15 Use Case I: Imaging Biomarkers in Neurological Disease. Focus on Multiple Sclerosis

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15.1 Introduction

Imaging is widely used for diagnosis and monitoring of neurological diseases. CT scans are routinely acquired in emergency units in patients with traumatic injuries or stroke. PET imaging has gained a strong foothold in oncology. MRI has become the standard of practice for the diagnosis, follow-up and management of numerous neurological and psychiatric conditions. All of these imaging techniques have in common that, in clinical practice, the images need to be interpreted visually by trained specialists, who are responsible for initial diagnosis or for interpretation of follow-up examinations.

Within the scientific literature there is increasing emphasis on the use of quantitative medical imaging biomarkers, i.e. relevant numerical values that can be extracted from 2D or 3D medical image data sets, using advanced image processing techniques. Many imaging biomarkers, such as volumetric assessment of brain structures, have been shown to have excellent sensitivity and specificity for diagnosis or prognosis of various neurological diseases.

In this chapter, we shall focus on the development of relevant MR imaging biomarkers for patients with multiple sclerosis (MS). However, several of the techniques described below can be generalised to other neurological conditions.

15.2 Imaging Biomarkers Relevant to MS

15.2.1 Background

Multiple sclerosis (MS) is a chronic, inflammatory demyelinating disease of the central nervous system (CNS) which is hallmarked by white matter lesions [9]. Since 2001, MRI has been formally incorporated in the diagnostic workup of patients with a clinical suspicion of MS [38]. Recently, MRI has become an important tool for assessing the extent of brain damage, which is used in the monitoring of disease progression and therapeutic efficacy. In current clinical practice, these assessments are based on visual inspection of MR images by expert neurologists and neuroradiologists, who evaluate the presence and distribution of focal white matter lesions. A huge body of research has been devoted to white matter lesions, since they are considered to be a hallmark of the disease (even though abnormalities

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also occur in the grey matter). Lesions (also known as "plaques") can be visualised with several MRI sequences:

- *T1-weighted MR images*: chronic stage lesions with axonal destruction and irreversible damage appear as dark spots ("black holes"), compared to the surrounding white matter (WM) tissue intensities (see Fig. 15.1).
- *Gadolinium-enhanced T1-weighted MR images*: "active" lesions taking up contrast material and indicating inflammation and breakdown of the blood–brain barrier; the presence of enhancing lesions indicates ongoing disease activity, since only new lesions (under 6 weeks old) enhance (see Fig. 15.2).
- *T2-weighted MR images*, *fluid attenuated inversion recovery* (*FLAIR*) *MR images and proton density* (*PD*) *MR images*: on these imaging sequences with a long repetition time (TR), lesions appear as hyperintense spots compared to the surrounding brain parenchyma (see Fig. 15.1).

The "lesion load", defined as the total volume of lesions in the brain, is one of the most important biomarkers in MS. Often, a distinction is made between T2 lesions (i.e. lesions that appear hyperintense on T2-weighted or FLAIR images), T1 lesions (i.e. lesions that appear hypointense on T1-weighted images, the so-called black holes), as well as contrast-enhancing lesions.

In addition to the lesion load, brain volumetry [25] and, more specifically, cerebral atrophy [5] and, in particular, grey matter (GM) atrophy [23] are currently considered to be essential biomarkers, since they are correlated with the speed of disease progression (see Fig. 15.3). Thus, apart from the detection of lesions, quantification of brain volumes and atrophy rates is increasingly important in the management of patients with MS.

15.2.2 Natural Course of the Disease

From a clinical point of view, MS starts with a first attack or a "clinically isolated syndrome" (CIS) suggestive of MS. CIS patients with a

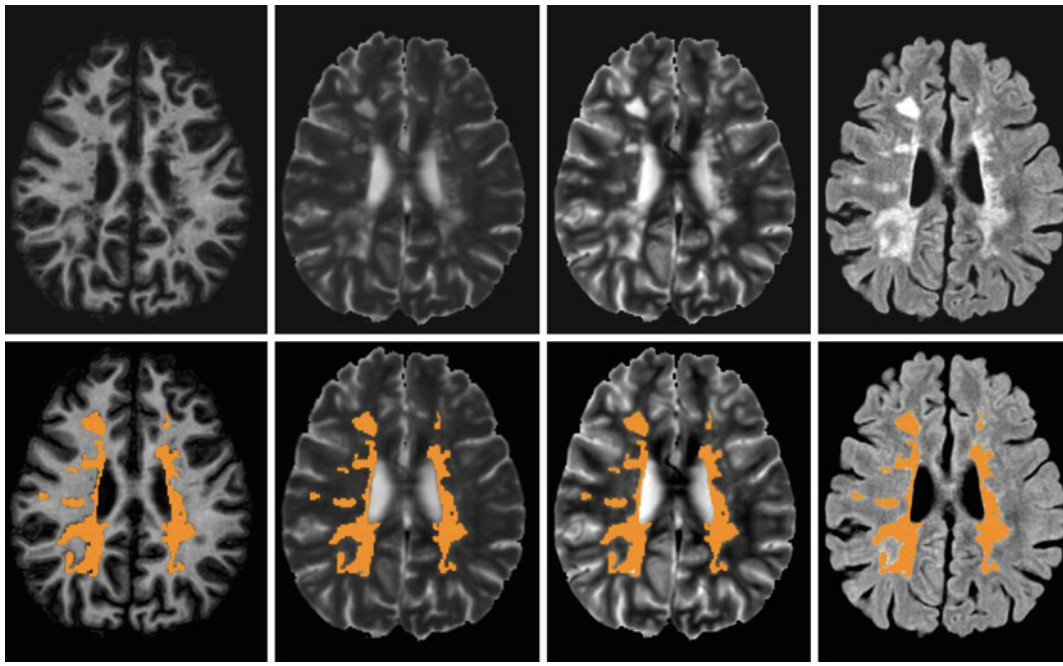

Fig. 15.1 *Top*, from left to right: T1-weighted, T2-weighted, proton density and FLAIR images, obtained in an MS patient. *Bottom*: same images overlaid with expert manual delineation of MS lesions (Data from the "MS longitudinal lesion segmentation challenge", ISBI 2015; training subject 02, time point 01.)

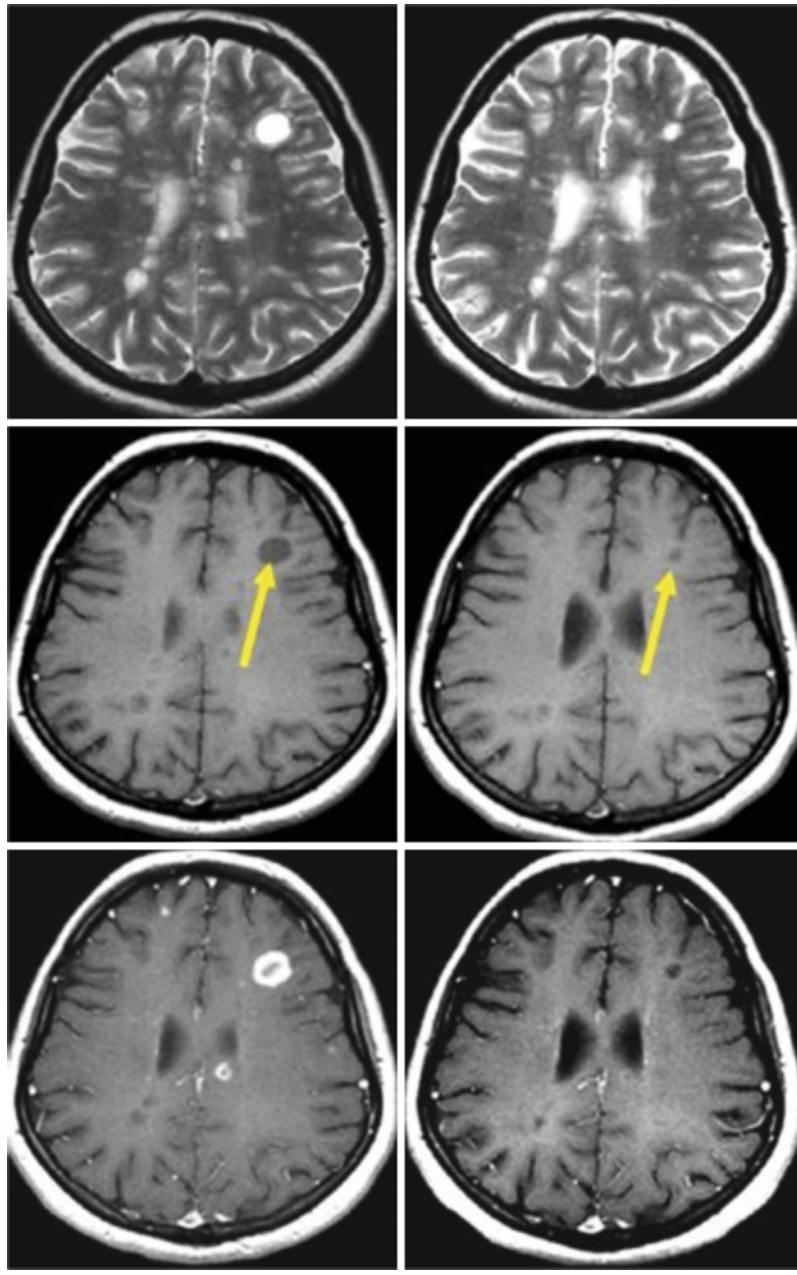

Fig. 15.2 Contrast enhancement in MS (Figure 5 from [45]; original caption: *Serial magnetic resonance imaging* (*MRI*) *scans obtained in a patient with relapsing-remitting multiple sclerosis. T2-weighted* (*upper row*), *unenhanced T1-weighted* (*middle row*) *and contrast-enhanced T1-weighted* (*lower row*) *MRI scans obtained at baseline* (*left*) *and 1 year later* (*right*). *Observe the active 'black hole' in the subcortical white matter of the left frontal lobe* (*arrow*), *which shows a ring-enhancement pattern of contrast uptake. After 1 year*, *the lesion decreased in size* (*arrow*), *but remained hypointense on T1-weighted images*, *indicating an irreversible black hole).* (Adapted from Reprinted by Permission from SAGE Publications, Ltd.: *Ther Adv Neurol Disord.* 6(5):298–310, copyright 2013

normal cerebral MRI at presentation have only a 5 % risk of subsequent clinical attack and thus of progression to clinically definite MS in the next 1–5 years [3]. Conversely, patients with cerebral lesions on MRI have a considerably higher risk, although the risk remains below 50 % when the total lesion volume does not exceed 1.2 ml [3].

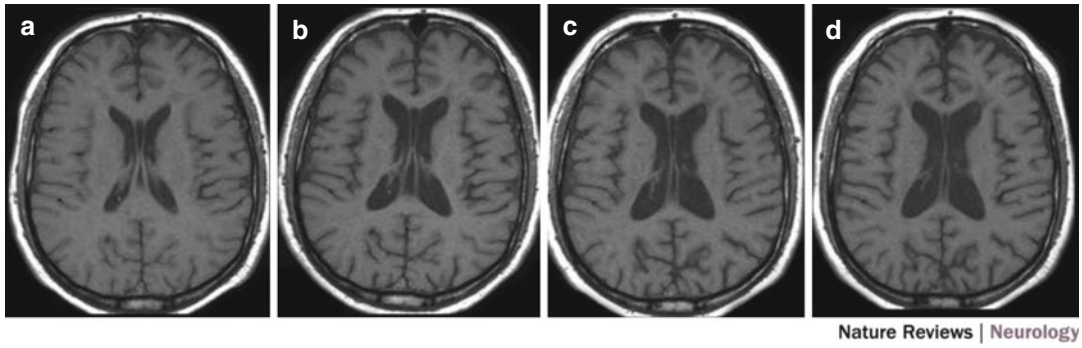

Fig. 15.3 Brain atrophy in an MS patient (Figure 2 from [60] with original caption: (***a***) *Baseline scan.* (***b–d***) *Regular scans over a 6-year follow-up period. Disease progression can be seen in the form of the increasing size of ventricular and subarachnoid spaces. These changes reflect brain volume loss over time, indicating progressive neurodegeneration*). (Adapted from Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Neurology* 11, 597–606, copyright 2015

When the disease evolves, it may take one of several forms: (1) relapsing-remitting MS (acute attacks are followed by remission periods), (2) primary progressive MS (steady worsening of neurologic functioning without any distinct relapses) and (3) secondary progressive MS (can follow relapsing-remitting MS and is characterised by a sustained build-up of disability, independent of any relapses). MR studies have confirmed the occurrence of lesions and the development of brain atrophy in all the MS types [14, 24, 29, 40].

Clinical evolution of MS is characterised by both motor and cognitive degradation [7, 41]. Pathological changes in the normal appearing white and grey matter are better correlated with progressive cognitive deficits than with visual, sensory and motor symptoms [4, 24, 51]. Brain atrophy, defined as the decrease in brain volume over time, is recognised as a typical consequence of MS [5]. The rate of brain volume loss in patients with MS exceeds the rate observed in healthy controls up to a factor of 2–8, that is, 0.5–1 % per year in MS patients versus 0.1–0.3 % per year in age-matched healthy controls [24, 48]. Formerly, it was believed that MS was defined pathologically as an inflammatory process confined to the white matter (WM). Nowadays, we know that in addition to white matter lesions, MS is also characterised by grey matter lesions and atrophy [15]. Moreover, MRI-based volumetric data have shown that grey matter atrophy better correlates with physical and cognitive disability than WM atrophy and T1 and T2 lesions [23, 24, 34].

Investigators have examined, and then confirmed, the unwritten rule that five new lesions (compared to the baseline MRI scan of the MS patient) are correlated with a higher risk of subsequent relapses [36]. A 10-year follow-up study on patients with relapsing-remitting MS confirmed the long-term clinical relevance of brain lesion evolution by showing that an accelerated clinical disability is particularly well correlated with the increase in T1 lesions [26]. Annualised lesion volume growth of 0.25 ± 0.5 mL ($+6.7 \pm 8.7$ %) for T2-weighted lesions and $+0.20 \pm 0.31$ mL ($+11.5 \pm 12.3$ %) for T1-weighted lesions was established over a period of 10 years [26]. In a 20-year follow-up of 107 MS patients, lesion growth was 0.80 mL/year in those who were relapsing-remitting but was 2.89 mL/year in secondary progression [18]. In another follow-up study comparing progressive and nonprogressive MS patients over 10 years, it was found that GM atrophy is a good candidate as a disease progression biomarker [30]. In addition, brain atrophy and lesion load have been shown to be correlated with long-term disability in a multicentre 10-year follow-up study [39]. Table 15.1 provides a summary of these investigations.

Table 15.1 Evidence for the relevance of lesion and atrophy biomarkers in MS

Biomarker	Findings	Study population	Reference
T2 lesions	Annualised change: +0.25 ± 0.5 mL	10-year follow-up RRMS	[26]
T1 lesions	Annualised change: +0.20 ± 0.31 mL	Idem	Idem
T2 lesions	Annualised change: +0.80 mL/year	20-year follow-up RRMS	[18]
T2 lesions	Correlated with disability progression	10-year follow-up	[39]
Total brain atrophy	Correlated with disability progression	Idem	Idem
Total brain atrophy	CIS = −0.40 % ± 0.47 %, RR = −0.49 % ± 0.65 %, SP = −0.64 % ± 0.68 %, PP = −0.56 % ± 0.55 %	>1-year follow-up 963 subjects	[12]
GM atrophy	Significant differences between progressive and nonprogressive	10-year follow-up	[30]

15.2.3 Treatment

MS researchers throughout the world acknowledge that, in addition to its well-established diagnostic value, MRI has an essential role in monitoring disease progression and therapeutic efficacy [2, 20]. Recent treatments, especially for the early stages of MS, focus on modifying the natural course of the disease and do not merely treat symptoms. Some of the more aggressive disease-modifying therapies can have serious side effects and are therefore not prescribed as first-line treatments. Criteria for switching from one treatment to another are still under active investigation. MRI-based monitoring of therapeutic effects becomes more and more essential in clinical trials and also in clinical practice. However, MRI-derived metrics are not yet standardised and still under development [2, 20, 27, 44].

In patients developing three or more active MRI lesions, in addition to a clinically active disease (relapses and disability progression), a change in treatment strategy is recommended [44]. During the course of disease-modifying therapy, new or enlarging lesions should be monitored on scans every 6 months to assess for change [43]. The presence of one or more Gd-enhancing lesions on a 6- or 12-month follow-up scan, or two or more new or enlarging lesions on a 12-month follow-up scan, should prompt consideration of therapy change [43].

Many lessons have been learned from clinical trials, for instance, that using lesion count and brain atrophy as endpoints might be more efficient than the Expanded Disability Status Scale (EDSS) [34]. Placebo-controlled trials in secondary progressive MS patients would require 32 subjects per arm to detect a 50 % treatment effect at 80 % power over 2 years, if MRI measures of brain atrophy (using the SIENA method [56]) were used as outcome measures [1]. Using EDSS as outcome measure, placebo-controlled trials would require about 150 patients per study arm to demonstrate statistically significant therapeutic effects for a study of 2–3 years [47].

The debate is ongoing whether whole-brain atrophy should be used as the gold standard for effective treatment of MS after the first year of treatment [48] or not [16]. A confounding factor is that whole-brain atrophy after 1 year of treatment might be an inaccurate parameter, due to the occurrence of pseudo-atrophy: the early reduction of brain volume as a result of a decreased inflammatory profile, rather than of the underlying disease [11]. Measuring GM atrophy, instead of whole-brain atrophy, is potentially more useful since pseudo-atrophy appears to affect white matter more than grey matter [48] and may persist for more than 2 years after treatment initiation.

Some clinical trials have shown that brain volume loss (or GM loss) is a good predictor for the

natural course of the disease [11]. However, when disease-modifying treatments (DMTs) are used, conflicting results have been observed in various clinical trials. Differences in the mechanism of action of the drugs, the patient populations, the quality of the MRI scans and the software packages used for the analysis could explain the occasional discrepancy between these results.

15.3 Acquisition Requirements

Widespread application of MR imaging biomarkers is hampered by issues such as non-standardised imaging protocols, imaging artefacts, lack of normative data and manual segmentations to interpret values in clinical practice. In order to mitigate such issues and promote MR imaging in MS clinical practice, the MAGNIMS study group published guidelines for the use of MRI in MS diagnosis [46], as well as recommendations to improve imaging and analysis of brain lesion load and atrophy in longitudinal MS studies [59, 60]. The group recommends that "images should be acquired using 3D pulse sequences, with near-isotropic spatial resolution and multiple image contrasts to allow more comprehensive analyses of lesion load and atrophy, across time points. Image artifacts need special attention given their effects on image analysis results" [59]. Image artefacts interfering with MRI readings include radiofrequency (RF) intensity non-uniformity, phase-encode ghosting, signal wrap and geometric distortion due to gradient non-uniformity and B_0 inhomogeneity.

Investigators of the Canadian MRI Consensus Group further specify that "a standardized MRI protocol is important during patient follow-up" [2]. The recommended brain MRI sequences are 3D FLAIR (or axial + sagittal FLAIR), post-gadolinium T1, axial T2 and/or PD, obtained with a minimum MRI field strength of 1.5 T and a slice thickness of 1 mm for T1 and ≤3 mm for FLAIR with no gap; total head coverage should include the entire brain and brainstem.

MSCare, the (US) Consortium of Multiple Sclerosis Centers [8], proposes a standardised MRI protocol that they regularly update (Table 15.2).

Table 15.2 MSCare guidelines for standardised MRI protocol

Standardized brain MRI protocol (diagnosis and routine follow-up of MS)	
Field strength	Scans should be of good quality, with adequate signal-noise ratio (SNR) and resolution (in-sections, pixel resolution of ≤ 1mm × 1mm)
Scan prescription Coverage Section thickness and gap	Use the subcallosal plane to prescribe or reformat axial oblique sections Whole brain coverage ≤3 mm, no gap (for 2D acquisition or 3D reconstruction)
Core sequences	Anatomic 3D inversion recovery-prepared T1 gradient echo (e.g. 1.0–1.5 mm thickness) Gadolinium single dose 0.1 mmol/kg given over 30 seconds[a] 3D sagittal T2-weighted FLAIR[b] (e.g. 1.0 to 1.5 mm thickness) 3D T2-weighted[b] (e.g. 1.0 to 1.5 mm thickness) 2D axial DWI (≤5 mm slices, no gap) 3D FLASH (non IR[c] prep) post-gadolinium[b] (e.g. 1.0 to 1.5 mm thickness) 3D series would be typically reconstructed to 3mm thickness for display and subsequent comparison for lesion counts
Optional sequences	Axial proton attenuation Pre-or post-gadolinium axial T1 spin-echo (for chronic black holes) Susceptibility weighted imaging (SW1) for identification of central vein within T2 lesions

Table 2 in [8]. Reprinted by permission from AMERICAN SOCIETY OF NEURORADIOLOGY: AJNR Am J Neuroradiol 37(3):394–401; copyright 2016

[a]Minimum 5-minute delay before obtaining post-gadolinium T1. The 3D sagittal FLAIR may be acquired immeaiately after contrast injection before the 3D FLASH series

[b]If unable to perform a 3D acquisition, then perform a 2D axial and sagittal FLAIR, axial fast spin-echo proton attentuation/T2, and axial post-gadolinium T1-weighted spin-echo at ≤3mm slice thickness

[c]Inversion recovery

These requirements are sufficient not only for visual assessment but also for automated image analysis software, since most packages performing MRI-based brain segmentation, atrophy computations or lesion segmentation work either on single MR images or on a subset of multiparametric images, simultaneously taken into account.

15.4 Analysis Methods

15.4.1 Cross-Sectional Biomarkers

15.4.1.1 Brain Volume Computations

The volume of the whole brain, or volumes of brain structures, can be easily computed through brain segmentation techniques. Brain segmentation implies that the whole brain, its constituent tissue types or individual brain substructures can be identified based on MRI(s). A typical first step is "brain extraction" or "skull stripping", a preprocessing step that ensures that only brain tissue is transmitted to the segmentation pathway. Various brain extraction methods, such as the brain extraction tool (BET) [55], brain surface extractor (BSE) [50], ROBEX [28], etc., are available. Approaches are diverse, including morphological, geometrical, image processing and modelling operations (hole filling, surface modelling, edge detection, intensity thresholding, atlas matching, deformable models, patch-based labelling, etc.). Moreover, the results of individual brain extraction methods can be enhanced by applying hybrid techniques, thus combining results from several individual methods.

After the first step of brain extraction, the process of brain segmentation can be started. This is typically based on a probabilistic modelling of voxel intensities, exploiting the fact that different tissue types have different MR image characteristics. Recent literature provides an excellent overview of brain segmentation methods [10]. Well-known and validated examples include FAST [61], SIENAX [56] and FreeSurfer [17]. Gaussian mixture models are popular; image intensities for each tissue type are modelled as a (sum of) Gaussian components. This modelling is usually performed using expectation–maximisation (EM), a well-known iterative parameter estimation algorithm. Spatial priors, serving as starting values and also as spatial constraints, can be obtained from appropriate brain atlases available in the literature [42]. The EM framework can be extended to intrinsically model some of the common distortions present in MR images, such as spatial inhomogeneity of image intensities known as bias field. Otherwise, such correction should be performed in preprocessing, e.g. using methods such as N3 or N4ITK [53, 58]. The EM results are probabilistic, i.e. each voxel is assigned probability of belonging to each of the classes of interest (WM, GM, CSF, etc.). These maps can be thresholded to obtain hard segmentations. Volumes in millilitres for each class can be computed either based on the hard or the fuzzy segmentation, by simply multiplying the sum of the tissue segmentation over all voxels by the voxel volume.

15.4.1.2 Lesion Detection and Volume Estimation

Some automatic lesion segmentation methods belong to the family of supervised classification methods, for which a representative training dataset, including expert segmentation, is required in order to build a model that can be used on new patients for lesion segmentation. Depending on the features extracted from images (local gradient intensity, mean intensity, spatial information, etc.) and on the type of classifier (k-nearest neighbours, artificial neural networks, Bayesian learning, support vector machines, etc.), many variants have been proposed ([22, 32, 33, 57]; see also García-Lorenzo et al. [21] and Mortazavi et al. [37] for overviews of algorithms and software solutions). Although excellent results can be obtained with supervised classification on the training dataset, these methods have two disadvantages. The first difficulty lies in building a training dataset that encompasses MS lesions of all possible shapes and intensities and is heterogeneously distributed in the WM. The second nontrivial problem lies in preprocessing a new image (acquired on a different scanner than

the one used for the training dataset), such that it matches the characteristics of the training dataset, e.g. by intensity normalisation. In other words, supervised methods perform well only when the new image to be segmented is well represented in the training dataset.

Another family of methods is based on unsupervised classification and does not require training images. These methods are usually based on stochastic modelling of voxel intensity distribution. They perform brain segmentation into GM, WM and CSF (with or without lesion detection) and often rely on post-processing approaches in order to segment lesions (e.g. lesion growing or pruning). The assumptions that are made in order to segment lesions have a great impact on the results. For instance, LST [49] and MSmetrix [31] detect FLAIR-hyperintense outliers, which are further promoted as lesions according to their spatial probability of being in the WM, where the WM segmentation is basically derived from T1-weighted image segmentation. LesionTOADS [52], on the other hand, employs a sophisticated mechanism of combining information from different MR sequences (T1-weighted, T2, PD or FLAIR) in order to simultaneously segment lesions and brain structures, while distance maps from the boundaries of structures such as CSF are used to confine the segmented lesions to typical locations.

15.4.2 Longitudinal Biomarkers

In contrast to cross-sectional approaches, longitudinal methods take into account two (or more) MRI scans of the same subject from different time points to calculate brain volume changes or atrophy. Typical preprocessing steps prior to longitudinal atrophy computations include [13] extraction of the intracranial cavity mask at baseline, correction of intensity inhomogeneities, rigid registration of follow-up scans on the baseline scan and differential bias field correction to correct for differences in intensity inhomogeneity artefacts.

Longitudinal methods for brain atrophy typically try to match two MRI scans using registration techniques and directly extract small changes in brain volume from this process. Approaches include brain edge motion analysis, voxel-based statistical analysis for voxel-based morphometry, statistical parametric mapping and local Jacobian determinant analysis after nonlinear matching between coregistered images [6, 19, 54, 56].

In what concerns lesions, many methods focus on segmenting MS lesions at a single time point, and there is not yet a single approach, according to the review of Lladó et al. [35], that can emerge as a standard in clinical practice for the analysis of lesion evolution over time.

15.5 How to Transmit the Information to the Clinician

Consistent with current clinical practice, MS patients are referred for an MRI examination by their neurologist. Good communication between neurologist and (neuro)radiologist is of paramount importance. According to recent recommendations [2], the radiologist should report back to the neurologist, qualitatively if not quantitatively, over the lesion status and the atrophy of MS patients, covering the following points:

- Comparison with previous scan(s)
- Evidence of new disease activity
- Number of new lesions (T2/T1)
- Lesion size
- Overall assessment, including the presence (definite/probable) and extent (number of new/enlarging lesions or gadolinium-enhancing lesions) of disease activity, change in T2 lesion volume and evidence of brain atrophy

Taking into account these recommendations, it is obvious that MRI biomarkers are already considered an important factor for making therapeutic decisions. Unfortunately, most MRI reports are written in prose and do not make use of the full potential embedded within the MRI datasets. Fortunately, communication regarding MRI findings between the (neuro)radiologist and the

neurologist can be improved with automatically computed, quantitative values for the relevant imaging biomarkers. To this end, the (neuro)radiologist should have easy access to approved tools for calculating these biomarkers.

In addition to having access to a structured radiology report, which includes quantitative data, the neurologist would benefit from having easy access, not only raw MRI scans, but also annotated image data sets. For example, the neurologist could examine overlays of tissue segmentations compared to previous MRI scans, overlays of individual brain structures or colour-coded overlays of lesions (new, enlarging or gadolinium-enhancing lesions).

For an adequate follow-up of patients with MS, it is essential to present the evolution of the imaging biomarkers in a relevant context. For instance, all available time points from a single patient should be used to plot the trend of each biomarker over time (see Fig. 15.4). These data could then be correlated with possible changes in treatment, or other events, over the same timeline. Furthermore, when following the evolution of changes in an individual patient, comparisons could be made of biomarker values against relevant populations (e.g. healthy controls, MS patients that respond well to therapy, etc.). Obviously, relevant confounding factors (such as age and sex) should be taken into account.

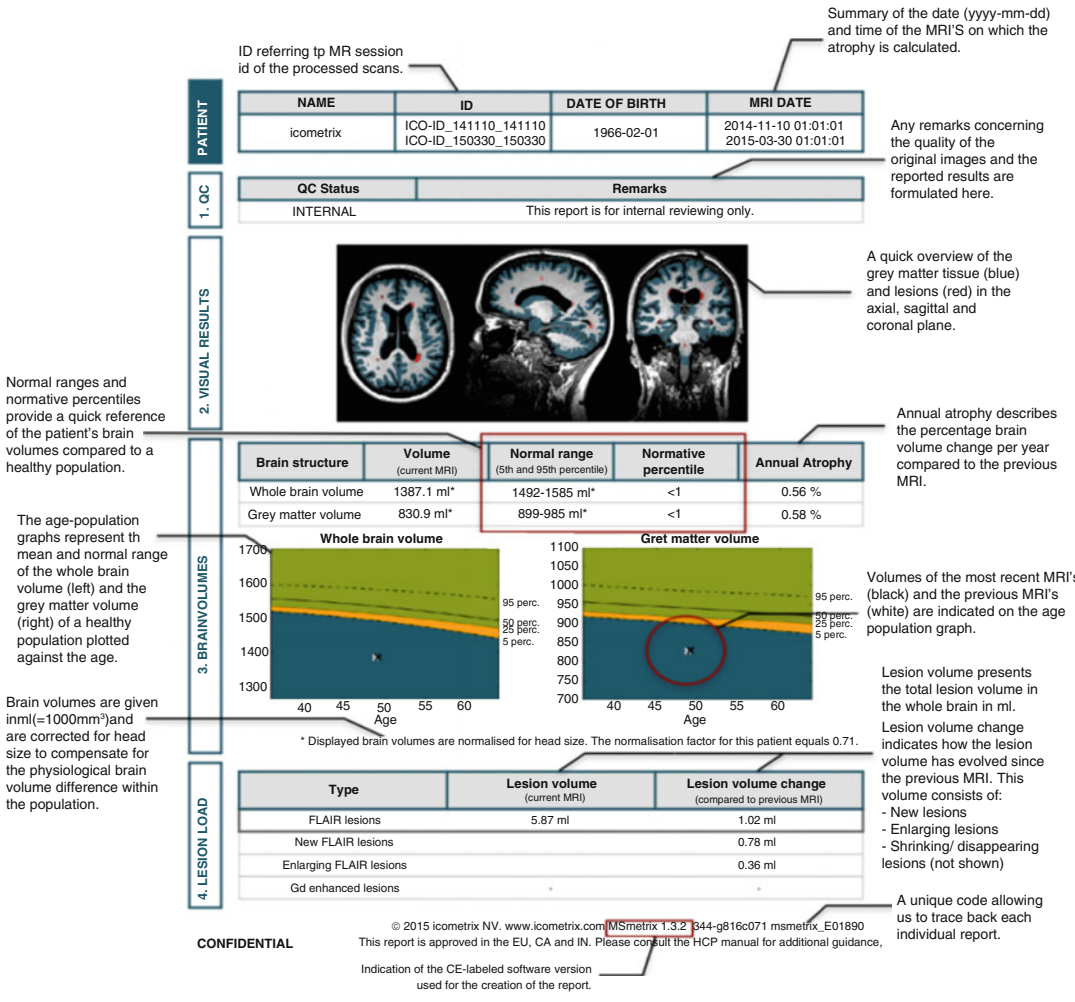

Fig. 15.4 Example of a typical MS imaging biomarker report (Image courtesy of ico*metrix*, Belgium)

The development of imaging biomarkers has led to a significant improvement in the diagnosis, management and follow-up of patients with MS. Standardisation of MRI acquisition protocols and improvement of quantitative reporting tools will provide better understanding of the natural history of MS and allow accurate treatment monitoring, for the greater benefit of patients.

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