regions with abnormally low k3 in patients with AD is less than NLS analysis, but it is greater than shape analysis. Computer simulation studies showed that RLS analysis provides a precise index of AChE in brain regions with low (for example, cortex) and moderate AChE activity (hippocampus and thalamus). Despite the performance of this new method are, in conditions of non advanced brain atrophy, much better than the ones of the shape analysis, RLS for [(11)C]-MP4A is not suitable for quantification in regions with high AChE activity.

1-11C-Methyl-4-piperidinyl n-butyrate, [(11)C]-MP4B, is a new radiopharmaceutical for in vivo assessment of butyrylcholinesterase (BuChE) activity using PET. It is the specific substrate for BuChE and is inert for AChE. The blood-brain barrier is impermeable to the hydrolyzed tracer 11C-MP4B-OH; thus, the tracer accumulates in the tissue in a rate determined by the local BuChE activity. Only a fraction of the injected tracer passes the blood-brain barrier, leaving most of the radioactivity in the lower parts of the body.

Investigators from several groups analyzed radiation dosimetry, biodistribution and blood metabolism of 11C-MP4B to understand whether it can be considered as a viable candidate for the estimation of cerebral cholinergic activity , in particular for patients suffering from AD [6].



Figure 3.2: Whole-body PET images of 22-y-old man, showing distribution of intravenously administered 11C-MP4B. Accumulation of tracer is clearly visible in urinary bladder, renal pelvis, stomach, liver, vertebral column, and salivary glands (arrows). (http://jnm.snmjournals.org/content/45/12/2032.figures-only)

In a study of Roivainen et al. (2004) they analyzed the distribution and kinetics of 11C-MP4B uptake in order to clarifying the metabolic fate and the whole-body distribution of the tracer [23]. They investigated the time-activity-course of radioactivity associated with 11C-MP4B and the analysis showed that the level of unmetabolized radioligand decreases rapidly at 15 min after injection.

In their studies there were large individual variation in the rate of plasma of 11C-MP4B hydrolysis but no significant differences in degradation of 11C-MP4B between male and female patients or between healthy controls and sick patients. For what concerns the whole-body distribution, they found the highest activity in, in order, urinary bladder, renal pelvis, stomach, salivary glands, liver, kidneys, spleen, vertebral column, and brain (Figure 3.2). In patients with AD, 11C-MP4B activity in the brain was highest in cerebellum, followed by striatum, pons, and thalamus. Lower 11C-MP4B activity was seen in cortical areas (Figure 3.3).

Eventually, biodistribution of 11C-MP4B in the brains of patients with AD appears to be in accordance with the distribution of BuChE seen in postmortem studies of human brain, except for the observed higher activity in striatum than in cortex.

In a study of Virta et al. (2008) they analyzed and estimated the radiationabsorbed doses of the 11C-MP4B in humans according to the guidelines of the International Commission on Radiological Protection [24]. They compared two different data acquisition protocols, dynamic organ-specific evaluation (DOSE) and whole-body scanning, both methods widely used for evaluation of radiation burden of 11C-labeled PET tracers. Their results showed that the organs with the highest radiation-absorbed were the liver and the urinary



Figure 3.3: (A) PET images showing radioactivity distribution in brain of AD patient (70-y-old man) after 731-MBq intravenous injection of 11C-MP4B. Images are summated from 20 to 40 min after injection and color coded according to amount of radioactivity, from dark blue (lowest) to hot red (highest). (B) Time-radioactivity curves for cerebellum, striatum, cortex and corresponding total and metabolite-corrected radioactivity curves of arterial plasma. (http://jnm.snmjournals.org/content/45/12/2032.figures-only)

bladder,followed by kidneys (renal cortex), upper large intestine, trabecular bone, salivary glands, and heart wall. More than 50% of the injected dose was excreted via the urinary pathway with a quite rapid clearance: 30% of the radioactivity excreted within 60 min after injection. Both DOSE method and simulated whole-body imaging approach gave same results. They concluded that 11C-MP4B causes less radiation burden than other 11C-labeled PET tracers and there were non intolerably absorbed doses on critical organs and this would allow multiple PET examinations during the follow-up of the disease for the same subject.

Next step is now the development of a kinetic model to quantify the cerebral cholinergic activity of BuChE with [11C]-MP4B through positron emission tomography images.

Chapter 4

Data sets and Methods

4.1 Data sets

In a typical PET study, PET data are sequentially obtained after the radioactive tracer is introduced (usually administrated intravenously) over time. By applying proper corrections for attenuation, dead-time of detector, physical decay of radioactivity and scattered photons, PET data represent the tracer concentration (in our case kBq/ml) at a certain time for the concerned subject.

Data sets were made available by a Finnish National Research Institute, the Turku PET Centre.

4.1.1 Subjects

A group of 8 subjects, 6 of them patients affected from Alzheimer's Disease, 2 of them normal control subjects, participated at the study. Of the 6 patients with AD, 2 of them were under medical treatment with Rivastigmine and we will refer to them as rivas01 and rivas07, 2 of them were under medication with Donepezil and we will reference them with the name donep02 and donep04 and finally the last 2 were not undergoing any treatment and their reference will be nomed04 and nomed08. The two controls' name will be contr03 and contr05.

4.1.2 Plasma data

For each subjects arterial blood samples were available, both the activity counts from entire blood (data with suffix $ap_comb.kbq$) and the activity counts from blood corrected for brain/collection site delay for tracer metabolism, that means that only counts from pure tracer are going to be taken in (data with suffix $ap_comp_pure_sinewf.delay.kbq$). All the blood samples were automatically collected through the radial artery of the patients according to a sampling protocol which envisaged a higher frequency at the beginning of the exam, when the tracer kinetic was faster, followed by a gradually decreasing frequency as the exam reached its end: 115 intervals of 5 seconds, 9/10 intervals of nearly 5 minutes. Each arterial sampling has a duration of about 50 minutes (Figure 4.1, Figure 4.2). All data are expressed in kBq/ml.



Figure 4.1: Activity counts of patient *contr03* from both the entire blood and the blood corrected for brain/collection site delay and tracer metabolism



Figure 4.2: Activity counts of patient *donep02* from both the entire blood and the blood corrected for brain/collection site delay tracer metabolism

4.1.3 PET images

PET images of [11C]-MP4B of the 8 subjects were available. All of them were performed with a GE Advance PET scanner (General Electric Medical Systems, Milwaukee, WI). Images were corrected for movement and both normalized to MNI space and left on their native space (Figure 4.3). At the start of the scan 11C-MP4B was injected in an antecubital vein as a constant bolus during 80 s and images were acquired in times gradually increasing. Radioactivity was measured in a consecutive series of 22 frames: 1x30 s, 4x15 s, 5x30 s, 2x60 s, 2x120 s,6x300 s, 2x600 s) with a total scan duration of 60 min.



Figure 4.3: PET images of, above, slice 20 and time 15 of subject contr03, below, slice 16 time 20 of subject contr03. Images on the left are in their native space, images on the right are normalized to the MNI space.

Chapter 5

Methods and Data Processing

5.1 Methods

Before describing the real work that has been done from the data at our disposal, we will discuss very briefly the methods that were used. First, we will describe the structures of Compartmental Models, their importance in the study of brain images, their development from data and their solution. After that we will briefly describe the types of quantification methods of PET images, based on Region of Interest or on Voxel, relating in particular to the method of the Basis Functions for non linear models solution.

5.1.1 Compartmental models

When trying to rationally explain the experimental data of a given biological system arises the need to divide the system into building blocks of the system itself, if they meet certain requirements, these compartments are enables not only to visualize the distribution of PET radiotracer, but also to quantify several biomedical functions. Compartmental model is the basic idea to analyze dynamic PET data [25].

A compartment, in order to be considered as such, must comply some requirements: its constituents must blend quickly, mixing must be uniform and constituents present in its volume must all have the same probability of moving into other compartments or outside. The requirement of homogeneity and identical behavior lead to an exponential law to express the kinetic output and allow to propose mathematical models consist of systems of first order linear differential equations with constant coefficients. The compartmental model of a system is defined by the various compartments and from the connections that bind them together, the transfer coefficient, that expresses the speed with which the process transfer from one compartment to another.

There are various types of compartmental models for the kinetic study of tracer. In principle, almost all the models are characterized by a compartmental group that describes the plasma kinetics and a set of compartments which describes the behavior of the tracer once entered into the tissue. Regarding the tissue we can have up to three different compartments, with their relative transfer constants: a compartment for the concentration of free tracer in the tissue, a compartment for the concentration of tracer non-specific ligated to receptors in the tissue and a compartment for the concentration of tracer bound to specific specific receptors in the tissue. In particular this latest one is of greater interest in the receptor study since it allows to locate the neurologically specific receptor sites for the radioligand. Regarding the plasma we can have up to two compartments, in which the first describes the concentration of free tracer in plasma, the second the concentration of tracer bound to blood proteins. However, very often these compartments collapse between them, because of lack of consistency or lack of sufficient data to estimate all the system variables, so we can have up to a unique blood compartment and a unique tissue compartment (Figure 5.1). What we can get from the study of these models is the only plasma-tissue transfer constant (K1), which expresses the rate at which the tracer crosses the blood-brain barrier and diffuses into the brain.



Figure 5.1: Mono compartmental model that describes the kinetics of a tracer. The constants K1 and k2 are the transfer constants of the tracer between the various compartments

The differential equations that describe the concentration of tracer in each of the compartments of a compartmental model have the structure of the ones below, which are the equations of the model in Figure 5.1:

$$dC_p(t)/dt = k_2C_t(t) - K_1C_p(t)$$
$$dC_t(t)/dt = K_1C_p(t) - (k_2)C_t(t)$$

 $dC_i(t)/dt$ is the change rate of the concentration of the tracer in the compartment. The constants units are generally in min-1 and describe the fraction of tracer that leaves the compartment in a time unit.

These equations can be solved with different methods, that we will discuss later. Each method leads to the quantification of the constants of transfer of the compartmental model or a composition of them. Such constants, in addition to having singularly their meaning, may give rise to other informative parameters: there are two parameters of interest to measure the kinetics of the tracer, the binding potential and the distribution volume, in the following we'll give their receptor-ligand kinetics definitions.

Binding potential is a combined measure of the density of available neuro-receptors and the affinity of a drug to that neuro-receptor.

Distribution volume of a radioligand is defined as the ratio between the radioligand concentration in tissue target region and radioligand concentration in plasma at equilibrium. The binding potential can be calculated for those tracers whose kinetic model is described by a 2- or 3-compartmental model, but not to those described by a kinetic mono-compartmental model, which does not distinguish between specific binding and non-specific. The distribution volume can be achieved, however, for all tracers. In theory, the binding potential is preferable to the distribution volume as we want to be sure that the differences we find are due to differences in the specific binding of the tracer with the receptors, not in non-specific binding. Although the distribution volume in itself has both specific binding and non-specific, its interest lies in the fact that it is still proportional to the concentration of receptors available for binding. It can be used when it is not possible to obtain accurate and reproducible parametric images of binding potential, or when the kinetics of the tracer is for most of the cases described by a mono-compartmental model.

A priori it is difficult to determine which is the most suitable model to describe the kinetics of a tracer, so it is necessary to test different models, validate them, and choose the one with best results and statistics.

5.1.2 Voxel based analysis: Basis Function Method (BFM)

The quantification of physiological information from dynamic studies is a fundamental aspect of PET. Starting conditions are, in fact, a combination of discrete spatial resolution of image acquisition combined with anatomical regions of reduced dimensions to be studied and it is therefore of fundamental importance that the estimates are accurate.

The quantification of the parameters can be done at the level of Region of Interest or at voxel/pixel level. The pros and cons that could tip the choice to a method rather than to another depend largely on what are the purposes of the study and on the kinetics of the tracer. To solve the equations of models such as those shown above, which are described by a non-linear kinetics, the state of the art would use the Weighted Non-Linear Least Square (WNLLS). This technique, although precise and effective, however, requires a heavy computational time, very often not compatible with the clinical requirements and, as a consequence, not useful. In the following lines we will explain the main features of the ROI- and voxel-based analysis, illustrating a particular type of voxel-level quantification method: the Basis Function Method.

ROI level analysis consists on divide the scanned image into sub-regions defined according to criteria that can be of functional or morphological type, make an average of the acquired signal in different voxels of the region and then proceeding with WNLLS to calculate the unknown kinetic parameters of the averaged signal. This technique allows to obtain reliable estimates since the averaged signal has a good SNR and does not require excessively long calculation times as the number of iterations is greatly reduced. In so doing it is also possible to investigate specific spatial regions, which may have relevant functional or morphological meaning. The analysis in terms of ROI, however, has its negative aspects, due primarily to the fact that it's going to miss the original spatial resolution of the image, secondly due to the problems arising from the previous statistical analysis. Voxel level analysis solves the model for each element of the image producing parametric maps having the same spatial resolution as the original PET image. Due to the low SNR of the voxel kinetics and very high number of voxels to be analyzed, that would require a high number of iterations, nonlinear estimators cannot be used. Voxel level analysis requires high computational time and, due to their sensitivity to initial estimates, convergence, in general, is not guaranteed.

When the study requires a voxel level quantification, to overcome the problem of nonlinear estimation, alternative methods can be used to combine the advantages of the voxel-based analysis and, at the same time, lightening the computational burden. One of this method is the Basis Function Method.

Basis Function Method (BFM)

Basis Function Method (Gunn et al., 1997) is a voxel-based method for quantification of tracer's kinetic parameters [26]. To explain how this method works we will apply it to the simple plasma- tissue compartmental model of Figure 5.1, that, once equations are solved, appears as follows:

$$C_{VOI}(t) = K_1 \int_0^t C_p(t) e^{-k_2(t-\tau)}$$

The equation can be written also as:

$$C_{VOI}(t) = K_1 C_p(t) \otimes e^{-k_2 t}$$

and, more in general:

$$C_{VOI}(t) = \theta_1 C_p(t) \otimes e^{-\theta_2 t}$$

where θ_1 and θ_2 represent the generic parameters or composition of parameters to estimate. To solve the equation above it must be used a nonlinear method, but if we renounce estimating k2, and we assign it a deterministic value, we can first solve the convolution operation and then go to estimate, through a linear method, the value of k1. Formally, defining θ_j the generic parameter for which we don't give a statistical description, we fix a range (i.e. a grid) of M possible values of θ_j and we pre-calculate for each time frame (i=1,...nT, with nT the number of frames) a set of M basis function (B_j) for the nonlinear term involving Cp(t) as:

$$B_j(t_i) = C_p(t_i) \otimes e^{-\theta_j t_i}$$
 j = 1,..., M

Once the (B_j) are solved we can use the Weighted Linear Least Square to evaluate the remaining kinetic parameters (θ_1 in the case above), solving the problem of calculation burden. The accuracy of the estimates will then depend, in large part, from the creation of the grid parameters, the real crux of this method.

5.2 Data Fitting

The procedure of quantification of PET images requires a known input signal that provides information on the concentration of tracer and metabolic residues of the tracer in the blood. It may happen that, when the samples are collected by an automatic machine, the data are too noisy to be used directly and they need to be fitted with an appropriate system. This is also useful for estimating the measurement error associated with the samples. For this purpose we used the software SAAM II.

5.2.1 SAAM II - Numerical

Simulation, Analysis, and Modeling Software II (SAAM II) is a software package available for tracer and pharmacokinetic analysis. The SAAM II system, a totally reengineered software system based on Berman's SAAM [27], is a powerful research tool to aid in the design of experiments and the analysis of data [28]. SAAM II deals easily with compartmental and numerical models, helping researchers create models, design and simulate experiments, and analyze data quickly, easily and accurately.



(a) Equation Builder tool.

(b) Parameters tool.

Figure 5.2: SAAM II Numerical Module tools

In the numerical module of SAAM II, user can enter algebraic equations manually or select them from a set of predefined templates as polynomials, exponential sums, sums of Gaussian, Michaelis Menten equations and Scatchard analysis. The software solves the equations and fits them to the data. The Equation Builder window (Figure 5.2 (a)) is used to select one of a predefined set of equations for the Numerical Module and if Sum of Exponentials is chosen, user must also select the type of experimental exogenous input.

As for the weights to be assigned to the data, where $d(t_i)$ (i = 1, ..., N) are the experiment data, SAAM II allows to assign the standard deviation of measurement error using one of the options in Table 5.1.

In addition, if a series of data have not to be considered, this assumption is specified in SAAM II typing, after the data, the sequence: (-).

SAAM II formulations	Standard Deviation
$(SD \alpha)$	$\sigma_i = \alpha$
$(FSD \ \alpha)$	$\sigma_i = \alpha d(t_i)$
(POIS α)	$\sigma_i = \sqrt{\alpha d(t_i)}$
(GEN a b c)	$\sigma_i = \sqrt{a + bd(t_i)^c}$

Table 5.1: Formulation of the standard deviations with SAAM II

The statistical information, available following a successful fit of the model to the set of data, are: best estimates for both the primary parameters and derived parameters (functions of the primary parameters), their estimated standard deviation, their fractional standard deviations and 95% confidence limits. The correlation coefficients were also provided. Additional options included the covariance matrix and information on the objective function.

5.2.2 Arterial and Metabolic Input Function

Starting from only visual inspection of the data it was possible to notice some irregularities in the descent curve of the arterial signal, both for the data relating to whole blood, and for those corrected for metabolic component (Figure 5.3).



Figure 5.3: Activity counts of patients *nomed04* and *donep02*. In the first one the "false" peak is visible, in the second not.

The nature of the irregularity detected was referred to be a noisy artifact. This "false" peak irregularity was more visible in some subjects than in others, with no distinction between sick patients and controls, in particular it was more pronounced in *rivas* and *nomed* groups than in others. We chose to delete the data related to these "false" peaks to allow the software to achieve a better optimization, an example on Figure 5.4.

Before solving, we set the initial values of the parameters specifying if they're adjustable or fixed and giving the bound within the quantification process can range, in particular to avoid that the optimization did not cross the frontier of the physiologically acceptable values (Figure 5.2 (b)).


Figure 5.4: Activity counts of patient nomed04 with and without "false" peak data

Data weights have been set as $SD = \sqrt{d_i}$ that corresponded at (*POIS* 1) in SAAM II standards (Table 5.1).

For choosing the best descriptive model, within various attempts proposed by the software, which were optimized on the available data and on the basic structure that each times we provided, we considered various factors. First we looked at the trends, more or less polarized, of the weighted residues, then the values, physiologically acceptable or not, of the hypothetical identified parameters and eventually we tryed to always prefer simple models.



Figure 5.5: Models attempts for rivas07 with and without "false" peak data

Models that showed an inferior polarization of residues were privileged, in particular we compared the performance of models built from the complete data set with models built on the data set without those data related to the "false" peak. In the case of the patient rivas07, for blood data corrected for brain/collection site delay and for tracer metabolism, it's possible to see (Figure 5.5) how, once the peak of the false data had been deleted, the program was able to identify a function that fit better the data and, in addition to visual inspection, this is also confirmed by the sharp decrease of polarization of the weighted residues.

The choice was more sensitive to those patients in whom the elimination of the peak would result in a substantial loss of data. In some cases, in fact, the elimination of a big portion of the data set has changed much of the result of the fit, leading to have more exponential components, low parameter values and therefore not very significant. In such cases, even the weighted residuals seemed to be less polarized, we prefered the model fitted on the original data set (Figure 5.6).



(a) Model with original data set for nomed04



(b) Model with data set without noisy data for nomed04.

Figure 5.6: Comparison between two plausible models for nomed04, in (a) the model optimized on the original data set, composed of two exponentials, and related weighted residues, in (b) the model optimized on the lighter data set, composed of three exponentials where a3 is very low, and related weighted residuals

Starting from these considerations, the models chosen to fit the arterial data from whole blood and from blood corrected for brain/collection site delay and for tracer metabolism for each patient are shown in Table 5.2 and Table 5.4.

Subject	Fitting optimization model for entire blood data
contr03	$ya(t) = 228.66e^{-2.157t} + 73.442e^{-0.42t} + 8.798e^{-0.069t} + 5.175$
contr05	$ya(t) = 470.663e^{-0.994t} + 18.426e^{-0.092t} + 7.594$
donep 02	$ya(t) = 171.885e^{-0.608t} + 14.536e^{-0.056t} + 5.693$
donep04	$ya(t) = 246.162e^{-0.938t} + 15.064e^{-0.126t} + 7.492e^{-0.006t}$
nomed04	$ya(t) = 1737.285e^{-1.983t} + 28.985e^{-0.127t} + 7.131$
nomed08	$ya(t) = 1085.433e^{-1.858t} + 19.671e^{-0.167t} + 9.815e^{-0.002t}$
rivas 01	$ya(t) = 1266.857e^{-1.869t} + 22.562e^{-0.169t} + 9.475e^{-0.005t}$
rivas07	$ya(t) = 463.317e^{-1.436t} + 14.078e^{-0.115t} + 6.456$

[ab	e 5.2:	Fitting	model i	for	\mathbf{entire}	bloc	od (lata :	for	each	l sul	oject	Ĵ
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Subject	A2	A3	A4	a1	a2	a3	A1-derived
contr03							
value	73.442	8.798	5.175	2.157	0.420	0.069	228.66
CV	18%	51%	20%	76%	20%	76%	200%
contr05							
value	18.426	7.594	-	0.994	0.092	-	470.663
CV	6%	5%	-	2%	12%	-	2%
donep02							
value	14.536	5.693	-	0.608	0.056	-	171.885
CV	7%	22%	-	3%	33%	-	1%
donep04							
value	15.064	7.492	-	0.937	0.126	0.006	246.162
CV	16%	48%	-	4%	44%	213%	3%
nomed04							
value	28.985	7.131	-	1.983	0.127	-	1737.283
CV	7%	10%	-	6%	12%	-	17%
nomed08							
value	19.671	9.815	-	1.858	0.167	0.002	1085.433
CV	14%	30%	-	7%	36%	345%	18%
rivas01							
value	22.562	9.475	-	1.869	0.169	0.005	1266.857
CV	10%	27%	-	5%	25%	130%	11%
rivas07							
value	14.078	6.456	-	1.436	0.115	-	463.317
CV	5%	5%	-	2%	*Fixed*	-	4%

 Table 5.3:
 Parameters statistics for entire blood AIF

Parameters statistic description is shown in Table 5.3 and Table 5.5. The A1-derived parameters are obtained from the setting of initial conditions of the equations: t_{max} , time of maximum value from which we have data

$\mathbf{Subject}$	Fitting optimization model for corrected blood data
contr03	$ya(t) = 45.126e^{-1.954t} + 8.571e^{-0.551t} + 0.575e^{-0.102*t} + 0.037$
contr05	$ya(t) = 105.99e^{-1.026t} + 2.688e^{-0.117t} + 0.140$
donep 02	$ya(t) = 59.307e^{-0.8t} + 3.108e^{-0.106t} + 0.221$
donep04	$ya(t) = 98.195e^{-1.136tt} + 4.325e^{-0.224t} + 0.639e^{-0.03t}$
nomed04	$ya(t) = 444.006e^{-2t} + 6.555e^{-0.185t} + 0.301$
nomed08	$ya(t) = 288.882e^{-2.017t} + 4.622e^{-0.274t} + 0.717e^{-0.028t}$
rivas 01	$ya(t) = 463.965e^{-1.634t} + 13.131e^{-0.189t} + 2.349e^{-0.017t}$
rivas 07	$ya(t) = 153.376e^{-1.531574t} + 3.65e^{-0.135t} + 0.352$

samples, $y(t_{max})$, sample value at t_{max} . Once the most suitable model have been found, we started the fitting process from time t=0 for each function.

 Table 5.4: Fitting model for blood data corrected for brain/collection site delay and for tracer metabolism for each subject

Subject	A2	A3	A4	a1	a2	a3	A1-derived
contr03							
value	8.571	0.575	0.037	1.954	0.551	0.102	45.126
CV	22%	35%	35%	22%	13%	30%	46%
contr05							
value	2.688	0.140	-	1.026	0.117	-	105.990
CV	11%	28%	-	2%	14%	-	2%
donep02							
value	3.108	0.221	-	0.800	0.106	-	59.307
CV	8%	16%	-	2%	11%	-	1%
donep04							
value	4.325	0.639	-	1.136	0.224	0.030	98.195
CV	17%	63%	-	3%	26%	61%	3%
nomed04							
value	6.555	0.301	-	2.000	0.185	-	444.006
CV	6%	15%	-	4%	6%	-	11%
nomed08							
value	4.622	0.717	-	2.016	0.274	0.028	288.882
CV	14%	39%	-	5%	20%	47%	14%
rivas01							
value	2.349	-	-	1.634	0.189	0.017	463.965
CV	54%	-	-	5%	22%	85%	9%
rivas07							
value	3.650	0.352	-	1.532	0.135	-	153.376
CV	7%	12%	-	2%	10%	-	4%

Table 5.5: Parameters statistics for AIF corrected for brain/collection site delay and for tracer metabolism

From these mathematical description of the data we evaluated the **metabolic component (Cmet)** making a subtraction from entire blood fitted data and the fitted data of blood corrected for brain/collection site delay and for tracer

metabolism. The purpose of this operation was to construct a function of the only blood metabolic component that we used in the following as the second input function requested in our kinetic model.

5.3 Modelling

To describe the dynamics of PET images we had to get from a complex physiological system to a highly simplified compartmental model. The state of art for [11C]-MP4B did not offer a compartmental model that could define the kinetics of the tracer, so the following step was to develop a suitable compartmental model. For this purpose it has been used the compartmental module of software SAAM II. In the following there are some short technical features of the software, useful to have a complete view of how it works.

5.3.1 SAAM II - Compartmental

In the compartmental module, the user can choose from a set of drag and drop model-building icons representing compartments, transfers, and delays to build a visual representation of a system on a drawing canvas. For each icon, attributes can be defined using dialogue boxes such as for the numerical module. Once the structure of the model is built, to simulate the behavior user must create an experiment. Even in this case, by exploiting the various toolbox the user inserts the various specifications related to the experiment, such as the functional form for the input function. Once the user specifies the structure and the inputs and outputs, SAAM II automatically creates the corresponding system of ordinary differential equations and simulates solutions given specific parameter values and input information.

There are two basic numerical computations performed by SAAM II, solving systems of ordinary (linear or nonlinear) differential equations and optimization. Parameters within the model that are not specified by an equation can be estimated by the optimization procedure.

SAAM II provides a choice of three integration methods: the Rosenbrock (the default one), the Runge-Kutta and the Pade integrator. Each integrator has different strengths and weaknesses, which will not influence the correctness of the results but may dramatically influence the running time of a solve or fit operation.

The optimizer is a modification of the Gauss-Newton method that handles the case of multiple data sets, where data sets can differ numerically by several orders of magnitude. The specific formulation of the objective function allows adjustment of the parameters not only to achieve an optimal fit to the data but also (in the case of model, relative weighting) to optimize the variance of the data with respect to the available information. Asymptotic parameter precisions are given by SAAM II for all available weighting schemes (data- or model-based and absolute or relative).

5.3.2 Compartmental Models for [11C]-MP4B

For the formulation of the model it is not possible to give general rules. The proposed model will depend on a number of factors related to physiological knowledge, pathological and pharmacological aspects, by analogy with similar models etc.

To propose a model for [11C]-MP4B kinetics we started from the general structure of a compartmental model for the kinetic study of tracers and we considered the fact that the metabolic component associated to this tracer was very significative and we took into account to include, in our development, a portion of the model that may describe the behaviour of the metabolic products.



Figure 5.7: Model proposed for [11C]-MP4A, Iyo et al (Lancet 349:1805-09, 1997)

We started from the model proposed for [11C]-MP4A (Figure 5.7), published by Iyo et al. (Lancet 349:1805-09, 1997) and we proposed for [11C]-MP4B two alternative suitable models with nearly the same structure: Model A (Figure 5.8) and Model B (Figure 5.9).

Model A

The structure of the model provides, at plasma level, a compartment for the tracer and a compartment for the metabolized product and, at tissue-level, a compartment for the tracer and a compartment for the ligated metabolite. None of the four compartmets distinguishes between free, or specific ligated or non specific ligated elements. Plasma and tissue compartments of the tracer are connected by constant transfer in both directions, those of the metabolite from the only plasma-tissue transfer constant. The two tissue and the two plasma compartments don't exchange substances to each other.

The equations that describe the model are the follows:

$$dC_3(t)/dt = k_{(3,1)}C_1(t)$$
$$dC_4(t)/dt = -k_{(1,2)}C_4(t) + (k_{(2,1)})C_2(t)$$

where the compartment 3 and 4 are respectively the tissue-tracer compartment (C_3) and the tissue-metabolite compartment (C_4) , while compartment 1 and 2 are respectively the plasma-tracer compartment (C_p) and the plasmametabolite compartment (C_{met}) . The transfer costant $k_{(3,1)}$ indicates the transfer rate of the tracer between plasma and tissue, the constants $k_{(4,2)}$ has the same meaning but it refers to the metabolite and the $k_{(2,4)}$ indicates the transfer rate of the metabolite return from tissue to plasma. From now we will refer to compartment 1 as the C_p compartment, to compartment 2 as the C_{met} compartment and the constant transfers $k_{(3,1)}$, $k_{(4,2)}$ and $k_{(2,4)}$ will be refered to as K_1 , k_{1met} and k_{2met} .



Figure 5.8: Model A proposed for [11C]-MP4B

The acquired signal is:

$$C_{VOI}(t) = (1 - V_b)(K_1 \int_0^t C_p(t) + k \mathbf{1}_{met} \int_0^t C_{met}(t) e^{-k\mathbf{2}_{met}(t-\tau)}) + V_b C_b$$

where it was introduced the C_b component of whole blood and the parameter V_B that is a correction for the vascular volume presence.

The parameters that are going to be estimated are K_1 , $k1_{met}$, $k2_{met}$ and V_B

Model B

The structure of the model is the same as the previous, the only difference is the adding of a transfer costant k_2 that quantify the rate of transfer of the [11C]-MP4B tracer from the tissue to the plasma.

The equations that describe the model are the follows:

$$dC_3(t)/dt = -k_{(1,3)}C_3(t) + k_{(3,1)}C_1(t)$$
$$dC_4(t)/dt = -k_{(1,2)}C_4(t) + (k_{(2,1)})C_2(t)$$

where the compartment 3 and 4 are respectively the tissue-tracer compartment (C_3) and the tissue-metabolite compartment (C_4) , while compartment 1 and 2 are respectively the plasma-tracer compartment (C_p) and the plasmametabolite compartment (C_{met}) . The transfer costant $k_{(3,1)}$ indicates the transfer rate of the tracer between plasma and tissue and the $k_{(1,3)}$ indicates the transfer rate of the tracer return from tissue to plasma. Transfer costants $k_{(4,2)}$ and $k_{(2,4)}$ have the same meaning but they refer to the metabolite. From now we will refer to compartment 1 as the C_p compartment, to compartment 2 as the C_{met} compartment and the constant transfers $k_{(3,1)}$, $k_{(1,3)}$, $k_{(4,2)}$ and $k_{(2,4)}$ will be refered to as K_1 , k_2 , k_{1met} and k_{2met} .

The acquired signal is:

$$C_{VOI}(t) = (1 - V_b)(K_1 \int_0^t C_p(t) e^{-k_2(t-\tau)}) + k \mathbf{1}_{met} \int_0^t C_{met}(t) e^{-k\mathbf{2}_{met}(t-\tau)}) + V_b C_b$$



Figure 5.9: Model B proposed for [11C]-MP4B

where C_b and V_B are, as the previous model, the component of whole blood and the correction for the vascular volume presence.

The parameters that are going to be estimated are K_1 , k_2 , k_{1met} , k_{2met} and V_B .

5.4 Cluster Analysis

Cluster analysis, or clustering, is the task of grouping a set of objects in such a way that objects in the same group (called cluster) are more similar (in some sense or another) to each other than to those in other groups [29]. It is a non-supervised classification method, that means that, a priori, you don't know the representative patterns of each class. In general, there are different methods of Cluster Analysis:

- **Connectivity-based clustering** is based on the core idea of objects being more related to nearby objects than to objects farther away. Typically, it uses the simple Euclidean distance and assesses the degree of closeness between clusters according to a rule of connection. It doesn't require the number of desired cluster as input, but require a termination condition, for example, a distance-threshold. It's simple from the implementation point of view but also the most computationally burdensome and it can be applied in imaging area only if the number of pixels is reasonable.
- **Centroid-based clustering** is based on the idea of using, to represent clusters, a central vector, which may not necessarily be a member of the data set. This method is fast and easy, but the number of clusters must be fixed a priori and because of the mean calculation it is sensible to noise and outliers.
- **Distribution-based clustering** is the most closely related to statistics and it is based on distribution models. Clusters can be defined as objects

belonging most likely to the same distribution. While the theoretical foundation of these methods is excellent, they suffer from one key problem known as overfitting, unless constraints are put on the model complexity.

Density-based clustering define clusters as areas of higher density than the remainder of the data set. Objects in these sparse areas - that are required to separate clusters - are usually considered to be noise and border points.

Among various methods we used the central-based clustering. In the next section we will describe this method more in detail and we will show the results of its application to our data.

5.4.1 Centroid-based clustering

Compared to other types of clustering algorithms, centroid-based algorithms are very efficient for clustering large databases and high-dimensional databases. The optimization problem itself is known to be NP-hard, and thus the common approach is to search only for approximate solutions. A particularly well known approximative method is Lloyd's algorithm, often actually referred to as "k-means algorithm" [30].

K-means clustering is a method of cluster analysis which aims to partition n observations into k clusters. The main idea is to define k centroids, one for each cluster. These centroids shoud be placed in a cunning way because of different location causes different result. So, the better choice is to place them as much as possible far away from each other. The next step is to take each point belonging to a given data set and associate it to the nearest centroid. When no point is pending, the first step is completed and an early groupage is done. At this point we need to re-calculate k new centroids as barycenters of the clusters resulting from the previous step. After we have these k new centroids, a new binding has to be done between the same data set points and the nearest new centroid. A loop has been generated. As a result of this loop we may notice that the k centroids change their location step by step until no more changes are done. Finally, this algorithm aims at minimizing an objective function, in this case a squared error function:

$$J = \sum_{j=1}^{k} \sum_{i=1}^{n} \|x_i^{(j)} - c_j\|^2$$

where $||x_i^{(j)} - c_j||^2$ is a chosen distance measure between a data point $x_i^{(j)}$ and the cluster center c_j .

The algorithm pseudo-code:

Inputs:

```
I{=}\{i1,\ldots,1k\ \} instances to be clustered , n number of clusters
```

Outputs:

```
C={c1,...,cn } cluster centroids ,
m: I ---> C cluster membership
```

```
Procedure
          of K-means:
           Set C to initial value (e.g. random selection of I)
           For each i_j in I
              m(i_j)= argmin distance(i_j,c_k)
                 k in {1...n}
           End %end for
           While m has changed
              For each j in \{1...n\}
                 Recompute i_j as the centroid of {i | m(i)=j}
              End %end for
              For each i_j in P
                 m(i_j)= argmin distance(i_j,c_k)
                    k in {1...n}
              End %end for
           End % end while
           Return C
```

K-means is a simple algorithm that has been adapted to many problem domains, by the way it has some problems:

- 1. the number of cluster must be specified before running
- 2. it can be sensitive to initial random choice of centroids
- 3. sometimes it doesn't find the absolute optimum but just a local one
- 4. it doesn't make any use of class information

In addition to these disadvantages, a feature of the k-means is making deterministic assignment type: each measure x_i is assigned to one and only one cluster assignment. A different clustering method, the **Fuzzy-C-means** (FCM), is a "soft" alternative that, instead of making a deterministic decision, considers the possibility for a measure to bind with more than one class.

The objective functions are virtually identical:

$$J = \sum_{j=1}^{k} \sum_{i=1}^{n} p_{nk}^{m} \|x_{i}^{(j)} - c_{j}\|^{2}$$

the only difference is the introduction of a vector p_{nk}^m which expresses the percentage of belonging of a given point to each of the clusters: the *fuzzy index*. This vector is submitted to a "stiffness" exponent aimed at giving more importance to the stronger connections (and conversely at minimizing the weight of weaker ones).

The algorithm is nearly the same, the only difference is that in the Fuzzy-C-means, at every step, they must be recalculated not only the clusters' centroids but also the probabilities. Beacuse of this, in terms of burden FCM is a little bit heavier, but it can take advantages from class information and it will never end on a local minimum.

We applied FCM and k-means to our datasets to get an automatic functional partition of subjects brain areas.

5.4.2 PET images Cluster Analysis for automatic generation of ROI

Cluster Analysis of the Time Activity Curve (TAC) provided the generation of functional ROIs. For each TAC it was made a parametrization of the curve features and then the Cluster Analysis was performed on the following parameters.



Figure 5.10: Simplified version of a Time Activity Curve where the following parameters are highlighted: peak value, tail value, Area Under Curve.

AUC identifies the Area Under Curve of Time Activity Curves (Figure 5.10)

mRise that identifies the slope of the rising phase of Time Activity Curves:

```
for i=1:n
   temp=data(i,:)';
   [y, pos]=max(temp);
   SlopeUP=polyfit(time(1:pos),temp(1:pos),1);
   mrise(i)=SlopeUP(1);
end
```

mWashout1 is a parameter description of the slope of the descent phase of the Time Activity Curve, found like this:

```
n=size(TACs,1);
l=length(time_PET);
m=zeros(n,1);
for i=1:n
   temp=data(i,:)';
   [y, posPeak]=max(temp);
   if posPeak<round(1/3)
      posMean=ceil((1-posPeak)/2);
      SlopeDown2=polyfit(time(posPeak:posMean),temp(posPeak:posMean),1);
      m(i)=SlopeDown1(1);
   else
      m(i)=0;
end
```

mWashout2 is a parameter description of the slope of the descent phase of Time Activity Curves, found like this:

```
n=size(TACs,1);
l=length(time_PET);
m=zeros(n,1);
for i=1:n
   temp=data(i,:)';
   [y, posPeak]=max(temp);
   if posPeak<round(1/2)
      posMean=round((1-posPeak)/2);
      SlopeDown2=polyfit(time(posPeak:posMean),temp(posPeak:posMean),1);
      m(i)=SlopeDown2(1);
   else
      m(i)=0;
end
```

peak identifies the peak value of Time Activity Curves (Figure 5.10)

tail identifies the tail value of Time Activity Curves (Figure 5.10)

peak/tail identifies the ratio between the peak and the tail values of each Time Activity Curve.

We moved, then, to cluster analysis by applying, in sequence, the fuzzy-C-Means and the k-means algorithms.



Figure 5.11: Centroids' trents of subject nomed04

It was possible to choose the parameters from which the clustering was applied, in the case where the carrier parChoice was equal to 0 the program performed the cluster analysis on PET TACs. Results of Clustering are shown in Figure 5.12 and in Figure 5.11.

The first image displays some slices of the patient nomed04 that show the partition of the scalp reaching up to a maximum of 12 clusters. The trends of the centroids of each cluster (dashed line), each with his band of variability (green band), are plotted in Figure 5.11.

Among the 12 clusters identified by the analysis, cortex, cerebellum, thalamic, hypotalamic and caudate nucleus regions are always visible and more or less partitioned into different clusters in each of the 8 subjects (Figure 5.12).



Figure 5.12: Slices of subject nomed04 that shows the clusterization of the scalp

Chapter 6

Results: Region of Interest quantification method

Quantification at Region of Interest level was applied in order to have estimates for unknown parameters K_1 , k_{1met} , k_{2met} and, only for Model B, k_2 . The analysis was performed with SAAM II, whose features and functioning were described in previous chapters.

The decision to perform a first quantification in terms of ROI had a dual purpose: in order to draw early considerations on which of the two proposed models could be the most appropriate to describe the kinetics of the tracer and in order to get first estimates of parameters values that we could use as *prior* in the later Bayesian quantification.

To get the estimates it was necessary to create an experiment, that means entering requested data and specify the equations system that SAAM II doesn't already create itself.

- Forcing Functions (FF) : the fitted function from arterial plasma tracer, C_p and the fitted function obtained for the metabolite C_{met} , have been specified as FF for compartment 1 and 2 and their values inserted in Data box tool.
- Measure equation (s1) : the signal acquired s1 have been associated to the specific data it refers to (ROI1, ROI2, etc) and its equation was made explicit in the SAAM II form:

$$s1 = (1 - Vb) * (q4 + q3) + Vb * lin(Cb)$$

where s1 is the acquired signal, Vb the vascular volume presence, q3 and q4 are the amount of substances in compartment 3 and 4 and Cb the entire blood sampling.

ROI data : data obtained from previous cluster analysis, on which the estimation was performed, were entered into a form compatible with the mode of SAAM II. For each patient and for each ROI, the estimated trends of the 12 centroids were inserted, the weighted were imposed as $SD = \sqrt{d(t_i)}$, (SD 1 in SAAM II), where $d(t_i)$ are the centroids data values. In that cases in which there were physiologically unacceptable values, we chose to impose zero weight, which corresponds to writing (-) in SAAM II.

The quantification returned the parameters values and the relative statistical information: SD, CV, 95% confidence interval, Correlation and Covariance matrix, value of the Objective Function and of the Akaike (AIC) and Schwarz (BIC) indexes.

6.1 Model A

Structure of Model A requires the parameters K1, $k1_{met}$ and $k2_{met}$ to be estimated:

$$C_{VOI}(t) = (1 - V_b)(K_1 \int_0^t C_p(t) + k \mathbf{1}_{met} \int_0^t C_{met}(t) e^{-k\mathbf{2}_{met}(t-\tau)}) + V_b C_b$$

The Table 6.1 shows the parameters values and their related Coefficient of Variation in the 12 ROIs for the 8 subjects.

In 18% of cases V_b cannot be considered valid, both because the software does not lead to a successful result and is not enable to return a value and a statistic description and because its estimated CV are higher than 100%. For the whole group, V_b in Region of Interest 8 is never estimated.

 k_1 shows acceptable values in *contr* and *donep* subjects groups, but for patients of *rivas* and *nomed* groups, the percentage of non estimated parameters riches more than 50% of values. In all subjects k_1 cannot be estimated in ROI 1.

Subject	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8	ROI 9	ROI 10	ROI 11	ROI 12
contr03		•	1		1	1		1				
Vb	0.0127	0.0170	0.0243	0.0269	0.0407	0.0158	0.0126	-	0.0307	0.0467	0.0303	0.0230
CV Vb	647~%	20%	29%	47%	20~%	29%	$81 \ \%$		25%	16%	21%	41%
k1	-	0.1169	0.0255	0.0907	0.1512	0.1871	0.7199	0.7357	0.2864	0.1327	0.2725	0.0953
CV k1		9%	3%	13%	6%	10%	2%	2%	6%	6%	3%	10%
k1met	0.1728	0.0304	0.3549	0.5407	0.1378	0.0408	0.3597	0.4958	0.0938	0.2595	0.2121	0.4052
CV k1met	93%	6%	2%	2%	5%	6%	3%	2%	5%	3%	2%	2%
k2met	0.6487	0.1175	0.2695	0.3335	0.2671	0.1022	0.3158	0.3645	0.1566	0.2802	0.2202	0.3009
CV k2met	79%	9%	3%	3%	7%	10%	4%	3%	8%	3%	3%	3%
contr05												
Vb	0.0330	0.0197	0.0212	0.0163	0.0248	0.0175	0.0252	-	0.0026	0.0073	0.0056	0.0317
CV Vb	9%	16%	10%	14%	7%	12%	19%		241%	69%	91%	11%
k1	0.0397	0.1217	0.0403	0.1163	0.0325	0.0993	0.0231	0.0447	0.3855	0.3356	0.1096	0.0501
CV k1	-	3 %	4%	2%	4%	2%	23%	8%	1%	1%	3%	5%
k1met	0.1878	0.0817	0.3542	0.2443	0.2800	0.1791	0.0324	0.0212	0.2910	0.2331	0.0337	0.1000
CV k1met	1%	3%	1%	1%	1%	1%	8%	10%	2%	2%	20%	3%
k2met	0.1763	0.1544	0.1950	0.1730	0.1805	0.1640	0.1136	0.1451	0.2469	0.2251	0.2931	0.1860
CV k2met	2%	5%	1%	1%	1%	2%	13%	17%	4%	4%	26%	4%
donep02												
Vb	0.0046	0.0070	-	-	0.0141	0.0190	0.0147	0.0130	0.0096	0.0253	0.0038	0.0059
CV Vb	43%	93%			14%	23%	$14 \ \%$	-	$22 \ \%$	12%	279%	60%
k1	0.0720	0.0332	0.1273	0.2678	0.0679	0.0262	0.0289	0.0271	0.0561	0.0351	0.0427	0.0830
CV k1	-	9%	5%	1%	2%	8%	4%	$14 \ \%$	4%	4%	11%	2%
k1met	0.1971	0.3252	0.5105	0.3041	0.1170	0.2353	0.0306	0.0325	0.0393	0.1118	0.4339	0.2881
CV k1met	1%	2%	3%	1%	1%	2%	4%	11%	4%	2%	3%	1%
k2met	0.1710	0.2268	0.2582	0.2365	0.1632	0.2234	0.1378	0.1420	0.1251	0.2065	0.2477	0.1986
CV k2met	1%	3%	4%	2%	2%	2%	5%	16%	6%	3%	4%	2%
donep04												
Vb	0.0148	0.0323	0.0054	0.0078	0.0108	0.0128	0.0187	0.0102	0.0073	0.0247	0.0126	0.0012
CV Vb	14%	13%	73%	32%	12%	$19 \ \%$	$13 \ \%$	-	27%	17%	31%	216%
k1	0.0558	0.0337	0.1510	0.1733	0.0907	0.0685	0.0568	0.0906	0.0324	0.0189	0.0104	-
CV k1	-	7 %	2 %	1%	1%	2 %	3%	1%	7%	14%	19%	
k1met	0.0886	0.1679	0.4040	0.2848	0.2297	0.3403	0.2417	0.1418	0.0354	0.0866	0.0425	0.0056
CV k1met	2%	2%	1%	1%	1%	1%	1%	1%	4%	4%	9%	13%
										Co	ontinued on	next page

6.1 Model A

Subject	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8	ROI 9	ROI 10	ROI 11	ROI 12
k2 met	0.1534	0.1917	0.2299	0.2053	0.1812	0.2203	0.2009	0.1608	0.1207	0.1713	0.2051	0.0425
CV k2met	3%	3%	2%	1%	1%	1%	1%	1%	7%	6%	11%	17%
nomed04												
Vb	0.0403	0.0494	0.0363	0.0285	0.0203	0.0447	-	0.0290	0.0357	0.0359	0.0418	0.0387
CV Vb	15%	$11 \ \%$	29%	41 %	20~%	$14 \ \%$		-	$19 \ \%$	15%	13%	17%
k1	0.0520	0.0096	0.1871	0.1906	0.0451	0.0229	0.0278	0.0281	0.0622	0.0137	0.0100	0.0603
CV k1	-	21%	3%	3 %	7%	10%	16%	13%	5 %	15%	21%	6%
k1met	0.1533	0.2990	0.3795	0.4883	0.0550	0.1469	0.0291	0.0490	0.3545	0.5276	0.4097	0.2460
CV k1met	3%	2%	3%	3%	5%	4%	9%	14%	2%	1%	1%	2%
k2met	0.1920	0.2627	0.2608	0.2921	0.1514	0.2426	0.1227	0.2110	0.2317	0.2879	0.2665	0.2032
CV k2met	4%	2%	4%	4%	8%	5%	14%	18%	3%	2%	2%	3%
nomed08												
Vb	0.0395	0.0120	0.0285	0.0574	0.0559	0.0450	0.0536	0.0583	0.0622	0.0700	0.0836	0.0847
CV Vb	18%	$21 \ \%$	$16 \ \%$	28%	39~%	$12 \ \%$	66%	-	59~%	7%	12%	13%
k1	0.0036	0.0247	0.0558	-	-	0.0729	-	0.0128	-	0.0011	0.2099	0.2664
CV k1	-	$18 \ \%$	$11 \ \%$			$11 \ \%$		44%		457%	5%	4%
k1 met	0.0679	0.0378	0.0700	0.1699	0.3028	0.1218	0.4256	0.2332	0.5133	0.3344	0.3588	0.4342
CV k1met	6%	4%	4%	8%	8%	3%	9%	1%	8%	1%	2%	2%
k2met	0.1481	0.1308	0.1510	0.2316	0.2816	0.1490	0.3067	0.1699	0.3069	0.1895	0.2046	0.2234
CV k2met	10%	7%	7%	8%	8%	5%	10%	2%	9%	2%	4%	4%
rivas01												
Vb	0.0252	0.0167	0.0309	0.0252	0.0135	0.0136	0.0369	0.0193	0.0273	0.0170	0.0224	0.0267
CV Vb	26%	26%	213%	63~%	$490 \ \%$	226~%	335%	-	342%	212%	18%	91%
k1	0.0011	0.0020	-	-	-	-	-	-	-	-	0.0023	-
CV k1	-	72%									79%	
k1 met	0.1098	0.1000	0.6926	0.3731	1.3632	1.0550	1.2349	0.9111	0.9563	0.5851	0.1533	0.3886
CV k1met	8%	5%	30%	8%	13%	7%	34%	20%	31%	12%	3%	16%
k2met	0.1751	0.1617	0.4091	0.2222	0.3681	0.2916	0.4571	0.3352	0.4259	0.2475	0.1492	0.3380
CV k2met	12%	8%	31%	8%	15%	7%	38%	22%	34%	13%	5%	16%
rivas07												
Vb	0.0280	0.0369	0.0244	0.0277	0.0245	0.0134	0.0352	0.0360	0.0261	0.0212	0.0186	0.0357
CV Vb	17 %	40%	$25 \ \%$	85%	45%	$18 \ \%$	20%	-	51 %	157%	18%	21%
k1	0.0060	-	-	-	-	0.0076	0.0509	0.0386	-	-	0.0067	-
CV k1	-					32~%	$10 \ \%$	15%			59%	
k1 met	0.1237	0.2338	0.2186	0.3470	0.1020	0.0435	0.3827	0.4973	0.3242	0.4645	0.0746	0.1407
CV k1met	2%	6%	2%	7%	11%	3%	2%	2%	3%	8%	3%	4%
k2met	0.1067	0.1902	0.1310	0.2088	0.2305	0.1146	0.1664	0.1887	0.1608	0.2315	0.1039	0.1715
CV k2met	4%	6%	2%	7%	11%	6%	3%	3%	4%	8%	5%	4%

Table 6.1: Results of estimation at ROI level of Model A for whole subjects

In Table 6.2 are displayed the Akaike indexes of the estimates of each ROI, that must be interpreted in their absolute value.

AIC indexes are higher in *rivas* group, immediately followed by *nomed* group and then *contr* and *donep* groups.

AIC	contr03	$\operatorname{contr}05$	donep02	donep04	nomed04	nomed08	rivas01	rivas07
ROI 1	2.1927	2.5462	-0.1557	-0.3525	0.6885	0.5575	0.8316	0.4387
ROI 2	-0.3077	0.4265	0.8992	0.2913	0.5073	-0.2980	0.2862	1.4294
ROI 3	0.1912	-0.0437	1.5888	0.2836	1.2575	0.1989	2.9737	0.7326
ROI 4	0.8319	0.1636	0.4422	-0.1385	1.3080	1.2946	1.7535	1.9286
ROI 5	0.2547	-0.1867	-0.2222	-0.7908	0.4187	1.5830	3.0792	1.0392
ROI 6	0.1745	0.0745	0.4549	-0.1083	0.5943	0.4019	2.3958	-0.2640
ROI 7	0.5926	0.7630	-0.4274	-0.2304	0.6254	2.0574	3.5846	0.8280
ROI 8	0.5820	0.5639	0.7480	-0.6470	0.9208	0.2050	3.1857	1.0587
ROI 9	0.5578	1.1495	-0.0847	-0.3118	0.8260	2.0858	3.3480	1.4620
$ROI \ 10$	0.3152	0.9013	0.0320	0.2549	0.5130	0.1686	2.6087	2.2570
ROI 11	0.2583	0.8551	1.3269	0.1344	0.5023	1.0435	0.4577	0.0835
ROI 12	0.4041	0.4436	0.3674	-0.2171	0.8256	1.0460	2.0033	0.7508

Table 6.2: AIC indexes of the 12 ROIs for the estimation of Model A for all subjects

6.2 Model B

Structure of Model B requires the parameters K1, k2, $k1_{met}$ and $k2_{met}$ to be estimated:

$$C_{VOI}(t) = (1 - V_b)(K_1 \int_0^t C_p(t) e^{-k_2(t-\tau)} + k \mathbf{1}_{met} \int_0^t C_{met}(t) e^{-k_2 met(t-\tau)}) + V_b C_b$$

The Table 6.3 shows the parameters values and their related Coefficient of Variation in the 12 ROIs for the 8 subjects.

In 21% of cases V_b cannot be considered valid, because the software does not lead to a successful result or because its estimated CV are higher than 100%. For the whole group, V_b in Region of Interest 1 is very low.

Subject	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8	ROI 9	ROI 10	ROI 11	ROI 12
contr03									•			•
Vb	-	0.0072	0.0233	0.0269	0.0314	0.0031	0.0036	-	0.0162	0.0430	0.0235	0.0229
CV Vb		13%	32%	48%	17%	37%	198%		14%	17%	19%	-
k1	-	0.2767	0.2697	0.0906	0.2609	0.4443	0.8517	0.7996	0.5449	0.1790	0.3921	0.0953
CV k1		2%	12%	13%	7%	1%	3%	4%	2%	16%	6%	11%
k2	-	0.0105	0.0009	-	0.0092	0.0100	0.0033	0.0016	0.0091	0.0050	0.0058	-
CV k2		3%	210%		14%	2%	19%	51%	4%	51%	17%	
k1met	_	0.0262	0.3550	0.5407	0.1427	0.0291	0.3634	0.4958	0.0849	0.2607	0.2091	0.4053
CV k1met		5%	2%	3%	4%	6%	2%	2%	3%	3%	2%	2%
k2met	-	0.3620	0.2728	0.3338	0.3471	0.3594	0.3606	0.3804	0.2763	0.2969	0.2512	0.3011
CV k2met		7%	4%	3 %	6%	9%	4%	4%	5%	5%	4%	3%
contr05			-,,,	- / 0	- / 0	- , 0		-, -	- , ,	- / 0		- / 0
Vh	_	0.0151	0.0211	0.0134	0.0242	0.0147	0.0204	-	_	0.0031	0.0051	0.0271
CV Vb		10 %	10 %	11%	8%	9%	26~%			124%	107%	8%
k1	0.0729	0.1712	0.0413	0.1488	0.0388	0.1310	0.0718	0.0646	0.4191	0.3757	0.1168	0.0878
CV k1	10%	3 %	15 %	4%	16 %	4%	23 %	17%	3%	3%	9%	6%
k2	0.0082	0.0048	0 0003	0.0036	0.0026	0 0039	0.0119	0.0052	0.0015	0.0020	0.0013	0.0080
$\frac{m2}{CV k2}$	16%	9 %	747 %	15%	86 %	14 %	25 %	51%	37%	24%	125%	12%
k1met	0 1830	0.0737	0 3541	0.2387	0 2790	0.1735	0.0242	0.0172	0.2872	0.2285	0.0326	0 0964
CV k1met	1%	2%	1%	1%	1%	1%	21%	21%	2%	2%	24%	2%
k2met	0 1921	0 2043	0 1953	0 1839	0 1825	0 1769	0 21 / 0	0 2192	0.2666	0 2510	0 3321	0.2304
CV k2met	2%	4%	1%	1%	1%	2%	40%	38%	4%	4%	39%	4%
donep02	270	170	170	170	170	270	1070	0070	170	170	5070	170
Vh	_	0.0070	_	_	0.0124	0.0190	0.0131	0.0086	0.0050	0.0242	0.0036	0,0059
CV Vh		95%			14 %	24%	16%	67%	17%	13%	298%	62%
k1	0.0720	0.0332	0.1273	0.2798	0.0867	0 0261	0.0437	0.0615	0 1046	0 0 4 4 0	0.0428	0.0830
CV k1	2%	9 %	5 %	4 %	8%	8 %	17%	24 %	3%	17%	11%	3%
k2	270			0 0008	0.0038		0.0059	0.0117	0 0088	0.0037	11/0	0/0
CV k2				78 %	33%		41 %	34 %	5%	72%		
k1met	0 1970	0 3252	0.5105	0 30 23	0 1137	0 2353	0.0278	0 0 2 9 3	0.0313	0 1112	0 4341	0.2881
CV k1met	1 %	20202	3%	1%	2%	2000	7%	23%	3%	3%	3%	1%
k2met	0 1708	0.2268	0.2582	0.2428	0 1759	0.2234	0 1673	0.2687	0.2320	0.2177	0.2480	0 1986
CV k2met	1%	3%	4%	3%	3%	0.2204 2%	12%	44%	6%	5%	4%	2%
donen04	170	570	470	070	070	270	1270	4470	070	570	470	270
Vh	_	0.0290	0.0055	0.0070	0.0108	0.0127	0.0185	0.0087	0.0029	0.0184	0.0113	0.0003
CV Vh		14%	74%	38%	12%	19%	14 %	16%	43%	18%	37%	3133%
k1	0.0873	0.0574	0 1510	0 1794	0.0907	0.0685	0.0589	0 1044	0.0720	0.0636	0 0195	0 0072
CV k1	5 %	20%	0.1010	4%	1%	0.0000 9 %	12 %	5%	6%	14%	48%	288%
k9	0 0058	0 0073	2/0	10005	1/0	2 70	0/ 41 0000 0	0.0020	0.00	0 0 1 4 5	0 0 0 0 0	0.0165
CV k9	10.000	250%	=	1170%	-	-	0.0000 20.20%	260%	Q.0032	110%	790%	30.40%
k1mot	10 /0	0 1631	0.4040	111/0	0.9907	0.3405	⊿90/0 0.9419	0 1370	0/0 0.09/9		1470 0.0410	0 0033
CV k1met	0.0001	20% 20%	10%	0.2000 10%	107	10%	107	10%	0.0242 70%	50%	110%	0.0033
k2mot	470 0.1896	0/0 0.9100	1/0 0.2200	1/0 0.20.82	1/0 0 1819	1/0	1/0 0.2021	1/0	1/0 0.2082	0.0386	11/0	∠0070 03089
CV k2mot	0.1020 90%	0.2100 g0%	0.2290 n0%	0.∠000 ∩0%	107	107.	0.2021 ე0%	0,1000 00%	1.2002	0.2300	0.2004	0.0200
UV KZIIIEL	J/0	J /0	4 /0	4 /0	T \0	1/0	4 /0	4 /0	1 4 /0	970	∠ 1 70	-

6.2 Model B

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CV k2met 3% 2% 4% 3% 6% 3% 46% 25% 2% 2% 2% 2 nomed08 - 0.0092 0.0247 0.0454 0.0617 0.0343 0.0489 0.0404 0.0554 0.0474 0.0585 0.056 CV Vb 23% 16% 23% 6% 14% 9% 9% 9% 10% 8% 100 k1 0.1310 0.0766 0.1202 0.1720 0.2795 0.4030 1.3709 0.7882 1.6441 1.1252 1.2472 1.513 CV k1 6% 16% 15% 16% 7% 1% 2% 1% 1% 1% 1%
nomed08 - 0.0092 0.0247 0.0454 0.0617 0.0343 0.0489 0.0404 0.0554 0.0474 0.0585 0.056 CV Vb 23% 16% 23% 6% 14% 9% 9% 9% 10% 8% 10° k1 0.1310 0.0766 0.1202 0.1720 0.2795 0.4030 1.3709 0.7882 1.6441 1.1252 1.2472 1.513 CV k1 6% 16% 15% 16% 7% 1% 2% 1% 1% 1% 1%
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CV Vb 23% 16% 23% 6% 14% 9% 9% 9% 10% 8% 10 k1 0.1310 0.0766 0.1202 0.1720 0.2795 0.4030 1.3709 0.7882 1.6441 1.1252 1.2472 1.513 CV k1 6% 16% 15% 16% 7% 1% 2% 1% 1% 1% 1%
k10.13100.07660.12020.17200.27950.40301.37090.78821.64411.12521.24721.515 CV k16 %16 %16 %15 %16 %7 %1 %2 %1 %1 %1 %
CV k1 6 % 16 % 16 % 15 % 16 % 7 % 1 % 2 % 1 % 1 % 1 % 1 %
k2 0.0206 0.0105 0.0083 0.0315 0.1228 0.1254 0.1230 0.1078 0.1295 0.1152 0.1558 0.170
CV k2 4 % 15 % 20 % 7 % 18% 20% 2% 7% 2% 5% 5% 4'
k1met 0.0591 0.0282 0.0589 0.1496 0.2069 0.0470 0.0217 0.0402 0.0299 0.0457 0.1025 0.120
CV k1met 9% 10% 8% 10% 7% 20% 8% 13% 7% 10% 4% 3
k2met 0.3803 0.1896 0.1999 0.3651 0.2212 0.0417 0.0145 0.0304 0.0183 0.0276 0.0383 0.038
CV k2met 13% 15% 13% 15% 8% 22% 18% 16% 14% 13% 5% 4
rivas01
Vb - 0.0082 0.0139 0.0214 - 0.0054 0.0012 0.0080 0.0050 0.0116 0.0140 0.023
CV Vb 29 % 28% 12% 98% 380 % 38% 66% 21% 18% 22'
k1 0.0287 0.0535 0.2338 0.0831 0.5686 0.3438 0.4487 0.2998 0.3428 0.1750 0.0682 0.085
CV k1 24 % 9% 2% 4% 6 % 10 % 1 % 1% 1% 2% 14% 5
k2 0.0238 0.3578 0.1017 0.0488 0.2041 0.2092 0.1165 0.0897 0.1056 0.0622 0.2261 0.078
CV k2 13 % 22% 2% 4% 2 % 4 % 2 % 2% 1% 3% 29% 6
k1met 0.0983 0.0603 0.3117 0.2502 0.4036 0.4958 0.4889 0.3968 0.3793 0.3020 0.0859 0.254
CV k1 met 17% 10% 5% 3% 10% 8% 4% 3% 4% 2% 21% 6
k2met 0.3472 0.0944 0.3644 0.2414 0.1477 0.1628 0.3785 0.2695 0.3564 0.2334 0.0846 0.345
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rivas07
Vb - 0.0322 0.0176 0.0256 0.0198 0.0090 0.0259 0.0252 0.0218 0.0209 0.0110 0.025
CV Vb 13 % 13 % 17 % 56 % 18 % 18 % 28 % 16 % 26 % 16 % 18 %
k1 0.1674 0.1698 0.1719 0.2457 0.0515 0.0604 0.1553 0.1446 0.2073 0.3248 0.1149 0.107
CV k1 6 % 7 % 8% 5% 44 % 9 % 12 % 16% 8% 4% 6% 11
k2 0.0204 0.0455 0.0282 0.0570 0.0408 0.0180 0.0136 0.0164 0.0402 0.0665 0.0194 0.035
CV k2 3 % 4% 2% 5 % 19 % 6 % 10 % 11% 4% 5% 3% 4
k1met 0.0749 0.1721 0.1584 0.2454 0.0931 0.0299 0.3579 0.4753 0.2436 0.3236 0.0436 0.118
$ CV k1 met \begin{vmatrix} 5\% & 3\% & 3\% & 3\% & 18\% & 7\% & 2\% & 2\% & 3\% & 6\% & 5' \end{vmatrix}$
k2met 0.1908 0.2271 0.1624 0.2209 0.3218 0.2205 0.1965 0.2193 0.1774 0.2343 0.2139 0.277
CV k2met 8% 6% 4% 29% 13% 3% 4% 4% 4% 11% 9

Table 6.3: Results of estimation at ROI level of Model B for whole subjects

Parameter k_1 has always good estimates, in each ROI. For patient nomed08 it assumes very high values in Region of Interest 7, 9, 10, 11, 12.

 k_2 shows very low values in patients of *contr* and *donep* groups, and for these subjects the percentage of non estimated, or with high CV, parameters riches the 50% of values. In *nomed* and *rivas* groups, k_2 assumes significant values with good CV.

In Table 6.4 are displayed the Akaike indexes relative to estimates of each ROI, that must be interpreted in their absolute value. AIC indexes have nearly the same values in all subjects.

Comparing the Akaike indices of the two models we can see that, overall, the values for Model B are 50% lower than those of model A and the difference

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AIC	contr03	$\operatorname{contr}05$	donep02	donep04	nomed04	nomed08	rivas01	rivas07
ROI 1		-0.1025	-0.1084	-1.0531	-0.2970	-0.3282	0.7045	-0.3481
ROI2	-1.7925	-0.3851	0.9447	0.2193	-0.0960	-0.4262	-0.5062	1.4407
ROI 3	0.2305	0.0017	1.6342	0.3291	0.6779	0.0089	0.1883	-0.3511
ROI 4	0.8785	-0.3792	0.4422	-0.1121	0.6813	0.7704	-0.0948	0.2303
ROI 5	-0.2219	-0.1732	-0.3411	-0.7456	-0.7868	-0.0900	0.7048	0.9710
ROI 6	-1.3947	-0.4547	0.5003	-0.1998	-0.5154	0.1807	0.4886	-0.7858
ROI 7	0.2048	0.7501	-0.4821	-0.1886	0.5715	-0.0088	0.3572	0.3562
ROI 8	0.5339	0.5422	0.7363	-0.7686	0.4475	-0.0559	0.0632	0.6254
ROI 9	-0.7915	1.0231	-1.1487	-0.9360	0.1622	0.1476	0.0451	0.0581
$ROI \ 10$	0.2847	0.6077	0.0358	-0.0996	0.4949	0.1128	-0.0357	0.4364
ROI 11	-0.1355	0.8783	1.3724	0.1468	0.1760	0.3000	-0.1502	-0.6540
ROI 12	0.4496	-0.1253	0.4128	-0.1732	-0.0912	0.2647	0.3647	0.1472

so marked is given by the indices of the *rivas* and *nomed* groups, extremely high for the model A.

Table 6.4: AIC indexes of the 12 ROIs for the estimation of Model B for all subjects

Chapter 7

Results: Voxel - based quantification method

The voxel-based quantification process main advantage is to maintain the original resolution of the PET images during the evaluation of parameters. By the way, the disadvantages are not negligible: first of all the signal-noise ratio (SNR) is worse than that measured for the ROI and then, having a lot of data to be analyzed, it is necessary to use fast algorithm for their identification. The gold-standard quantification method, Weighted Non Linear Least Square, it's not applicable in this context because the computational burden would require processing time clinically unacceptable.

The Basis Function Method stands as an alternative to a non-linear quantification method. As we described on Chapter 4, this method switches from a nonlinear parameter dependence to a linear parameter dependence model.

The equations of our two models both have one or two parameters related to the kinetics with a non-linear relationship.

Model A:

$$C_{VOI}(t) = (1 - V_b)(K_1 \int_0^t C_p(t) + k \mathbf{1}_{met} \int_0^t C_{met}(t) e^{-k\mathbf{2}_{met}(t-\tau)} + V_b C_b$$

where $k2_{met}$ dependence is nonlinear:

$$\int_0^t C_{met}(t) e^{-k2_{met}(t-\tau)}$$

Model B:

$$C_{VOI}(t) = (1 - V_b)(K_1 \int_0^t C_p(t) e^{-k_2(t-\tau)} + k \mathbf{1}_{met} \int_0^t C_{met}(t) e^{-k\mathbf{2}_{met}(t-\tau)} + V_b C_b$$

where k_2 and k_{2met} dependencies are nonlinear:

$$\int_0^t C_p(t) e^{-k_2(t-\tau)} \quad , \quad \int_0^t C_{met}(t) e^{-k_{2met}(t-\tau)}$$

For those non-linear components, next step will be the evaluation of the Basis Functions through which we will provide the parameters estimates using Linear Least Squares.

7.1 Parameters grids

The development of the Basis Function starts from the construction of the grids for the parameters k2 and k2met. This is the most delicate step of the whole procedure and what is the most appropriate grid is still an open question that must be evaluated case by case. During the construction phase of the grids we must take into account two critical issues: the number of elements of the grid, which is closely related to the computational burden of the algorithm and the accuracy of the final estimates, and the upper and lower extremes of the grid within which it makes sense to assume that the parameter can take value.

Starting from two different model structures, that requires different features, we have chosen to split the problem in a way to create ad hoc grids for each of the two cases in analysis.

7.1.1 Model A: $k2_{met}$ parameter grid

The application of the BFM to Model A, having this model a unique nonlinear component to elude, requires a computational burden linearly proportional to the number of elements of the grids. We chose to dimension the grid with N = 300 elements, a fairly large number, which will allow us a good accuracy in the precision of the estimates. The range of values of the grid must be chosen in order to cover all the possible values that the parameter could assume. Based on the results obtained from ROI-level k2met estimates, we decided to fix the lower edge to 0 and the upper edge to 1. Grid was developed in MATLAB :

```
%Construction of k2_met grid from 0 to 1, N=300
grid=logspace(log10(0.008),log10(1),299)';
k2met_gridA(:,1)=[0; grid];
```

Results from ROI-based quantification return that a little less than 50% of values were included from 0 and 0.2, another little less than 50% of values were included from 0.2 and 0.5 and the remaining 1% of values was higher than 0.5. Therefore, we chose a distribution of logarithmic type in order to have elements more dense at lower values and elements more widely spaced at higher values, as we can see in Table 7.1: the more the value is high the more the gap from previous element is big.

value	0	0.0126	0.0205	0.0333	0.0541	0.0880	0.1431	0.2327	0.3783	0.6150	1
index	1	30	60	90	120	150	180	210	240	270	300
		_		1 0 · ·	1 6 1 0	. 1	. 1		0.0		

Table 7.1: Grid of k2met values taken 1 every 30

7.1.2 Model B: k_2 and k_{2met} parameters grids

The construction of parametric grids for Model B required a more delicate process. The structure of the model provides, in fact, two parameters with non-linear dependence, this implies that if each grid is composed of M values, the number of iterations that the algorithm will have to make are at least M x M. At this point, it becomes crucial to ease the burden of the algorithm building grids with a contained number of elements. However, it is equally important that the grids could be able to adequately describe all the possible values that the parameters could take.

We decided to act through two different paths: the first provided that the grids were structured with a few values wisely chosen from previous quantifications at ROI-level, and the second sacrificed the burden of the algorithm in favor of a wider description of the range of possible values.

ROI grids

Starting from the estimates obtained from the cluster analysis we developed the grids for the parameters k2 and k2met. The first set of grids was constructed for each ROI of each patient, while the second was constructed from the average of the values of the 12 ROIs of each patient, both in terms of value of the parameter and in terms of CV. In the following we will describe how both groups of grids were built.

Grids constructed on each ROI In order to contain the burden of the algorithm we chose to size grids of k_2 and k_{2met} both with 20 values each, suitably constructed on the basis of ROI-level quantification.



Figure 7.1: Example of development of a grid for parameters k2 and k2met from ROI 1 for patient *nomed*04

For each subject and for each ROI we took the estimated value and the Coefficient of Variance obtained from ROI-based estimation and we get the relative Standard Deviation. We derived the lower and upper limits of the range as $[ROIvalue \pm 2SD]$ and we divided it into 20 equally spaced elements (Figure 7.1).

Grid constructed on ROIs mean For each subject we calculated the related grid through the average of the 12 parameters values of the 12 ROIs. Upper and lower extremes were calculated by adding 3meanSD and subtracting 3meanSD from the average value already found: $[meanROIvalue \pm 3meanSD]$. The meanSD was in turn calculated as the mean of the 12 Standard Deviations of each ROI for the parameter in exam.

In this case we chose to size both grids with 30 elements each, considering that the range values would be more extended than in the previous case, but trying anyway not to burden the algorithm.

Extended grids

The extended grids are constructed as an alternative to those developed on ROI. These grids, in fact, sacrifice the computational burden for greater accuracy in the estimates. Each grid is composed of 90 elements spacing between a wide range.

The same grid, covering a large range of values, was used to calculate the Basis Functions indistinctly for each ROI and for each patient.

7.2 Model A

The development of Basis Functions, once you have the parameters grids, is the next step. In the case of Model A, the functions to be estimated are those derived from the portion of non-linear kinetics for the parameter $k2_{met}$. Considering that the grid has N=300 elements, the Basis Function for the i-th element of the grid has the form:

$$BF_{i}(t) = C_{met}(t) \otimes e^{-(k2_{met})_{i}t}$$
 $i = 1, ..., N$

and, always for the i-th element, the equations of the predicting model becomes:

$$C_{VOI}(t) = (1 - V_b)(k_1 \int_0^t C_p(t) + k \mathbf{1}_{met} BF_i(t)) + V_b C_b$$

We can now define, given M the number of PET scans:

$$A_{i} = \begin{bmatrix} C_{p}(t_{1}) & BF_{i}(t_{1}) & C_{b}(t_{1}) \\ C_{p}(t_{2}) & BF_{i}(t_{2}) & C_{b}(t_{2}) \\ \vdots & \vdots & \vdots \\ C_{p}(t_{M}) & BF_{i}(t_{M}) & C_{b}(t_{M}) \end{bmatrix} \in \Re^{Mx2}$$

$$par = \begin{bmatrix} (1-V_b)k_1\\ (1-V_b)k_1_{met}\\ V_b \end{bmatrix} \in \Re^{3x1} \quad C_{pred} = \begin{bmatrix} C_{pred}(t_1)\\ C_{pred}(t_2)\\ \vdots\\ C_{pred}(t_M) \end{bmatrix} \in \Re^{Mx1}$$

The equation that relates the three components is:

$$C_{pred} = A_i par$$

And through the Weighted Linear Least Square we achieve the parameters estimation:

$$p\hat{a}r = [A_i^T W A_i]^{-1} A_i^T W C_{pred}$$

where the $W = diag(w) \in \Re^{MxM}$ is the diagonal matrix of weight described further.

The procedure is as follows:

For
$$i = 1, ..., N$$

 $k2_{met} = (k2_{met})_i$, $BF = BF_i$
 $\begin{bmatrix} ((1 - V_b)k_1)_i \\ ((1 - V_b)k_1_{met})_i \\ (V_b)_i \end{bmatrix} = [A_i^T W A_i]^{-1} A_i^T W C_{pred}$
 $WRSS_i = \sum_{k=1}^M w_{kk} [C_{VOI}(t_k) - C_{pred}(t_k)]^2$

end

The final optimal estimation of the parameters is obtained by:

$$\begin{bmatrix} ((1-\hat{V}_b)k_1)_i \\ ((1-\hat{V}_b)k_{1_{met}})_i \\ (\hat{V}_b)_i \end{bmatrix} = minWRSS_i$$

The data weight was managed through the relative weights. The use of relative weights instead of absolute ones, did not affect the estimation of the parameters, however it was important to get the absolute weights to know the precision of the estimates. Since these weights were unknown, we proceeded in this way:

1. We build the diagonal matrix of the weight on centroids values:

Sigma=diag(sqrt(mean_centroids./scan_sec));

2. Negative values have been imposed with zero weight:

```
inv_Sigma=(Sigma)^-1;
inv_Sigma(find(Cvoi<0),find(Cvoi<0))=0;</pre>
```

- 3. Estimates where then calculate through WLLS
- 4. The new Sigma, which provides the precision of the estimates, has been calculated:

```
data_number=sum(diag(inv_Sigma>0));
gamma=WRSS(optimal_index)/(data_number-par_number);
Sigmanew=gamma.*Sigma;
Sigmap=(GGi'*(Sigmanew^-1)*GGi)^-1;
```

where the GG_i corresponds to the previously defined A_i at optimal index

7.2.1 Results Model A

We applied the Basis Functions Methods at Model A for each patient.

For what concern the statistics of the obtained results, we evaluated the number of acceptable estimations and for the valid ones we calculated the mean value, the maximum and the minimum, of parameters V_b , k_1 and k_{1met} . For the statistic of parameter Weighted Residuals Sum Squares (WRSS) we only evaluated the mean value.

In Table 7.2 are shown the results for each subject, fields *Estimates TOT* and *Estimates valid* represent the number of estimations that the program calculated and the number of which we considered valid in terms of physiologically acceptable values (non negative) and statistic variance (CV < 200%).

Subject	k1	k1met	Vb	WRSS	CV k1	CV k1met	CV Vb	Estimates	Estimates
					<200%	<200%	<200%	101	
contr03									
mean	0.1988	0.2271	0.0803	159	93135	111932	69123	117233	54293
\max	12.102	0.9229	0.4934						
\min	0.0024	0.0003	0.0086						
contr05									
mean	0.0777	0.1499	0.0449	328	76121	102850	79611	107083	53057
\max	0.5467	0.7834	0.3797						
\min	0.0018	0.0002	0.0031						
donep02									
mean	0.0567	0.1557	0.05	311	91042	124135	96910	129578	62940
\max	0.4161	0.8316	0.5209						
\min	0.0019	0.0002	0.0028						
donep04									
mean	0.0626	0.1634	0.0479	200	74527	98832	81286	106335	55514
\max	1.022	65.683	1.3932						
\min	0.0004	0.0002	0.002						
nomed04									
mean	0.0537	0.2018	0.0528	278	80086	118402	96771	121078	61482
\max	0.3443	0.8822	0.4852						
\min	0.0026	0.0004	0.0029						
nomed08									
mean	0.0928	0.1683	0.0622	163	42817	93733	73612	101674	27156
\max	0.5439	0.9787	0.386						
\min	0.0017	0.0003	0.0045						
rivas01									
mean	0.0235	0.1143	0.0408	238	3925	104231	113527	123675	3203
\max	0.0681	0.9568	0.289						
\min	0.0032	0.0002	0.0019						
rivas07									
mean	0.0389	0.1524	0.0432	176	40273	100755	77465	111112	25090
\max	0.2058	0.6327	0.3887						
min	0.0037	0.0008	0.0032						

Table 7.2: Statistics of the estimated parameters for Model A

At least half of the estimates are not acceptable. In particular for patients treated with *rivastigmine* percentage of acceptable estimates is extremely lower than that of the estimates to reject. The drastic reduction in the number of acceptable estimates is, as can be noted, due to the difficulty of estimating the parameter k1, particularly for patients in the group of *nomed* and *rivas*. To report the maximum value of k_{1met} of subject *donep04* absolutely in contrast with the habitual values of kinetic parameters, probably an outlier.

In the next pages are reported the histograms of the distribution of the values of optimal k_{2met} in each subject (Figure 7.2).

The distribution of the indices values shows that many of the optimal k2met fall within the range [0.04 - 0.4] and another good part of them within [0.025 - 0.035]. In particular, patients treated with *rivastigmine* manifest a very high peak of optimal k2met on the lower values range, however the majority of these values will not be taken into consideration by the program because it will provide inconsistent estimates.

Kinetic cerebral trends of indices k_1 , k_{1met} , k_{2met} , V_b and Vt_{met} , where Vt_{met} is the Distribution Volume of the metabolite, are shown in Figures 7.3, 7.4, 7.5.

The free tracer and the metabolite enter the brain first from the area of the cerebellum after which, while the metabolite diffuses freely in all brain areas, the tracer remains confined within this zone. Figure 7.3 and Figure 7.4 show this behavior for the subjects in which the amount of acceptable estimates allowed a qualitative and quantitative analysis of the performance of the parameters: contr03, contr05, donep02, donep04.

In subjects from *contr* group the tracer can also spread in thalamic areas while in those belonging to *donep* group the signal coming from thalamic areas is very low and therefor not significative, as shown in Figure 7.5. As previously said, the remaining groups are difficult to examine because valid results are poor.

The metabolite presents significant k_{1met} values in all brain regions and moreover those values are proportional to the ones of k_{2met} , therefore, in turn, significant.

Consequently Vt_{met} is uniform throughout the scalp. The values of V_b are maintained for the most part within physiologically acceptable values for all patients of which it is possible to provide a visual interpretation of the performance.

These results are in agreement with those obtained from the ROI analysis for the model A.

In subjects *nomed*04, *nomed*08, *rivas*01, *rivas*07 the number of acceptable estimations was very low and it was not possible to make some considerations about the kinetic trends of parameters.



Figure 7.2: Histograms of distribution of k2met for all subjects



(b) Parameter trends for contr05.

Figure 7.3: Visualization of tracer trend in cerebellum for subject contr03 and contr05







Figure 7.4: Visualization of tracer trend in cerebellum for subject donep02 and donep04



(b) Parameters trends for donep02.

Figure 7.5: Visualization of tracer trend in thalamic areas for subject contr03 and donep02

7.3 Model A Bayesian estimation: MAP

We then applied the Bayesian approach for parameter estimation of the Model A. The starting point of this approach is based on the fact that, even before estimation of the parameters, we have expectations for them, that is information, summarized in the *a priori* probability density:

 $f_p(p)$

These expectations are changed after seeing the data z, therefore we speak of the *a posteriori* probability density, conditioned by z:

$$f_{p|z}(p|z)$$

This is the function key of Bayesian estimation, from that we get estimates and confidence intervals.

A Bayesian estimator is the *Maximum a Posteriori* (MAP).

Defined z the data, Σ_v weights matrix, G(p) the model matrix defined as A_i in Section 7.2, Σ_p the covariance matrix of the *prior* information and eventually μ_p the *a priori* information vector, the estimations were calculated through:

$$\hat{p_{map}} = (G^T \Sigma_v G + \Sigma_p^{-1})(G^T \Sigma_v z + \Sigma_p^{-1} \mu_p)$$

 Σ_p is a square matrix that has on the diagonal the standard deviation of the *a priori* information:

$$\Sigma_{p} = \begin{bmatrix} SD_{k_{1}} & 0 & 0\\ 0 & SD_{k_{1_{met}}} & 0\\ 0 & 0 & SD_{V_{b}} \end{bmatrix}$$
$$u_{p} = \begin{bmatrix} k_{1_{prior}}(1 - Vb_{prior}) & k_{1_{met_{prior}}}(1 - Vb_{prior}) & Vb_{prior} \end{bmatrix}$$

We built our μ_p and Σ_p through the results from ROI-based analysis and then we applied the MAP Estimation to our data.

Summarizing, this method solves the compartmental model with the following procedure: model is linearized through the Basis Function Method and then solved using a linear Maximum A Posteriori (MAP) estimator, priors were previously automatically generated from the data, using the estimates obtained solving the compartmental model at the region level (functional clustering).

7.3.1 Results MAP

Bayesian estimation has been applied only to model A, in order to see if, providing a *prior* information, the results of the estimation improved, especially for those patients in which the valid estimates were few.

The overall results are given in Table 7.3. The number of valid estimates, *Estimates TOT*, increased for all patients, especially for those in which we found some difficulties in the previous model. By the way, for subject rivas01 number of not acceptable estimates is nearly the 80%, and the parameter whose estimation hardly depress this number is k_1 .

Subject	k1	k1met	Vb	WRSS	CV k1	CV k1met	CV Vb	Estimates	Estimates
					<200%	<200%	<200%	101	valid
contr03									
mean	0.2065	0.3277	0.0733	234	97990	93515	45707	117233	43149
max	0.4912	0.6769	0.3779						
\min	0.0049	$< 0.1 e^{-006}$	0.0134						
contr05					1				
mean	0.1132	0.1628	0.0405	392	75657	74757	63222	107083	56445
\max	0.557	0.749	0.2908						
\min	0.0029	$< 0.1 e^{-006}$	0.0048						
donep02									
mean	0.0995	0.1656	0.0418	382	99867	108099	86701	129578	72210
max	0.3772	0.7	0.5002						
\min	0.0059	$< 0.1e^{-006}$	0.0047						
donep04									
mean	0.0991	0.1127	0.0425	227	93928	65932	78037	106335	73599
\max	0.3373	0.6178	0.2533						
\min	0.0036	$< 0.1e^{-006}$	0.0049						
nomed04									
mean	0.0913	0.2078	0.0518	345	100139	81569	94541	121078	88495
max	0.3031	0.8577	0.3531						
min	0.002	$< 0.1 e^{-006}$	0.0036						
nomed08									
mean	0.1627	0.2283	0.0549	305	67417	83316	68094	101674	44333
\max	0.5424	0.9782	0.2913						
\min	0.0089	$< 0.1 e^{-006}$	0.008						
rivas01					1				
mean	0.0409	0.1419	0.0424	499	26117	113382	115500	123675	24687
\max	0.1291	0.8086	0.2755						
\min	0.0026	$< 0.1 e^{-006}$	0.0017						
rivas07									
mean	0.0925	0.1585	0.0417	249	79801	87454	81704	111112	67481
max	0.2061	0.6673	0.2465						
\min	0.0048	$< 0.1 e^{-006}$	0.0039						

Table 7.3: Statistics of the MAP estimations of Model A

Parameters values of k_1 , k_{1met} and V_b are all within acceptable range.

WRSS are a little bit higher than in the previous model but this is a standard result while performing Bayesian estimation.

Histograms of the distribution of k_{2met} are shown in Figure 7.6.



Figure 7.6: Histograms of distribution of k2met for all subjects with MAP estimator

The distribution of the parameters is much more homogeneous than in the

analysis without prior. Moreover, the peak values that occurred at around the 100-th index appears now much reduced and in some subjects, even not visible.

These findings have their feedback when we analyze PET images. In Figure 7.7 and in Figure 7.8 are shown results from *contr*03 and *donep*02 to give visually confirm to what we said about the homogeneity of the found parameters: images appears smoother and anatomical distinct regions are more visible.



(a) Parameters trends for contr03.



(b) Parameters trends for donep02.

Figure 7.7: Visualization of smoother tracer trends for subject *contr03* and *donep02* from MAP estimation. Focus on cerebellum area



(b) Parameters trends for donep02.

Figure 7.8: Visualization of smoother tracer trends for subject *contr03* and *donep02* and from MAP estimation. Focus on thalamic areas
While analyzing those patients which couldn't afford good estimates in the previous quantification, we can see a great improvement in the quality of the results. By visual inspection it is now possible to evaluate that even in subjects of *nomed* group there is a localized diffusion of the tracer in the cerebellum and in the thalamic areas, while the metabolite freely diffuses in the whole brain (Figure 7.9, 7.10). For what concern *rivas*07, cerebellum and thalamic activity of parameter k_1 are not so evident, while they shows high values for k_{1met} that it seems to spread mainly in those areas.

As we said previously, subject *rivas*01 didn't provide good estimates, and the number of valid ones was so low that we couldn't make any considerations about its kinetics.



(a) Parameters trends for nomed04.



(b) Parameters trends for rivas07.

Figure 7.9: Visualization of smoother tracer trends for subject *nomed04* and *rivas07* and from MAP estimation. Focus on cerebellum.



(b) Parameters trends for rivas07.

Figure 7.10: Visualization of smoother tracer trends for subject nomed04 and ri-vas07 and from MAP estimation. Focus on thalamic areas

7.4 Model B

In the case of Model B, the functions to be estimated are those derived from the two portions of non-linear kinetics for the parameters k_2 and k_{2met} . Considering that the grids have both N=20 or N=30 or N=90 elements, depending on the case, the Basis Function for the i-th element of the grid has always the form:

$$BFk2_i(t) = C_p(t) \otimes e^{-(k_2)_i t} \qquad i = 1, ..., N$$
$$BFk2met_j(t) = C_{met}(t) \otimes e^{-(k_2met)_j t} \qquad j = 1, ..., N$$

and, always for the i-th element, the equations of the predicting model becomes:

$$C_{VOI}(t) = (1 - V_b)(k_1 BF k_{2i}(t) + k_{1met} BF k_2 met_j(t)) + V_b C_b$$

We can now define, given M the number of PET scans:

$$A_{ij} = \begin{bmatrix} BFk2_{i}(t_{1}) & BFk2met_{j}(t_{1}) & C_{b}(t_{1}) \\ BFk2_{i}(t_{2}) & BFk2met_{j}(t_{2}) & C_{b}(t_{2}) \\ \vdots & \vdots & \vdots \\ BFk2_{i}(t_{M}) & BFk2met_{j}(t_{M}) & C_{b}(t_{M}) \end{bmatrix} \in \Re^{Mx2}$$

$$par = \begin{bmatrix} (1-V_b)k_1\\ (1-V_b)k_1_{met}\\ V_b \end{bmatrix} \in \Re^{3x1} \quad C_{pred} = \begin{bmatrix} C_{pred}(t_1)\\ C_{pred}(t_2)\\ \vdots\\ C_{pred}(t_M) \end{bmatrix} \in \Re^{Mx1}$$

The equation that relates the three components is:

$$C_{pred} = A_{ij} par$$

And through the Weighted Linear Least Square we achieve the parameters estimation:

$$p\hat{a}r = [A_{ij}^T W A_{ij}]^{-1} A_{ij}^T W C_{pred}$$

where the $W = diag(w) \in \Re^{MxM}$ is the diagonal matrix of **weight** described further.

The procedure is as follows:

$$\begin{array}{l} For \ i = 1, ..., N \\ for \ j = 1, ..., N \\ k2_{met} = (k2_{met})_i \quad , \quad BFk2met = BFk2met_i \\ k_2 = (k_2)_j \quad , \quad BFk2 = BFk2_j \\ \left[\begin{array}{c} ((1 - V_b)k_1)_{ij} \\ ((1 - V_b)k_1_{met})_{ij} \\ (V_b)_{ij} \end{array} \right] = [A_{ij}^T WA_{ij}]^{-1} A_{ij}^T WC_{pred} \\ WRSS_{ij} = \sum_{k=1}^M w_{kk} [Cvoi_{ij}(t_k) - Cpred_{ij}(t_k)]^2 \\ end \\ end \end{array}$$

The final optimal estimation of the parameters is obtained by:

$$\begin{bmatrix} ((1-\hat{V}_b)k_1)_{ij} \\ ((1-\hat{V}_b)k_1_{met})_{ij} \\ (\hat{V}_b)_{ij} \end{bmatrix} = minWRSS_{ij}$$

Data weight was managed through the relative weights as for Model A. The estimation was performed using the relative weights, defined by:

Sigma=diag(sqrt(mean_centroids./scan_sec));

while the absolute weights were calculated only "a posteriori" and used to provide the actual precision of the estimates.

7.4.1 Results for grid built on each ROI

We applied BFM at Model B using the grid built on each ROI and we analyzed the results as for Model A.

Table 7.4 shows the estimations of k_1 , k_{1met} and V_b maximum and minimum values in addition to the average. Weighted Residuals Sum Squares is also reported to allow a comparison with the related values of following models. Number of estimates *Estimates TOT* and number of acceptable estimates *Estimates valid* were calculated in order to understand how many values have been deleted or because in contrast with physiological acceptable values or because the estimation had a CV higher than 200%.

For what concern this latest parameter, *Estimates valid*, we can see that in *rivas* group it is very low. In *rivas*01 only the 22% of values is taken in consideration and in *rivas*07 the 27%, while in other subjects the percentage of valid estimation is always higher than 45%, reaching 63% in *donep*04. Parameter that seems more difficult to estimate is V_b , it's the one with the least number of valid values. In *rivas*01 the value of valid estimates of k_1 it's very low too.

By the way, comparing these results with those of Model A, Table 7.2, the acceptable estimates are globally higher in Model B, for each patient.

Results for k_1 , k_{1met} and V_b are in line with the values obtained for these parameters from other kinetic tracer analyzing models available in literature.

The histograms of the distribution of k_2 and k_{2met} values are shown in Figure 7.11 and in Figure 7.12.

The distribution of the values of k_{2met} tends to concentrate to the extreme bound of the interval, in particular for patients rivas01 and rivas07 and the percentage of values falling in the central part of the interval is very low. This behavior is accentuated even more for the distributions of the parameter k_2 , where it seems that the histogram try to overcome the edge of the range.

It's still not clear if the edge values are those that are deleted at a later time, by the way it could be significative to enlarge the range of parameters k_2 and k_{2met} to evaluate if this trend might change (Subsection 7.4.2).

Subject	k1	k1met	Vb	WRSS	CV k1	CV k1met	CV Vb	Estimates	Estimates
					$<200%$	${<}200\%$	${<}200\%$	TOT	valid
contr03									
mean	0.3204	0.2068	0.0791	101	79685	74556	54060	117233	52217
\max	1.4249	0.7419	0.4919	-					
min	0.0071	0.0008	0.0127	-					
contr05					1				
mean	0.1065	0.1408	0.0443	166	71175	71834	60862	107083	57347
\max	0.7119	0.7432	0.3748	-					
\min	0.0037	0.0030	0.0030	-					
donep02									
mean	0.0850	0.1354	0.0549	127	81566	81738	74244	129578	68197
\max	0.5108	0.6457	0.3912	-					
\min	0.0027	0.0036	0.0035	-					
donep04					1				
mean	0.0866	0.1289	0.0496	129	77149	76273	70177	106335	67203
\max	0.4755	0.5340	0.2980	-					
\min	0.0060	0.0072	0.0028	-					
nomed04									
mean	0.1028	0.1950	0.0526	133	80697	83451	75179	121078	68222
max	0.7858	0.9451	0.3427	-					
\min	0.0059	0.0056	0.0047	-					
nomed08									
mean	0.519	0.0573	0.0689	100	68081	60628	47068	101674	45271
\max	2.3013	0.8576	0.3899	-					
\min	0.0008	0.0806	0.0082	-					
rivas01									
mean	0.203	0.2104	0.0771	203	37636	55285	45039	123675	28083
\max	0.9059	1.3403	0.3645	-					
\min	0.0106	0.0215	0.0032	-					
rivas07									
mean	0.1341	0.1027	0.0522	115	59904	68563	32926	111112	29461
\max	0.8014	0.6945	0.2833	-					
min	0.0061	0.0009	0.0053	-					

Table 7.4: Statistics of the estimations: Model B, grids built on each ROI



Figure 7.11: Histograms of distribution of k2met for all subjects



Figure 7.12: Histograms of distribution of k2 for all subjects

Kinetic cerebral trends of indices k_1 , k_{1met} , k_2 , k_{2met} , V_b and Vt_{met} , where Vt_{met} and V_t are the Distribution Volume of, respectively, the metabolite and the tracer, are shown in the following figures.

As regards the vascular volume presence, V_b this always takes values within a rage of eligibility.

The Distribution Volume of the metabolite, Vt_{met} assumes values between 1[%] and 5[%], homogeneously distributed within the whole brain volume.

The Distribution Volume of the tracer, V_t , has a very unbalanced distribution, in correspondence with the area of the cerebellum values touch peaks between 50[%] and 200[%], and, except for *donep* group and subject *nomed*04 that show high values also in correspondence of thalamic areas, the remaining parts of the brain assume very low Distribution Volume values.



(a) Parameters trends for contr03.



(b) Parameters trends for donep02.

Figure 7.13: Visualization of tracer trend in cerebellum for subject contr03 and in thalamic areas for donep02 from results of Model B analysis on ROI grids



(a) Parameters trends for contr03.



(b) Parameters trends for donep02.



(c) Parameters trends for nomed04.

Figure 7.14: Visualization of tracer trend in cerebellum for subject *contr03*, *donep02* and *nomed04* from results of Model B analysis based on ROI grids



(a) Parameters trends for donep02.



(b) Parameters trends for donep04.



(c) Parameters trends for nomed04.

Figure 7.15: Visualization of tracer trend in thalamic area for subject control, donep02 and nomed04 from results of Model B analysis based on ROI grids

For what concern k_1 , k_2 , k_{1met} and k_{2met} parameters, results are shown in Figure 7.14 .

In patients of *contr* and *donep* groups the behavior of the parameters is very similar.

Parameter k_1 is maintained, with even higher values, within specific regions, particularly in the cerebellum for the control subjects, moving even in the thalamic areas, clearly visible in the results of *donep* patients. In the group of *nomed* and *rivas* only subject *nomed*04 presents estimates appreciable and behaviour similar to *donep* patients.

Parameter k_2 shows low values for all the patients examined.

Parameter $k1_{met}$ spreads initially in the area of the cerebellum, then enters freely within the entire brain.

Parameter k_{2met} is homogeneous throughout the brain and with reasonable values.

In patients from rivas group and subject nomed08 is not possible to analyze qualitatively and/or quantitatively the results because, as for previous models, images are still not appreciable because of the lack of valid data.

7.4.2 Results for grid built on ROIs mean

Starting from the considerations that had been made for grids built on each ROI we developed new grids, based on ROI analysis too, but evaluated on the mean of ROIs values and enlarged until 3SD on the extremes. In Table 7.4 the results for each subject.

Fields *Estimates TOT* and *Estimates valid* represent the number of estimations that the program calculated and the number of which we considered valid in terms of physiologically acceptable values and statistic variance (CV < 200%).

In this case the value of EstimatesValid is quite homogeneous for all subjects without much differences: *contr*, *donep*, *nomed* groups and *rivas*07 percentage of valid estimates is largely upper than 50%, the only subject whose percentage is inferior at 50% is *rivas*01 reaching just the 40% of acceptable estimates.

Parameters k_1 , k_{1met} and V_b Coefficients of Variance are for the major part under the threshold value of 200%. The most problematic parameter to estimate is, even in that case, V_b .

Comparing the number of valid estimates with the ones obtained from previous results we can see that this value is higher than the value obtained for Model A and for Model B using the grid built on each ROI.

Parameter WRSS is higher than the one of results obtained from Model B using grid built on each ROI.

The histograms of the distribution of k2 and k2met values are shown in Figure 7.11 and the tracer and metabolite kinetics trends are shown immediately after.

Subject	k1	k1met	Vb	WRSS	$ig egin{array}{ccc} { m CV} & { m k1} \ { m <200\%} \end{array}$	${ m CV~k1met}\ {<}200\%$	$egin{array}{ccc} { m CV} & { m Vb} \ {<}200\% \end{array}$	Estimates TOT	Estimates valid
contr03									
mean	0.3192	0.1823	0.0800	162	100926	88076	63360	117233	61889
max	2.0989	0.5299	0.4936						
min	0.0033	0.0066	0.0095						
$\operatorname{contr}05$									
mean	0.1012	0.1073	0.0471	340	90844	84682	78095	107083	71493
\max	0.5975	0.6952	0.3756						
\min	0.0014	0.0023	0.0031						
donep02									
mean	0.0805	0.1150	0.0537	328	106366	99865	95947	129578	88664
\max	0.5417	0.7535	0.4291						
\min	0.0018	0.0129	0.0034						
donep04					1				
mean	0.0917	0.1165	0.0502	208	88716	76775	75432	106335	72158
\max	3.3193	11.3965	1.0104						
\min	0.0004	0.0002	0.0216						
nomed04									
mean	0.0909	0.1598	0.0531	269	94798	98405	93028	121078	79711
\max	0.6557	0.8260	0.3690						
\min	0.0014	0.0014	0.0030						
nomed08									
mean	0.5814	0.0658	0.0748	169	96254	72011	73400	101674	70392
\max	3.1622	0.5161	0.3917						
\min	0.0018	0.1957	0.0071						
rivas01									
mean	0.2156	0.1928	0.0534	363	79087	99672	78325	123675	51391
\max	1.1449	1.2501	0.3677						
\min	0.0023	0.0004	0.0019						
rivas07									
mean	0.1511	0.1050	0.0511	176	94621	78143	62752	111112	59600
\max	1.0323	0.6851	0.3696						
\min	0.0015	0.0001	0.0033						

Table 7.5: Statistics of the estimations: Model B, grids built on ROIs mean

Parameter values seem to distribute a little more homogeneously on the interval. By the way it still remains the trend to exceed the upper and the lower limit of the range, in particular the parameter k2 seems to have the tendency to distribute nearly the upper edge of the interval.



Figure 7.16: Histograms of distribution of k2met for all subjects



Figure 7.17: Histograms of distribution of k2 for all subjects



(a) Parameters trends for contr03.



(b) Parameters trends for donep02.



(c) Parameters trends for nomed04.

Figure 7.18: Visualization of tracer and metabolite trends in cerebellum area for subject contr03, donep02 and nomed04 from results of Model B analysis based on ROI means grids



(a) Parameters trends for contr03.



(b) Parameters trends for donep02.



(c) Parameters trends for nomed04.

Figure 7.19: Visualization of V_t , Vt_{met} and V_b trends in cerebellum area for subject contr03, donep02 and nomed04 from results of Model B analysis based on ROI means grids



(a) Parameters trends for nomed08.



(b) Parameters trends for rivas01.



(c) Parameters trends for rivas07.

Figure 7.20: Visualization of tracer and metabolite trends for subject nomed08, rivas01 and rivas07 from results of Model B analysis based on ROI means grids



(a) Parameters trends for nomed08.



(b) Parameters trends for rivas01.



(c) Parameters trends for rivas07.

Figure 7.21: Visualization of V_t , Vt_{met} and V_b trends for subject nomed08, rivas01 and rivas07 from results of Model B analysis based on ROI means grids

For subjects of *contr* and *donep* groups and for *nomed*04 the trends of parameters are very similar to the ones obtained from previous model. That means the tracer enters mainly in cerebellum and thalamic areas but doesn't spread in the other regions: k_1 is very high in that regions and very low elsewhere. The metabolite, instead, diffuses in the whole brain: k_{1met} has significant values everywhere (Figure 7.18).

For the same patients, also, Distribution Volume of tracer V_t shows highest peaks in cerebellum areas and very high values in thalamic zones while the Distribution Volume of metabolite Vt_{met} is quite constant in the whole brain (Figure 7.19).

For what concern *rivas* group and *nomed*08 they seem to have a different behaviour. That means that the k_1 parameter associated to the kinetic of the tracer indicates high values of diffusion in the whole brain (especially for *nomed*08), and the k_2 values are, in those cases, relevant. Parameters that describe the trends of the metabolite, k_{1met} and k_{2met} also diffuse in quite the whole brain but with lower values (Figure 7.20). These results are in agreement with those obtained from the ROI analysis for the model B.

Distribution Volume of tracer appears homogeneous, V_t values stay between 0[%] and 50[%], Distribution Volume of metabolite stays between 0[%] and 5[%] (Figure 7.21).

7.4.3 Extended grids results

Starting from the consideration that it seemed that k_2 and k_{2met} had always the tendency to distribute near the upper and lower values of the range, we decided to built some extended grids, of 90 elements each. This solution had been tested to understand if a wider range, with the increase of computational burden that follows, could provide further information about which of the two types of model better describes the data and about the trends of kinetic parameters.

Analyzing the histogram of the distribution of values of k_2 and k_{2met} it can be noted that there is always a tendency for a good portion of values to accumulate near the extremes of the interval of the grid.

At visual inspection, from the obtained images of quantification of parameters, it is also possible to see that parameter k_1 assumes very high values anywhere within the section, both for patients in which the estimation had encountered difficulties for this parameter (*rivas* and *nomed* groups) and in those in which the estimates were found to be good (*contr* and *donep* groups).

The big difference, in this case, is that for patients of *rivas* and *nomed* groups we get a trend well distributed throughout the brain, while for subjects *contr* and *donep* groups, the extremely high values of k_1 are not concentrated in particular regions, but appear by way of salt and pepper noise inside the brain (Figure 7.22).

Parameter k_{1met} shows good estimations and in line with those obtained from previous analysis, except for *rivas* group where high sporadic peaks of values are present (Figure 7.22).

Distribution Volume of the tracer V_t shows the same noisy behaviour of k_1 , with high peaks reaching values since 100[%] while the Distribution Volume

of the metabolite Vt_{met} is quite homogeneous in the whole brain and with values between 0[%] and 5[%], except, of course, for patients *rivas*01 and *rivas*07 (Figure 7.22).



(a) Parameters trends for contr03.



(b) Parameters trends for rivas01.

Figure 7.22: Visualization of different tracer trends for subject contr03 and rivas01 from BFM with Extended grids estimation. Subject contr03 shows salt and pepper noise. Subject rivas01 shows good estimates and high values of k_1 .

Chapter 8

Conclusions

Recent findings have shown that the study of brain cholinergic system through the instrument of PET with *ad hoc* synthesized tracers can be a valuable tool for early diagnosis of Alzheimer's disease. In particular, we wanted to study the possibility of investigating for this purpose through a brand new tracer, 11C-MP4B, of which it still had not been developed any kinetic model for quantification, and thus its efficacy had not yet been proven.

We started from the data of arterial sampling for 8 subjects, 2 controls and 6 affected from AD. The first step was to eliminate the noisy components of the sampling in order to construct the functions, developed by fitting a model of exponential sums on the data, which acted as arterial input functions of the kinetic model that we would develop later.

The first consideration that we made was about the trends of those arterial sampling data. We noticed that for subjects of *rivas* and *nomed* groups there was an irregularity, a sort of second peak of concentration that was refered us as to be a noisy artifact. In light of the subsequent analyzes, that have seen the patients of this groups form a sort of subgroup at the level of the kinetic behavior, and in view of the fact that this false peak is not manifested in the same way for all patients, leaving room for doubt that it may be not a noisy artifact. This will certainly be a reason for further future investigations.

We started the analysis of the images themselves and we decided, as a first step, to begin our analysis by quantifying the cholinergic activity through a region-based method rather then a voxel-based one. We performed then a cluster analysis.

The algorithm was constructed so that it performed clustering on the TACs suitably parameterized into 6 features. We got the division into 12 subregions which in turn reflected some precise anatomical areas: cerebellum, thalamus, hippocampus, etc etc. This leads us to conclude that at functional level, different anatomical regions have different kinetic behavior.

We then skipped to the evaluation of parameters and we did it for two model structures appropriately constructed on the basis of state of art of tracers kinetic models and on the basis of models already tested for tracers with similar substrate.

Performing ROI-level quantification, the results of the kinetic parameters show complementary behaviour between a first group composed by subjects *contr*03, *contr*05, *donep*02 and *donep*04 and a second group composed by *rivas*01, *rivas*07, *nomed*04 and *nomed*08. For the first group the most suitable model resulted to be Model A, because of the higher valid values and accuracy of estimates and the lower WRSS, while, for the same reasons, for the second group resulted to be Model B.

Finally, the last and most complex step, it was that of quantification at voxel-level. We performed it through Basis Function Method. This estimation method requires that were linearized the components with a non-linear dependence with the measure equation: for the parameters in question we built specific grids, for each element of the grid we calculated the related measurement equation and we estimated the parameters, then finally we choose the combination of grids values that has provided the lowest WRSS.

The application of the BFM returned results from which we could draw the following conclusions regarding the grids suitability and the best model structure to be used.

In light of the number of valid estimates and the goodness of the estimation, Model A is the most suitable model for the quantification of cholinergic activity in subjects of *contr* and *donep* groups while for patients of *nomed* and *rivas* groups it doesn't afford acceptable estimates.

Using a priori information improves the goodness of the estimation in terms of estimates precision and number of valid estimates. In patients where the quantification didn't lead to a significant number of valid estimates, MAP estimator allowed better performance. By the way subject rivas01 was still problematic.

Bayesian estimator allow a more qualitative analysis thanks to the *prior* vector which gives a preliminary partitioning of the image into sub-regions.

Model B seems to be the most suitable model for the quantification of the activity of *nomed* and *rivas* groups, where k_2 assumes significant values, while for *contr* and *donep* ones the values of the estimated k_2 were so low that we can say it might not be necessary to include it in the model structure. Eventually, even voxel-based analysis returned that for those latest subjects, the most suitable model could be Model A.

The most suitable grids structure for parameter k_2 and k_{2met} is still an open question. From the results obtained using three different types of grids, we could evaluate that the extension of the grid, increasing however the computational burden, can not significatively affect the improvement of the estimate. In our case, in fact, the use of extended grids has given rise to a kind of compensation of the algorithm, for all subjects, even if related to different parameters. By the way, it is important to report the fact that only with those Extended grids we succeeded good results for *rivas*01. Between the two grids built on ROI, the most suitable was the one built on the average of the 12 ROIs values, because of a little bit higher number of elements and of the wider range.

Finally, as regards the behavior of the tracer, we can draw the following conclusions. 11C-MP4B has not provided the desired results, especially since, in the majority of subjects analyzed, the tracer remains confined in some areas of the brain and does not diffuse freely.

Moreover, in those patients where k_2 had very low values it seems there is a trapping region where the tracer enters and cannot exit anymore.

Metabolite product, instead, diffuses in the whole brain, with a good estimated kinetics, without trapping zones. It could be a reason for new studies the possibility to extract information on cholinergic activity from metabolite product rather than from the tracer.

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