DELIVERABLE 6.1

Normal ageing brain models – Initial phenomenological model and links to mechanical modelling framework

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EXECUTIVE SUMMARY

In order to develop a novel clinical decision support system, which is one of the key objectives of the VPH-Dare@IT project, understanding changes in brain morphology and function owing to normal ageing or to different types of dementia is of utmost importance.

This deliverable describes our first efforts to provide insight into the normal ageing brain by analysing MR imaging data from a large population-based imaging study. Imaging biomarkers, characterising different brain tissues, and the presence of white matter lesions, are extracted from 5,000 healthy individuals and presented in percentile curves. The curves, generated for the whole brain and per brain lobe and central region, can be used in a diagnostic setting by comparing an individual to the reference data (to measure deviation from normal), or can be used as input for or evaluation of mechanistic modelling approaches (WP5). Most degeneration is found in the white matter. In the near future we will, therefore, also include imaging biomarkers extracted from diffusion tensor MRI data; diffusion tensor MRI analyses can be used to investigate integrity of the white matter. Furthermore, we will generate similar reference curves using image processing pipelines developed by consortium partners, as described in WP3.
1. **INTRODUCTION**

Interpretation of brain MRI images made in the diagnostic workup for dementia can be challenging for the radiologist, as early pathological brain abnormalities can be hard to detect visually, especially in the early stages of the disease, and hard to confidently distinguish from ‘normal’ age related brain changes. Age- and gender-specific reference data derived from brain MRIs of healthy, non-demented, ageing individuals using automated image analysis techniques may aid the radiologist in distinguishing dementia-related brain changes from changes due to normal ageing. This may improve the detection of more subtle pathological brain changes, leading to an earlier diagnosis of dementia and facilitate disease subtyping. In addition, models of normal brain ageing and models of disease development can be used as input for simulation studies.

In the last two decades, research has seen major technological advances in imaging for studying the ageing brain, and the role of imaging in studying normal ageing and neurodegeneration has increased accordingly, (Frisoni et al., 2010). Advances in multi-modality image acquisition have gone hand-in-hand with sophisticated and efficient image analysis techniques, and appropriate computing infrastructure, which has led to a range of quantitative imaging biomarkers. Imaging biomarkers are characteristics measured in medical images which may be used as an indicator of biological condition or disease.

1.1. **REFERENCE DATA**

The objective of this deliverable is to provide statistics on quantitative imaging biomarkers related to brain morphology in healthy ageing individuals. The reference data of healthy ageing individuals is obtained from the Rotterdam Scan Study (RSS) (Ikram et al., 2011), a prospective large population-based cohort study, in which multi-spectral MR imaging data of over 5,000 individuals are collected at multiple time points. Also, a rich set of potential risk factors (lifestyle, demographics, education etc.) is available. This will provide the opportunity to obtain imaging biomarkers for different subgroups: smokers/non-smokers, diabetics/non-diabetics, APOE-e4 carriers/non-carriers, etc.

The reference data from the RSS were used to create reference curves, i.e. scalar models, of specific biomarkers. These curves can then be used to compare biomarkers extracted from individuals with the reference data. Once it is known how neurodegeneration develops in normal ageing, it can be determined if and how much the data of an individual deviates from normal ageing. This is important information for diagnostics on neurodegenerative diseases like Alzheimer’s disease.
1.2. LINK TO MECHANICAL MODELLING

Understanding how certain morphological imaging biomarkers develop over age is important knowledge for the mechanical modelling framework in WP5. The generated reference curves can be used as input for the mechanical modelling framework (as boundary conditions) but they can also be used to validate the developed mechanical model: the brain atrophy predicted by the mechanical model should be close to the atrophy measured in the reference data of the RSS.

1.3. RELATION TO OTHER TASKS AND WORK PACKAGES

Besides the above mentioned link to the mechanical modelling framework, this work is related to work package 3 and task 3.3, in which construction of image analysis pipelines are specified, optimised and developed. The developed pipelines in this task will also be applied to the data of the RSS. The extracted curves derived using the pipelines of task 3.3 can be compared to the initially extracted curves, which are generated using a pipeline developed by the Erasmus MC.
2. MATERIALS AND METHODS

Structural brain MRI scan sets of healthy ageing individuals (age range: 45.73 - 96.70 years; mean age: 64.12 years) from the population-based RSS were used to create the age- and sex specific percentile curves. All participants of the RSS were non-demented at time of MRI, as this was part of the study exclusion criteria (De Leeuw et al., 2001, Ikram et al., 2011, Hofman et al., 1991).

The RSS participants are divided into three different cohorts: a first cohort having participants between the age of 55 and 106 from the start of the study in 1991, a second cohort having participants that became 55 years since the start of the study or those of 55 years or over that migrated into the study district and a third cohort having participants that aged 45 years and over living in the study district that had not been examined already (i.e., mainly comprising those aged 45 – 60 years). Figure 2 shows an overview of the Rotterdam study and the different sub-cohorts. For generating the curves, the sub-cohorts, RS-I-5, RS-I-X, RS-II-2 and RS-III-1 where combined, to get a total of 5,000 subjects. The age histograms of the subcohorts are shown in Figure 2. Sixty percent of the data originate from the ‘youngest’ cohort, RS-III-1.

Automated image analysis techniques were applied to the brain MRI scans obtained in the RSS to support research into understanding the normal ageing brain and patterns in neurodegenerative disease, and early and differential diagnosis of neurodegenerative disease.

![Histograms of the age distribution of the cohorts used in this study.](image)

Imaging data of the RSS was pre-processed in order to spatially align the different MRI sequences, and to correct for MR intensity inhomogeneities. Subsequently, the following imaging biomarkers are extracted, over the whole brain and per brain lobe:

1. Grey matter (GM) volume
2. White matter (WM) volume
3. Cerebrospinal fluid (CSF) volume
4. White matter lesion (WML) volume
2.1. Imaging Sequences

All brain scans were performed on a 1.5 T MRI system (General Electric Healthcare, Milwaukee, Wisconsin). The imaging protocol included a T1-weighted (T1w) sequence, a proton density–weighted (PDw) sequence and a fluid-attenuated inversion recovery (FLAIR) sequence. The T1w sequence was a fast radio-frequency spoiled gradient–recalled acquisition in steady state with an inversion recovery pre-pulse sequence. The details of all sequences are shown in Table 1.

2.2. Image Processing Pipeline for Imaging Biomarker Extraction

The automated MR brain data processing pipeline, available at the Biomedical Imaging Group Rotterdam (BIGH; Erasmus MC) was used to process the MR data of the RSS. A well-defined workflow is implemented to warrant integrity of the raw and processed data.

The processing pipeline consists of the following steps:

1. Pre-processing of the input scans
2. Skull stripping and lobe segmentation (4 right lobes, 4 left lobes and a central area)
3. Tissue segmentation into GM, WM and CSF
4. WML detection

The different steps are explained in the sections below. A scheme of the complete pipeline is shown in Figure 4.

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2.2.1. Pre-processing

The first step in the processing pipeline at Erasmus MC is pre-processing of the images. All input scans are co-registered to the space of the T1w scan using a rigid transformation model. For the registration, the open source registration software Elastix was used (Klein et al., 2010). Due to RF excitation field inhomogeneities, non-uniform reception coil sensitivity and eddy currents a smooth variation in intensity can arise in MR images. This intensity variation needs to be corrected before further automatic processing and that is why all co-registered scans are corrected for the non-uniformity in intensity with the Non-parametric Non-uniform intensity Normalisation (N3) algorithm (Sled et al., 1998).

2.2.2. Skull-stripping and lobe detection

After pre-processing, the brain mask is created using non-rigid multi-atlas registration. The registration approach is based on free-form deformations parameterised by a cubic b-spline, (Rückert et al., 1999). For the lobe detection, six in-house manually-labelled T1w scans, in
which brain lobes were manually labelled, are registered to the individual subjects, resulting in six lobe annotations for the individual. Using majority voting, all voxels in the individual are assigned one label. An example of a manually labelled T1w scan is shown on the right of Figure 3.

2.2.3. Brain tissue segmentation

The brain tissue segmentation in our pipeline is based on an automatically-trained k-Nearest-Neighbour (kNN) classifier with k = 45 as described by Cocosco et al. and Vrooman et al. (Cocosco et al., 2003, Vrooman et al., 2007). The method does not require user interaction. For fully automated training, the method utilises non-rigid atlas registration. By aligning the data set to be segmented with a probabilistic anatomical atlas, i.e. probability maps for background, CSF, GM and WM, samples that very likely belong to a certain tissue class are automatically generated. An example of a tissue atlas is shown on the left of Figure 3. The generated samples are used by the kNN classifier for the tissue classification of the entire 3D brain data set. The advantage of this brain tissue segmentation method is that it is relatively insensitive to the distribution of voxel intensities in the image, i.e. this method imposes few restrictions on the exact scanning protocol or scanner type.

2.2.4. White matter lesion detection

The WML-detection step is based on an automatically-selected threshold on the FLAIR scan (de Boer et al., 2009). First, the tissue segmentation from the previous step is used to localise the grey matter voxels in the FLAIR scan. Assuming that the voxels with the highest intensity in the histogram are white matter lesion candidates, a threshold is set on a predefined number of standard deviations higher than the location of the top of the smoothed histogram. Upon thresholding, a number of regions are wrongly classified as WML, such as regions that are located inside the grey matter. For each lesion, the ratio of number of neighbouring WM voxels and the number of neighbouring CSF+GM voxels is calculated. A 3D 18-connectivity is used to define a group of connected voxels. If the ratio is smaller than 0.26, the lesion is relabelled as GM. For WML that are classified in the background, a similar approach is used. In this case, the ratio is defined as the number of neighbouring non-background voxels divided by the number of background voxels. If this ratio is larger than 0.4 the voxels are relabelled as background. Intracranial volume (ICV) was calculated by summing WM, GM, WML and CSF volumes. All resulting volumes were subsequently corrected for ICV.

2.2.5. Statistical analysis

Age- and sex specific percentile curves were generated for each quantified parameter (whole brain volume, GM/WM/WML volume and lobar tissue volumes) derived from all reference subjects using the LMS method (Cole et al., 1992). The LMS method estimates the Box-Cox power transformation (λ), mean (µ) and co-efficient of variation (σ) for the appropriate volume at each value of the covariate age. From the parameters λ, µ and σ, percentile lines can be estimated for the appropriate age range.

The Box-Cox power transformation assumes the input values to be positive. Because the distribution for the power-transformation is assumed to be a normal distribution, a logarithm is applied to the WML volume distribution. After this mapping, the input values are not positive anymore. The Yeo-Johnson power transformation (Yeo et al., 2000) can have negative input values. That is why, for the generation of all the reference percentile curves, the Box-Cox transformation is replaced by the Yeo-Johnson power transformation. The LMS estimation is performed using a penalised maximum likelihood estimator. For this statistical analysis the R-library VGAM was employed (Yee et al., 2002).
2.2.6. Error checking

Since the generation of reference curves includes processing of a very large dataset, manual error checking of all results is not feasible. Therefore, a pragmatic approach is adopted, in which all cases are checked if they are outside the 1.5 times the interquartile range (IQR). This means that all subjects with a biomarker larger than median + 1.5 times IQR or smaller than median – 1.5 times IQR are manually checked.
3. RESULTS

GM, WM, CSF and WML volumes were calculated for the following regions: whole brain (total), left hemisphere (left) and right hemisphere (right) and for each of these regions the volumes are calculated for the following sub regions: all regions (all), frontal lobe (fl), parietal lobe (pl), occipital lobe (ol), temporal lobe (tl) and the central region (rest). Curves presented in this deliverable show statistics for males and females but owing to the available metadata such curves can also be derived for subgroups such as smokers/non-smokers, diabetic/non-diabetic and APOE-e4 carriers/non-carriers.

Using the method described in Section 2.2.6, 196 outliers were found based on the tissue and lobe segmentation and they were manually checked. Outliers with a highly improbable segmentation (i.e., the segmentation failed) or with an anomalous pathology were excluded for generating the curves. From the 196 manually-checked outlier subjects, 41 were not included.

Below examples of the generated curves are shown, for the whole brain (Figures 5, 6 and 7) and for the frontal lobe (Figures 8, 9, 10 and 11). The curves are presented as percentage of ICV (%ICV). Figures 5 – 7 show that there is a decrease in GM and WM and an increase in CSF and WML volumes over age in the whole brain for both female and male. Most decrease is found in the WM volume, which increases interest in research on diffusion measures in normal ageing. Figures 8 – 11 show a decrease in GM, WM and CSF volumes and an increase in WML volume over age in the frontal lobe for both female and male subjects. Again, the greatest decrease is found in the WM volume.

Figure 5 GM volume of the whole brain for female (top) and male (bottom) in %ICV, a pink point represents a female individual, a black point a male individual.
Figure 6 WM volume (top) and CSF volume (bottom) of the whole brain for female (left) and male (right) in %ICV.

Figure 7 WML volume in the logarithm of %ICV of the whole brain for female (top) and male (bottom).
Figure 8 GM volume in the frontal lobe for female (top) and male (bottom) in %ICV.

Figure 9 WM volume in the frontal lobe for female (top) and male (bottom) in %ICV.
Figure 10 CSF volume in the frontal lobe for female (top) and male (bottom) in %ICV.

Figure 11 WML volume in the logarithm of %ICV in the frontal lobe for female (top) and male (bottom).
4. DISCUSSION AND CONCLUSION

Using fully-automated techniques to analyse 5,000 MRI brain imaging data of the population-based Rotterdam Scan Study, we could derive statistics of several quantitative imaging biomarkers related to brain morphometry (grey matter, white matter, CSF) and vascular pathologies (white matter lesions) as a function of normal ageing. Since we also have access to many other metadata, our framework will enable the generation of customised curves, e.g. showing the typical change in these imaging biomarkers in subgroups (smokers, carriers of a certain risk allele). The presented scalar models give a first insight into the process of degeneration in normal ageing. This information can be used to assist with early and differential diagnosis of dementia (e.g. detecting deviation from normal). It can also be used for initialising or validating biomechanical models (WP5).

One of the limitations of the fully automated image processing that is used in this method is that there may be undetected errors in the data that will constitute the reference curves. Another limitation of the method is that when the curves are used for clinical decision-making, the data that make up the reference curves should be representative for the patient group for which they are used.

Future work will also include the estimation of percentile curves of other quantitative imaging biomarkers. This will include brain structures such as the hippocampus and the ventricles. For these further analyses, we will collaborate with WP3 and apply image processing pipelines on the RSS data including methods from consortium partners University College London (UCL) and Imperial College (ICL). UCL has already provided imaging pipelines for imaging biomarker extraction as part of Task 3.3 (see Deliverable 3.1).

The results on brain tissue indicated that most of the neur-degeneration is found in the white matter. Since the white matter comprises of glial cells and myelinated axons, it indicates that a more detailed analysis of how the microstructure of the brain evolves as a result of ageing can lead to interesting results. Diffusion-tensor imaging (DTI) allows for mapping of the diffusion direction of water in the brain, which indicates the direction of the axons. That is why, besides structural-based curves, percentile curves of DTI-based measures, such as mean diffusivity and fractional anisotropy will be estimated in future work.

Within the project, we will not limit ourselves to scalar models. In Task 6.2, we will develop full 4D ageing brain models, i.e. models that capture the 3D brain structure as a function of ageing (full image models), enabling a higher-dimensional analysis. Instead of comparing single numbers with reference data, entire deformation fields can be used as a comparison method. This information will be shared with other work packages for e.g. simulating processes of brain ageing and degeneration, providing a strong link between the activities in this WP and WP5.
REFERENCES


