Magnetic resonance guidance for the multidisciplinary management of pelvic malignancies

Clinical potential and technical limitations

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1 Preface

1.1 Abbreviations
ADC  Apparent diffusion coefficient
ADT  Androgen deprivation therapy
AE1/AE3  Anti-Epithelial
AJCC  American Joint Committee on Cancer
APR  Abdominoperineal resection
CAP  College of American Pathologists
CAPRA  Cancer of the Prostate Risk Assessment
CD31  Cluster of differentiation 31
CNR  Contrast-to-noise ratio
CR  Complete response
CRM  Circumferential resection margin
CRT  Chemoradiotherapy therapy
CT  Computer Tomography
CTV  Clinical tumour volume
DCA  Dynamic conformal arc
DCE  Dynamic contrast enhanced
DF  Denonvilliers fascia
DFS  Disease free survival
DRE  Digital rectal examination
DSC  Dynamic susceptibility contrast
DTI  Diffusion tensor imaging
DVH  Dose-volume histogram
DW  Diffusion weighting
EAU  European Association of Urology
ELAP  Extralevator abdominoperineal excision
EMD  Extramural depth
EMVI  Extramural venous invasion
EPE  Extraprostatic extension
EPI  Echo planar imaging
ESUR  European Society of Uroradiology
ETME  Extended total mesorectal excision
F-MISO  18F-Fluoromisonidazole
FDG  Fluorodeoxyglucose
FDP  Fast diffusion pool
FIGO  Federation of Obstetricians and Gynaecologists
FLOX  Fluorouracil Leucovorin Oxaliplatin
GE  Gradient echo
gEUD  generalized equivalent uniform dose
GIST  Gastrointestinal stroma tumour
GTV  Gross tumour volume
Gy  Gray - unit of ionizing radiation dose
H&E  Hematoxylin and eosin
HDR  High-dose-rate
HeLa  Henrietta Lacks
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>HMRS</td>
<td>Hydrogen magnetic resonance spectroscopy</td>
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<tr>
<td>ICRU</td>
<td>International Commission of Radiation Units and Measurements</td>
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<tr>
<td>IM</td>
<td>Internal margin</td>
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<tr>
<td>IMRT</td>
<td>Intensity-modulated radiation therapy</td>
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<td>ITV</td>
<td>Internal tumour volume</td>
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<td>IVIM</td>
<td>Intravoxel incoherent motion</td>
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<tr>
<td>LAPC</td>
<td>Locally advanced prostate cancer</td>
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<td>LAR</td>
<td>Low anterior resection</td>
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<tr>
<td>LARC</td>
<td>Locally advanced rectal cancer</td>
</tr>
<tr>
<td>LARC-RRP</td>
<td>Locally Advanced Rectal Cancer – Radiation Response Prediction</td>
</tr>
<tr>
<td>LF</td>
<td>Levator ani fascia</td>
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<tr>
<td>LR</td>
<td>Local recurrence</td>
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<tr>
<td>MDT</td>
<td>Multidisciplinary treatment</td>
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<td>MERCURY</td>
<td>Magnetic Resonance Imaging and Rectal Cancer European Equivalence</td>
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<td>MR</td>
<td>Magnetic resonance</td>
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<td>MRCP</td>
<td>Magnetic resonance cholangiopancreatography</td>
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<td>MRF</td>
<td>Mesorectal fascia</td>
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<td>NACT</td>
<td>Neoadjuvant chemotherapy</td>
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<td>NGICG</td>
<td>Norwegian Gastrointestinal Cancer Group</td>
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<tr>
<td>NIN</td>
<td>Number of infiltrated nerves</td>
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<td>NVB</td>
<td>Neurovascular bundle</td>
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<td>OARs</td>
<td>Organ at risk</td>
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<td>OS</td>
<td>Overall survival</td>
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<td>PACS</td>
<td>Picture archiving and communication system</td>
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<td>PD</td>
<td>Proton density</td>
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<td>PR</td>
<td>Partial response</td>
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<td>PD</td>
<td>Progressive disease</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>PF</td>
<td>Prostatic fascia</td>
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<tr>
<td>PI-RADS</td>
<td>Prostate Imaging-Reporting and Data System</td>
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<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
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<td>PTV</td>
<td>Planning target volume</td>
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<tr>
<td>PW</td>
<td>Perfusion weighting</td>
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<tr>
<td>RALP</td>
<td>Robotic-assisted laparoscopic prostatectomy</td>
</tr>
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<td>RECIST</td>
<td>Response evaluation criteria in solid tumours</td>
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<td>RCRG</td>
<td>Rectal cancer regression grade</td>
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<td>RF</td>
<td>Radio frequency</td>
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<tr>
<td>RT</td>
<td>Radiotherapy</td>
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<td>SAR</td>
<td>Specific absorption rate</td>
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<td>SBRT</td>
<td>Stereotactic body radiotherapy</td>
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<td>SD</td>
<td>Stable disease</td>
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<td>SDF</td>
<td>Slow diffusion pool</td>
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<td>SE</td>
<td>Spin echo</td>
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<td>SIB</td>
<td>Simultaneous integrated boost</td>
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<tr>
<td>SM</td>
<td>Set-up margin</td>
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<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<tr>
<td>SPCG</td>
<td>Scandinavian Prostate Cancer Group</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<td>---------</td>
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<tr>
<td>SRS</td>
<td>Stereotactic radiosurgery</td>
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<tr>
<td>T</td>
<td>Tesla</td>
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<tr>
<td>TEM</td>
<td>Trans-anal endoscopic microsurgery</td>
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<tr>
<td>TME</td>
<td>Total mesorectal excision</td>
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<td>TNM</td>
<td>Tumour-Node-Metastasis</td>
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<tr>
<td>TRG</td>
<td>Tumour regression grade</td>
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<tr>
<td>TRUS</td>
<td>Transrectal ultrasonography</td>
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<tr>
<td>UCSF</td>
<td>University of California, San Francisco</td>
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<tr>
<td>UICC</td>
<td>Union for International Cancer Control</td>
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<tr>
<td>VHF</td>
<td>Very high frequency</td>
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<td>VMAT</td>
<td>Volumetric-modulated arc therapy</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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1.2 List of papers

Papers included in the thesis

Paper 1

Paper 2

Paper 3

Paper 4
*Submitted to European Radiology*
1.3 Other relevant publications not included in the thesis


2 Introduction

Magnetic resonance (MR) has evolved to become the preferred method to map the local extent of most pelvic malignancies. Clinical decision-making is to a large extent based on imaging: patient stratification, treatment planning, treatment response assessment and detection of recurrence exploit the opportunities provided by imaging.

Multidisciplinary approach to cancer care has emerged over the past decade. Multimodal treatment is planned and evaluated in multidisciplinary teams of oncologists, surgeons, radiologists and pathologists, a collaboration in which imaging is essential.

The final outcome of multimodal cancer treatment is based on interdependent decisions where the impact of image guidance is difficult to isolate. Considered that MR provides the premises of decisive treatment choices, it is essential to closely scrutiny the method itself. This thesis seeks to explore the potentials and explain the limitations of MR guidance, studying mainly surgical materials where correlation to histopathology can demonstrate the limitation of MR as a method. The two frequent pelvic malignancies, prostate cancer and rectal cancer were chosen. The discussion analyses the findings with emphasis on clinical significance and technical limitations, and considers the conditions for transferability of the results.

Histopathology is the reference standard for MR interpretation and is also widely used as a short-term endpoint of cancer treatment. Consequently, the assessment of multidisciplinary cancer management in general, and the accuracy of MR in particular depend critically on the histopathologic interpretation. Therefore, the thesis also requests limitations of histopathology as a method.

In the context of multidisciplinary management of pelvic malignancies, this thesis explores MR guidance of:

- **Surgery**
  - Resection of T3-prostate cancer
  - Resection of T4-rectal cancer

- **Oncology**
  - Neoadjuvant chemotherapy: tumour volume changes
  - Chemoradiotherapy: tumour downstaging and volume changes
  - Radiotherapy: adaptive radiation dose escalation (or de-escalation)

- **Histopathology**
  - Sampling of specimens
  - Orientation in sections
3 Background

3.1 Evaluating image guidance in multidisciplinary management

3.1.1 Variable and conflicting results of image guidance

Imaging has over the past two decades evolved from a supplementary diagnostic tool to a premise provider of multidisciplinary cancer management. While some cancer treatment communities were critical, others quickly incorporated MR into patient stratification and treatment planning. The early results from staging of rectal cancer with MR were promising [1, 2]. When later confirmed in a large multicenter study[3], MR became the method of choice for treatment stratification of rectal cancer and technical requirements for the MR examination were established.

Encouraged by the promising results in staging rectal cancer and cervical cancer, our institution began staging prostate cancer using MR in 2005. However, the results were substantially inferior. Our disappointing experiences were in accordance with the literature, reporting a very wide range of staging accuracies and no consensus regarding technical standard.

What are the causes for these differences in staging performance? Possible explanations may be differences in tumour biology, technical limitations or higher precision requirements needed for surgical planning.

Following neoadjuvant multimodal treatment of rectal cancer, the staging accuracy decreased[4]. Some researchers claimed however that complete tumour regression at MR was a reliable biomarker of pathologic complete response (pCR) [5], whereas others considered that small clusters of residual tumour remains a problem [6]. Functional imaging techniques such as diffusion weighting and fluorodeoxyglucose positron emission tomography (FDG-PET) were introduced leading to early promising results in the assessment of treatment response [7], but these methods are still not regarded as reliable predictors of pCR. What are the causes for the modest staging accuracy following neoadjuvant treatment?

Understanding the causes of inaccurate MR results are critical for the appropriate use of MR in treatment planning and a prerequisite for further technical developments.
3.1.2 Choice of material

Exploring the potentials and limitations of image guidance requires a patient material where image findings may lead to modification of the treatment. Locally advanced tumours were chosen since individual image guidance often is needed to determine the appropriate dissection plane and neoadjuvant oncologic treatment may induce sufficient shrinkage to allow adjustments in the subsequent treatment.

3.1.3 Long-term and short-term endpoints

In evaluation of cancer treatment, overall survival (OS), disease-free survival (DFS) and local recurrence rates (LR) are well established and the most important endpoints, assessed against treatment morbidity and cost-benefit considerations. These endpoints are particularly long-termed if the tumour growth is slow as in prostate cancer or if the efficacy of the treatment is substantial, but not curative, such as for rectal cancer treatment [8].

When introducing new surgical techniques, new oncologic treatment regimens or improvements in imaging, short-term endpoints are warranted as surrogate markers of the long-term outcome. As histopathology is widely acknowledged as the golden standard of short-term endpoints, correlation to histopathology is generally used to evaluate the staging accuracy of imaging.

3.1.4 Methodological challenges in the assessment of multidisciplinary treatment

Interrelationship is an inherent challenge to research on multidisciplinary treatment (MDT). Improvement of one factor does not necessarily lead to better outcome because other factors may limit or mask the impact of the improvement. Although the ultimate goal is to improve OS and LR, it is necessary to assess the efficacy of each single step in the complex package of multidisciplinary decisions. Individualized treatment is another inherent challenge of research on multidisciplinary management. It is methodologically challenging to compare outcome of treatments that are initially customized, and subsequently adjusted as a result of individual response. Due to these methodological challenges in the assessment of MDT, it is important to investigate the clinical potentials and technical limitations of image guidance, as a part of the multidisciplinary management.

3.1.5 Explaining the limitations of image guidance

The ambition of this work was to understand the limitations at a sufficiently basic level to transfer the knowledge into other tumour entities and applications in MDT. The explanations of the limitations are searched in three main areas: In technology, i.e. voxel size and tissue contrast, in tumour biology, i.e. growth pattern and treatment response at a cellular level, and in the clinical treatment, i.e. needed precision based on anatomical relationships. Explaining the limitation is important to understand how and in which direction MR can be developed to improve MDT.
3.1.6 Exploring the potentials of image guidance

When exploring the potentials of a method there are two ways to go other than improving the method itself. The most evident is to test the method on new application areas, but also a close scrutiny of the reference standard is fundamental. A method could not be proven to be better than its reference standard; really true findings will be regarded as false if the reference is negative. Hence, shortcomings inherent in the reference standard may disregard the detection of improvements. In this thesis the potentials are explored by critical review of histopathology as a reference and by application of MR guidance to new treatment strategies such as simultaneous integrated boost (SIB) and volumetry of neoadjuvant chemotherapy.

3.1.7 Generalizability and transferability

Generalizability means that the results of research in one situation are extended to similar situations. In the context of this thesis, this applies to whether the results from a specific patient cohort are representative for all patients in the same situation. This may be interpreted either as for patients with the same diagnosis or as for patients with equally advanced stage of the same disease. The terms generalizability and transferability are somewhat interrelated and not mutually exclusive. In this thesis most considerations to what extent observations are applicable to other patients and other situations are generally understood under the term transferability.

Transferability means applying the results of research in one situation to other similar situations. In the context of this thesis, transferability can be divided into two categories: whether the results are transferable to other types of treatment or to different tumour stages and types.

3.1.8 Modality-specific research questions

Surgery
Surgical treatment of locally advanced cancers is particularly dependent on image guidance. Tumours extending beyond the organ must be reliably detected and charted preoperatively to provide adequate patient treatment. When a tumour no longer is organ-confined, it is crucial to identify the appropriate surgical dissection plane in order to remove tumour completely with minimum morbidity. Hence, to what extent can MR be relied upon to guide surgical treatment of locally advanced cancer?

Oncology-chemotherapy
Neoadjuvant and concomitant multimodal treatment are advancing in the search for reduced morbidity and improved outcome, particularly in the management of locally advanced cancers. These developments place new demands on imaging. Even if MR precisely and robustly delineates the extent of tumour in pre-treatment staging of rectal cancer, one might ask: does this accurate staging performance also apply to the assessment of residual tumour following chemo- and radiotherapy?
Oncology-radiotherapy

Radiotherapy planning must balance the demands of a high dose to the target volume with a low dose to the neighbouring structures at risk. MR is increasingly used, co-registered with computer tomography (CT), to optimize radiation treatment planning: to define the target volume and to outline the organs at risk. As advances in treatment planning and delivering techniques enable more complex and sharply defined 3D radiation volumes, the demands for, and the challenges of image guidance increase correspondingly. Could MR-guided reduction of the target volume (as the tumour shrinks during the treatment), enable a higher dose to tumour without increasing the dose to the organs at risk?

Histopathology

The information from histopathology is highly dependent on adequate sampling. The strength of histopathology lies in superior details whereas the advantages of imaging are overview and anatomical relationships. Histopathologic sectioning and sampling are particularly challenging if the surgical specimens are bulky. Neoadjuvant and concomitant treatments pose further challenges as considerable decrease of tumour load may aggravate the sampling difficulties. Hence, may the overview provided by MR guidance improve the microscopic interpretation?

3.1.9 Limitations of the thesis

This thesis does not explore MR guidance of interventional procedures in the work-up and treatment of pelvic malignancies. However, the thesis discusses whether the findings from image guidance of surgical and oncological treatment are transferable to interventional procedures. This applies to guiding a number of procedures, such as biopsy sampling, embolisation and focal ablative treatment options, such as hyperthermia (focused ultrasound, laser and radiofrequency), hypothermia (cryotherapy), local instillation of cytotoxic agents (ethanol etc) as well as high-dose-rate (HDR) brachytherapy and radiosurgery.

3.2 MR

3.2.1 From proton to anatomical information

The signal in MR originates from the nucleus of the hydrogen atom, which consists of a single proton. Because the proton is electrically charged and rotates around its own axis, it possesses magnetic properties; i.e. all hydrogen nuclei are small magnets. Normally their orientation is random so there is no net magnetic force. In the presence of an external magnetic field, the protons align either parallel or anti-parallel to the external field with small excess in the parallel direction that creates a net magnetic force. The protons, and thereby the net magnetism, can be brought into synchronised motion by electromagnetic waves. Once the magnetism of the tissue is moved away from the strong external field, it can be measured and a MR signal can be produced. The signal is captured in receiver coils, which are placed close to, or on the patient.
Placed in an external magnetic field, protons precess around the direction of the external field, and also rotate around its own axis. The frequency of this precession, termed the Larmor\(^1\) frequency, is proportional to the strength of the external magnetic field. This frequency is essential in MR since the frequency of the transmitted electromagnetic waves must match the precession frequency of the protons in order to exchange energy and achieve magnetic resonance.

Currently, clinically used scanners typically have field strength of 1.5 or 3 tesla (T). The transmitted pulses necessary to achieve magnetic resonance are within the range of radiofrequencies\(^2\) and accordingly termed radiofrequency (RF) pulses. The frequencies are slightly below the frequencies used in cell phones and far below ionising radiation; consequently there is no danger of causing irradiation injury by MR imaging. Local or global heating can however be a problem, so the specific absorption rate (SAR) is monitored during MR scanning.

The tissue contrast in MR imaging depends on the size of the molecule that hydrogen is part of. In addition to the share density of the protons (PD), there are two main mechanisms causing signal differences in MR; the energy that the tissue has absorbed from the transmitted RF-pulse is released to the neighbouring protons (T1-relaxation) and loss of synchronized motion (coherence) of each proton causing loss of net magnetism (T2-relaxation).

**T1-relaxation**

The efficacy of the energy release is a function of the tumbling rate of the molecule, which in turn, is given by the size of the molecule. When the tumbling rate is close to the Larmor frequency (the precession frequency of the hydrogen nuclei) the energy exchange is fast. Fatty tissue is composed of medium-sized molecules yielding effective energy exchange, whereas large molecules (in proteins) or very small molecules (water) tumble at rates very far from the Larmor frequency, causing the energy exchange to be slow. T1-weighted (T1W) MR sequences are designed to maximize differences in T1-relaxation, leading to high signal from fat, low signal from solid tissue and very low signal from water.

**T2-relaxation**

In order to obtain a signal in MR, the protons must move synchronised (in phase) so that the very small magnetic force of each proton adds up to a measurable magnetic force. As soon as the transmitted RF-pulse is turned off, the protons inevitably begin to point in different directions caused by magnetic field inhomogeneities and consequently the net signal will decay. Local differences in the magnetic field may be due to imperfection of the external field or introduced by air-tissue interfaces, haemorrhage or magnetic implants etc. However, differences in molecule size also determine an inherent, “true” T2-contrast of the tissue. Large molecules with slow tumbling rates are thus influenced in time by relatively static magnetic forces from neighbouring atoms leading to lasting differences in precession frequency and rapid loss of net signal. Conversely, small water molecules that

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\(^1\) Named after Joseph Larmor, Irish physicist and mathematician (1857-1942)

\(^2\) Very high frequency (VHF) radio frequencies are between 30 and 300 MV.
tumble extremely fast are exposed to rapidly changing neighbouring magnetic forces that do not accumulate to substantial effect; hence, the net magnetic force is maintained. T2-weighted (T2W) MR sequences are designed to produce different grades of tissue contrast ranging from subtle signal differences, reflecting nuanced tissue composition, to high-contrast “water only” images as in MRCP\(^3\) and MR-urography. Moderately T2W images most closely resemble the tissue contrast of hematoxylin- and eosin-stained histopathology sections and are thus the preferred MR sequences for morphologic correlation studies.

**Pulse sequences**

There are two main types of imaging sequences used in MR: spin echo (SE) and gradient echo (GE), possessing different assets and drawbacks. SE uses trains of RF-pulses to minimize artefacts due to magnetic field inhomogeneities, which enables high signal intensity, however at the cost of long scan times. Typically, SE is used to produce high-resolution morphologic images. GE basically generates lower signal magnitude and is much more prone to artefacts. As the main advantage lies in much faster acquisition times, GE is commonly used in examinations of moving anatomic structures and for functional imaging. The T2W produced by a GE sequence is termed T2*W and is considerably more sensitive to (static) magnetic field variations.

**Coils**

The distance from the tissue to the receiving coil(s) is crucial for the signal strength. For high-resolution MR the built-in body coil is too far from the patient and therefore local coils must be placed on or inside the patient. Phased array coils are composed of several small coils combined to record the signal simultaneously and independently, resulting in better signal-to-noise ratio (SNR) than from one large coil. The array of coils also enables parallel imaging in which the spatial information of each coil is utilized to reconstruct an undersampled image, resulting in shorter scan times and potentially less artefacts. Increasing the number of coils may reduce the scan time. However, smaller coil elements introduce signal inhomogeneities because the depth penetration decreases. The use of an internal coil, e.g. endorectal coil, can compensate for these geometry-dependent signal inhomogeneities, however with substantial drawbacks. In addition to patient discomfort, increased costs and prolonged preparation time for the examination, an endorectal coil introduces technical challenges. The anatomical displacement caused by the coil is not appropriate for image guidance of radiotherapy planning, and the compression of tissue may lead to underestimation of surgical margins. Furthermore, image fusion with other modalities, such as PET/CT for tissue characterization or ultrasound for biopsy guidance is hampered by the presence of an endo-luminal coil.

3.2.2 From proton to functional information

While morphologic imaging reflects the anatomy, functional imaging exhibits additional, mainly time-dependent information. Diffusion, perfusion and hydrogen spectroscopic

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\(^3\) Magnetic Resonance CholangioPancreatography
imaging (HMRS) are well established and commonly used functional MR techniques in oncologic imaging.

**Diffusion weighting**

Image contrast in diffusion weighting (DW) depends on the spatial motion of water molecules; the more motion the smaller signal. The mechanisms behind limited water diffusion in biologic tissue are complex and not fully understood (Fig 3.1). The terms *restricted* and *hindered* are often used, but not consistently defined. According to one of the pioneers of diffusion MR, Denis Le Bihan, *restricted* refers to water molecule movements limited to confined geometries, whereas *hindered* covers the multifactorial mechanism [9]. Some authors use these terms synonymously.

**Fig 3.1** A) Elementary mechanisms of hindered diffusion. Diffusion is restricted (green) when boundaries prevent molecules from moving freely. Barriers may be strictly reflecting, partially absorbing or semi-permeable (red). Tortuosity (blue) is the concept where water, in the presence of obstacles must travel longer paths to cover a given distance. B) The observed biphasic behaviour of diffusion in biological tissues. (Images: Le Bihan [10])

The overall observed effect (Fig 3.1 B) of (hindered) diffusion in biological tissue is however a biphasic behaviour; a fast diffusion pool (FDP) and a slow diffusion pool (SDF) (Ackerman, Le Bihan, book). The two pools are observed in both the intra- and extracellular compartments. The slow diffusion pool is probably caused by layers of structured water adjacent to cell membranes (Fig 3.2) (and to lesser extent to the proteins) resulting in reduced diffusion parallel to their surfaces [9]. The structured water layers are of particular interest to cancer imaging because the diffusivity of water molecules correlates (negatively) with cellular membrane density, making DW a suitable imaging tool for visualization and characterisation of high cellular tissues, such as cancer. Also other conditions characterised by high cellularity, such as cellular inflammation and especially abscess formation (granulocyte rich) give rise to high signal intensity in DW images, as do protein rich fluids.
Fig 3.2 Illustration of bulk water in fast exchange with structured water, a concept that might correspond to the observed slow and fast diffusion pools of water in biological tissues. There is a strong interaction of the cellular membrane (green) with its first hydration layer [11].

Diffusion weighting is achieved using a water sensitive sequence, i.e. T2W, combined with a diffusion gradient. The magnitude of the diffusion weighting is determined by the strength and the duration of the gradient, denoted as \( b \)-value. Heavier weighting increases the tissue contrast but reduces the total signal strength. The appropriate degree of weighting is therefore a balance between contrast and signal. DW is mainly performed using SE echo-planar (EPI) sequences, which allow for very rapid acquisitions, but are highly susceptible to artefacts and geometric distortions. Despite extensive signal averaging, DW is generally low in signal strength.

In cancer imaging, two approaches to DW are generally used; qualitative and quantitative. Qualitative DW refers to heavily DW where mainly low diffusion pool gives rise to signal, causing cellular tissue to appear bright against low signal background. Quantitative DW refers to the use of more \( b \)-values (minimum two) to calculate the diffusivity measured as the apparent diffusion coefficient (ADC). The term *apparent* refers to the fact that the mathematical method used is quite simple compared to the complex multifactorial, not fully understood mechanisms of diffusion. Since high cellular tissue has low water diffusivity, cellular tumour is displayed with low signal on ADC maps. Hence, tumour is not as conspicuous as on heavily DW images.

Where capillaries are randomly distributed in space, movement of water (blood) in microvasculature might mimic diffusion. Dependent of vessel size, microperfusion might contribute to the DW signal up to \( b \)-values of 600 (s/m\(^2\)) [9]. The term *intravoxel incoherent motion* (IVIM) was introduced to cover all molecular displacements to which DW could be sensitive, and is used to describe DW imaging using low, perfusion-sensitive \( b \)-values. IVIM-DW is a potential tool for tumour characterisation and evaluation of (antiangiogenetic) treatment response, but not covered in this thesis.
The major asset of quantitative DW is that the ADC correlates to the cell membrane density as well as to a change in cellularity, thereby facilitating characterisation of primary tumours and assessment of treatment response, respectively. Following treatment, ADC vary depending on the response mechanism: In use of cytotoxic therapies, tumour lysis leads to disruption of the cell membranes and abrupt increase in ADC reflecting bulk water diffusion of liquefying necrosis. In use of anti-angiogenic therapies, cellular ischemia induces abrupt decrease in ADC reflecting coagulative necrosis. In anti-tumour treatment inducing apoptosis (Fig 3.3), ADC changes more slowly reflecting the gradually decreased cellular density, however ADC may increase or decrease [12]; good treatment response eventually leads to replacement of tumour cells, either by reparative tissue such as fibrosis or re-establishment of the normal tissue. Fibrotic tissue has very low signal on T2W images, consequently also at ADC maps. As both fibrosis and tumour have low signal, they may be indistinguishable at ADC maps. Therefore, ADC is best suited for assessment of early treatment response.

![Fig 3.3 Illustration of cellular response to anti-tumour treatment inducing apoptosis.](image)

**Perfusion weighting**

Perfusion weighting (PW) refers to imaging of temporal changes in tissue signal as a result of blood flow. The temporal signal changes originate from the kinetics of the intravenously administered contrast agent (gadolinium). At low concentrations the most pronounced effect of gadolinium is shortening of T1-relaxation, and hence increased signal intensity on T1W sequences, whereas at high concentrations, as following an arterial bolus, the shortening of T2-relaxation predominates, leading to signal decrease or complete dropout on T2W sequences. Accordingly, there are two categories of perfusion imaging; dynamic contrast enhanced (DCE) imaging that utilizes the T1-effect of gadolinium, and dynamic susceptibility\(^4\) contrast (DSC) that makes use of the T2-effect. DSC images a transient bolus and reflects the vasculature itself; blood flow, blood volume and mean transit time, whereas DCE images not only the through-pass, but also the permeability-dependent exchange of contrast between the intravascular and the extravascular spaces. Accordingly, DCE is also termed permeability imaging. Perfusion imaging is always a compromise between temporal and spatial resolution. While DCE is performed by standard GE sequences facilitating spatial resolution, DSC utilizes very fast EPI-based (GE or SE) sequences enabling high temporal resolution. Traditionally, DSC is used in brain imaging where the superior sensitivity of head coils provides strong signals, whereas DCE is

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\(^4\) Magnetic susceptibility indicates the degree of magnetization in response to an applied magnetic field
preferred in body imaging. Nevertheless, rapid parallel imaging techniques make high temporal resolution possible for T1-perfusion (DCE), and high field strengths enable high-amplitude T2*-perfusion (DSC) for body imaging.

The promising technique of arterial spin labelling may produce bloodflow-dependent tissue characteristics without the use of intravenous contrast; however, it has yet not reached clinical routine, at least not outside the brain.

**Spectroscopy**

Hydrogen spectroscopy (HMRS) was not used in this thesis and is generally not used in the management of pelvic malignancies. HMRS is a technique to visualize the biochemistry (the metabolites), not the structure of the tissue. The basic principle is that hydrogen nuclei precess at different frequencies depending on which molecule they are bound to. The molecule-dependent alterations of the precessing frequencies (chemical shifts) are characteristic of the metabolites. After suppressing the much more abundant signal from water and fat, metabolites present in sufficient concentrations can be measured. As an element of the cell membrane, choline is the most widely used metabolite in clinical oncologic HMRS, reflecting the increased cell membrane density of many cancerous tissues.

### 3.2.3 Nature of digital imaging

The detectability of a structure on MR depends on three basic factors: image resolution, tissue contrast and SNR.

**Image resolution**

The *image resolution* (of a digital image) is given by the smallest sample or data point, known as a *pixel*, i.e. a square or a rectangle. In MR and all medical imaging, the image represents a three-dimensional volume because the image is generated from a tissue slice of a specific thickness (Fig 3.4). The *tissue resolution* (smallest sample) is therefore given by a cube or cuboid known as a *voxel*. The slice thickness, field-of-view and the image matrix determine the size (extent) of the voxel. The spatial resolution is limited by the voxel because all signals from any tissue within the voxel are added to one single (gray scale) value. In 2D-imaging sequences, intersection gaps may further decrease the spatial resolution.
Fig 3.4 The pixel size determines the image resolution, whereas the voxel size determines the spatial resolution.

Nyquist-Shannon sampling theorem
As the pixel represents a sampled (discrete) signal of a continuous (analog) frequency, the sampling rate limits the highest frequency that can be correctly reconstructed (Fig 3.5). According to the Nyquist-Shannon sampling theorem, this maximum frequency is half of the sampling rate of a discrete signal processing system (Nyquist frequency). Translated to digital images of anatomy, this implies that the minimal lesion or distance that reliably can be discriminated must be twice the pixel size (sampling rate).

Fig 3.5 Illustration of the Nyquist-Shannon sampling theorem: the relationship between sampling rate (red dots) and reconstruction of continuous frequencies. At sampling rates ≥2x, the reconstructed frequencies are correct. At sampling rates <2x, the reconstructed frequencies are incorrect (aliased).
**Image contrast - tissue contrast**
Contrast refers to the difference in signal intensity between tissue compartments. There must be a minimum of signal difference in order to discern two tissue types; neighbouring voxels with similar gray scale values cannot be discriminated.

**Signal-to-noise ratio**
SNR is the ratio between the signal intensity of the tissue and the inherent electronic noise that adds up to the voxel signal. The ratio is usually determined by the magnitude of the tissue signal since the amount of electronic noise is fairly constant in MR.

**Contrast-to-noise ratio**
The combined significance of tissue contrast and SNR for the image quality is expressed as the contrast-to-noise ratio (CNR); the relative brightness of different tissues divided by the (standard deviation of the) noise. CNR equals the visibility of structures in an image (Fig 3.6).

![High SNR vs Low SNR](image)

**Fig 3.6** Illustrates the relationship of image contrast, SNR, and CNR; high contrast lesions in the upper row and low contrast lesions in the lower row, high SNR to the left and gradually lower to the right. The conspicuity of the lesion (CNR) depends clearly on both image contrast and SNR; high-contrast images are less susceptible to noise degradation than low-contrast images.

**Intravoxel tissue mixture - partial volume effect**
The gray scale value of a pixel reflects the sum of signals from all tissues within the corresponding voxel; intravoxel tissue mixture is therefore a fundamental source of error. The *partial volume effect* in tissue characterisation and lesion detectability may be substantial. Structures smaller than a voxel may disappear or be displayed with the size of the whole voxel. Depending on the signal intensity of the structure and its surroundings, this leads to reduced or improved conspicuity of a subvoxel structure. Even a fairly large structure with low signal will be outshined if the surroundings have high signal (Fig 3.7 A). Conversely, a structure that is smaller than a voxel could still be detectable if it has high signal and the background has low signal (Fig 3.7 B). Weightings that produce such tissue contrast are hereafter referred to as *black background* sequences.
Fig 3.7 Illustration of the partial volume effect on the detectability of small structures. A low-signal subvoxel structure is not detectable within high-signal surroundings, whereas a high-signal subvoxel structure is detectable within low-signal surroundings.

Adding up the signals from a voxel containing different tissues may result in a grey scale value that is not characteristic of any of the tissue components. T2W is particularly prone to such effects, especially in the assessment of tumour treatment response, because water and fat are bright, fibrosis is black, whereas tumour is medium grey. A composite voxel of fat and fibrosis or water and fibrosis may therefore appear medium grey with the risk of being misinterpreted as tumour (Fig 3.8).

Fig 3.8 Illustration of the partial volume effect on tissue characterisation. Separately characteristic signals from different tissues are added up to a gray scale value not characteristic of either. This may occur in high-signal surroundings as well as in low-signal surroundings.

**Tone mapping**

Tone mapping is the image processing technique that defines the relationship between the digital value of a pixel (signal intensity) and its greyscale tone (brightness). In digital imaging, tone mapping generally causes information loss because pixels with nearby digital values are mapped to the same brightness level, indiscernible to the interpreter. The main underlying explanation is two-fold: (I) Computers can record considerably more signal intensities than normal monitors can display and (II) computers record pixel values linearly, whereas the human eye has a logarithmic response to brightness [13].
The dynamic range is the ratio from the maximum to the minimum measurable values above noise [14]. Most MR imaging systems digitize the signal with up to 12-bit precision\(^5\), which translates into \(2^{12} = 4096\) possible greyscale tones. This recordable dynamic range (input) is substantially wider than the displayable dynamic range (output), which is limited by the 8-bit capacity of the computer monitor\(^6\), displaying only \(2^8 = 256\) greyscales. The wide recorded tone range is thus compressed into the displayable 256 tones (Fig 3.9). Consequently, all brightness levels in a typical MR image cannot be discriminated on a standard monitor. To counteract this limitation, picture archiving and communication systems (PACS) allow changing the window/level setting of the images, so that the available 256 tones of the monitor can be applied to a particular section of the tone range of the image.

\[\text{Fig 3.9 Illustration of tone mapping of high dynamic range MR signals (input) to the low dynamic range of the monitor (output). The total tone range is usually linearly compressed (globally equalized) to the narrow tone range of the monitor. By applying a selective window/level setting, a section of the tone range, corresponding to an area of interest in the image, can be re-mapped (locally adapted) to the tonal range of the monitor.}\]

The human vision can adapt to a huge range of light intensities, much wider than the tone range of normal MR imaging. Due to the adaptive manner of human response to physical stimuli, the ability to discriminate levels of sensation is relative. This relative sensorial response does not match the linear encoding of digital signal intensities. Digital images, also photography, are therefore tone mapped to a greyscale that visually is perceived as evenly distributed (\textit{gamma correction}\(^7\)). Due to the exponential behaviour of T1- and T2-relaxation, the high signal intensities occupy a disproportionately large portion of the signal dynamic range [14] (Fig 3.10). Consequently, disproportionately few pixels occupy most of the high brightness tones, causing the image to appear dark (Fig 3.11).

\(^5\) Recorded at 12-bit precision and stored in 16-bit files.

\(^6\) High dynamic range display monitors exist, but are not routinely used in reading MR-images

\(^7\) output = input \(^\frac{1}{\gamma}\). Usually \(\gamma\) is 2.2 or 1.8
Fig 3.10 Illustration of linear versus exponential tone mapping. In the exponential illustration, the 10% highest signal intensities are distributed throughout half of the greyscale range (bright tones), whereas half of the signal intensities (low values) are compressed to only 10% of the greyscale tones. In the linear illustration, the signal intensities are evenly distributed over the whole range of greyscale tones.

Fig 3.11 Illustration of linear versus gamma corrected images. Left: Linear encoded T2W image with corresponding histogram (number of pixels displaying each tone). Right: Gamma corrected T2W image with corresponding histogram.

The tone mapping from a physical to a perceptual greyscale leads to a very uneven distribution of data (bits) along the grey scale tone range that explains the limited discrimination of pixel values in the low brightness range (Fig 3.12).
Fig 3.12 Illustration of the relationship between tones and their allocated bits. When the linear encoding (A) is stretched (B, gamma correction) a large range of low brightness tones is represented only by very few bits (C) [15].

The gamma encoded image does not hold enough information in the dark areas to allow window/level adjustments without introducing image *posterization* (Fig 3.13) and noise [13].

Fig 3.13 Illustration of *posterization* or banding-effect of bit depth truncation.

If too few bits are spread over a too wide tone range, the visual discrimination may not improve because nearby pixel values are all given the same digitized value (Fig 3.14 D). This may occur if a narrow window/level setting is applied to “magnify” details in the dark image areas. In the bright image areas however, a selective window/level setting can visualize otherwise blown-out details because there are lots of bits allocated to the highlights (Fig 3.14 E).
Fig 3.14 Illustration of tone mapping in a set of MR sequences typically used in modern pelvic cancer imaging. In the globally tone mapped first pass DCE image (B), all details within the contrast enhancing recurrent tumour (white arrows) was burned out, whereas local adaption (E, white rectangle) revealed fine details. At the T2W image (A) however, limiting the tone mapping to a small area of the recurrent tumour did not reveal substantially more details (D). The highly DW image occupied an in-between position, but still benefitted from local tonal adaption (F). See Fig 7.1 legend for clinical details.

3.2.4 Pelvis and MR

Compared to CT, MR is particularly well suited for imaging the pelvis. In general, limited respiratory motion enables high-resolution images, array of local coils facilitates good SNR, and the superior soft tissue contrast yields good CNR. High quality morphologic images are particularly important in the lower pelvis where fine anatomical details distinguish a preserved function from severe treatment morbidity.

3.3 Histopathology

3.3.1 Grossing of pathology specimens

Gross examination (grossing) of the pathology specimen is the process where formalin-fixed tissue specimens are examined, described and cut into a set of tissue blocks. Carefully and systematically selected tissue blocks are subsequently placed into processing cassettes and embedded in paraffin, from which very thin sections are cut by a microtome and mounted onto microscope glass slides. Commonly two sizes of glass slides are used: standard (25 x 75 mm²) and large (51 x 75 mm²). The term whole-mount refers to the glass slide being sufficiently large to include the entire (cross) section of an organ, however, the term is also used to describe large-sized microscope glasses.
3.3.2 Staining of histopathology

Staining is the process where tissue sections, after clearing the paraffin, are stained in order to visualize features that are otherwise indistinguishable. While a variety of different staining techniques have been developed, two contrasting dyes are used in routine histological work: hematoxylin and eosin (H&E). Hematoxylin stains nuclei of the cells blue and eosin counterstains most other tissue structures in various shades of red, pink and orange. Finally, the sections are permanently mounted under a cover glass slide.

3.3.3 Immunohistochemistry

Immunohistochemistry (IHC) staining refers to detection of antigens in a histological tissue section by the principle of specific antibody binding. This is a method that provides supplemental information to the morphological assessment. The antibody-antigen interaction is visualized (immunodetection) by incubation with enzymes that catalyse colour-producing reactions. A second counterstain is often applied to make the antigen stain stand out. Immunodetection can also be performed by means of fluorescence labelling. Various antigen amplification methods are available for increasing the sensitivity of the technology.

3.4 Methods for assessment of treatment response

Different methods are used for the assessment of treatment response: Response Evaluation Criteria in Solid Tumours (RECIST) [16] is based on the tumour size, thus often referred to as downsizing. TNM (ref) is based on the tumour stage and often referred to as downstaging. Tumour Regression Grade (TRG) reflects the relationship between treatment changes and residual tumour at histopathology of surgical specimens.

3.4.1 Response Evaluation Criteria in Solid Tumours: RECIST

This system is based on the measurement of the longest diameter of one target lesion or the sum of diameters of two or more target lesions. The assessment is reported in categories: Complete Response (CD), Partial Response (PR), Stable Disease (SD) or Progressive Disease (PD). PR requires at least 30% decrease in the sum of longest diameters of target lesions, whereas 20% increase in the sum of diameters of target lesions, or new lesions, are considered as PD.

3.4.2 Volumetry

The size of the tumour can also be measured using volumetry. The RECIST Working Group developing RECIST 1.1 [16] considered whether it was appropriate to move from anatomic unidimensional assessment of tumour burden to volumetric anatomical assessment or to
functional assessment with PET or MRI. It was concluded that, at present, there is not sufficient standardisation or evidence to abandon anatomical assessment of tumour burden. Volumetry is undoubtedly an anatomical assessment. Compared to uni-dimensional assessments, volumetric measurements are inherently more sensitive to small volume changes and less sensitive to measuring errors caused by non-spherical tumour shape. RECIST overestimates volumes even of spherical tumours as compared to volumetry, whether segmented or calculated from bi-dimensional orthogonal diameters (World Health Organisation criteria[\textsuperscript{17}])[\textsuperscript{18}]. Volumetry is essentially a sensitive variant of the present RECIST, that admittedly needs standardisation, but do not introduce the non-anatomical methodological challenges of the functional imaging techniques. Of note, volumetry is a continuous variable, whereas RECIST is categorical.

3.4.3 Tumour-Node-Metastasis classification system: TNM

This system categorizes the extent of the disease into stages that reflect the prognosis of mainly epithelial tumours. T for tumour denotes the primary tumour, based on size, depth of penetration and presence of peritoneal spread (pelvic malignancies). N for node denotes the presence of lymph node metastases and may be sub-classified based on node number and location. M for metastasis denotes the presence of distant metastases and is often sub-classified according to location: non-regional lymph node, the skeleton or parenchymal organs. Although principally uniform, the detailed TNM-classification is specific for site of the tumour. Some prefixed modifiers are defined, such as c for clinical assessment and p for histopathologic evaluation of surgical specimens. Imaging is basically included in clinical evaluation, but prefixes denoting the image modality are commonly used in research and advocated also for clinical use [\textsuperscript{19}]. In this thesis, the prefix mr denotes MR based assessment. The prefix y denotes that the evaluation is performed following radiation- or chemotherapy. TNM is maintained by the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC). Currently, the UICC and AJCC staging systems are unified into a single staging system. It is updated according to new prognostic insights and treatment achievements, so a TNM-classification should state the edition used. The system also includes a classification for the evaluation of the resection margin of surgical specimens: R0 denotes free margin, R1 denotes microscopic and R2 denotes macroscopic involvement of the resection margin. R-classification is commonly used for short-term evaluation of surgical treatment.

3.4.4 Tumour Regression Grade: TRG

In 1994 Mandard \textit{et al} [\textsuperscript{20}] categorized histopathologic assessment of regressive changes following chemoradiotherapy (CRT) in esophageal carcinomas. In 1997 Dworak \textit{et al} [\textsuperscript{21}] used the same principle when describing histopathologic features after CRT in rectal cancer, but reversed the order of regression categories. In 2002 Bouzourene \textit{et al} [\textsuperscript{22}] applied the system described by Mandard \textit{et al} in LARC. All systems are based on the presence of residual tumour cells and the extent of regressive changes, i.e. fibrosis. The extremes are no regression and complete regression, each comprising one category. Near-complete response, where residual cancer cells are rare and difficult to find at microscopy,
constitutes one category. Partial response is subdivided into two categories based on whether regressive changes outgrow residual tumour or vice versa (Fig 3.14)

More recently, modified TRG systems have been introduced (Table 3.1). In 2002, Wheeler et al proposed a simplified measurement of tumour regression [23]. TRG1+2 were combined to rectal cancer regression grade (RtCG1). RtCG2 consisted of marked fibrosis but macroscopic disease present and RtCG3 of little or no fibrosis, with abundant macroscopic disease. In 2005, Ryan et al demonstrated better reproducibility using a modified 3-point scale if TRG1+2 and TRG4+5 were combined [24]. In 2005 Rödel et al described a 5-point scale based on the degree of tumour regression ranging from grade 0 (no regression) to grade 4 (complete regression) in <25, 25-50, >50% intervals [25]. The 7th edition (2010) of the AJCC (not UICC) recommends recording the histopathologic response to neoadjuvant treatment according to the guidelines from the College of American Pathologists (CAP) - Protocol for the examination of specimens from patients with primary carcinoma of the colon and rectum. They modified the 3-point scale from Ryan et al [24] by adding a separate grade for complete response, resulting in a 4-point scale: (0) complete response – no viable cells, (1) moderate response – single cells or small groups of cancer cells, (2) minimal response - residual cancer outgrown by fibrosis, and (3) poor response – minimal or no tumor kill; extensive residual cancer [26]. Furthermore, in 2008, Taylor et al [27] proposed a Tumor Regression Grade as seen on MRI, based on the system of Dworak [21].

![Fig 3.14 Illustration of tumour regression grading system described by Mandard and Bouzourene](image)
Table 3.1 Different tumour regression grading systems for assessment of treatment response to chemoradiotherapy. The grading systems written in grey differ from the system used in papers 3 and 4.

<table>
<thead>
<tr>
<th>Tumor Regression Grading systems</th>
<th>Complete regression</th>
<th>Rare/scattered residual cancer cells</th>
<th>Fibrosis outgrowing residual cancer</th>
<th>Residual cancer outgrowing fibrosis</th>
<th>No regression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mandard 1994</strong> Esophageal carcinoma</td>
<td>TRG1</td>
<td>TRG2</td>
<td>TRG3</td>
<td>TRG4</td>
<td>TRG5</td>
</tr>
<tr>
<td><strong>Dworak 1997</strong> Rectal carcinoma</td>
<td>TRG4</td>
<td>TRG3</td>
<td>TRG2</td>
<td>TRG1</td>
<td>TRG0</td>
</tr>
<tr>
<td><strong>Bouzourene 2002</strong> LARC</td>
<td>TRG1</td>
<td>TRG2</td>
<td>TRG3</td>
<td>TRG4</td>
<td>TRG5</td>
</tr>
<tr>
<td><strong>Wheeler 2002</strong> Rectal cancer</td>
<td>RCRG1</td>
<td></td>
<td>RCRG2</td>
<td></td>
<td>RCRG3</td>
</tr>
<tr>
<td><strong>Ryan 2005</strong> LARC</td>
<td>TRG1</td>
<td></td>
<td>TRG2</td>
<td></td>
<td>TRG3</td>
</tr>
<tr>
<td><strong>Rödel 2005</strong> Rectal cancer</td>
<td>TRG4</td>
<td></td>
<td>TRG3</td>
<td></td>
<td>TRG0</td>
</tr>
<tr>
<td><strong>AJCC 2010</strong> Colorectal cancer</td>
<td>TRG0</td>
<td></td>
<td>TRG1</td>
<td></td>
<td>TRG3</td>
</tr>
</tbody>
</table>

An additional classification system from the *Federation of Obstetricians and Gynaecologists* (FIGO) is used for gynaecologic malignancies [www.figo.org]. Parallel to TNM, this system can also be used for assessment of treatment response.

### 3.5 Principles of radiotherapy

#### 3.5.1 Planning

The biological effect of radiation on tumour as well as normal tissues is dose dependent. The dose-response curves are typically sigmoid-shaped. Curative radiation treatment requires that the respective curves are sufficiently parallel and separated to control cancer without damaging normal tissue. However, the slope of tumour control curves is frequently less steep than for normal tissue, possibly limiting the ability to deliver curative doses [28]. Specific target volumes for RT planning are defined in two reports from the *International Commission of Radiation Units and Measurements* (ICRU): ICRU 50 from 1993 and ICRU 83 from 2010. There are two main types of volumes to be considered in RT planning; those based on anatomy and those based on geometry. The anatomical volumes are particularly relevant to diagnostic imaging, whereas the geometrical volumes are mainly related to the delivery of RT. In line with technological developments, the
geometrical volumes are further refined in ICRU 83 [29], but no changes were made to the definitions of the anatomical volumes.

**Anatomical tumour volumes**
Gross Tumour Volume (GTV): *the gross demonstrable extent and location of the tumor.*
Clinical Target Volume (CTV): *GTV and/or subclinical malignant disease with a certain probability of occurrence considered relevant for therapy.*

**Geometrical target volumes**
Planning Target Volume (PTV): Margin needed for uncertainty in planning and delivery. PTV depends on both the CTV and the delivery technique used. In ICRU 83, PTV is subdivided into Internal Margin (IM): margin accounting for physiological movements and Set-up Margin (SM): margin accounting for uncertainties in patient positioning and alignment of therapeutic beams throughout all sessions (Fig 3.15).

Internal Target Volume (ITV): CTV and IM
Planning Target volume (PTV): CTV+IM+SM

Treated Volume: Volume enclosed by an isodose surface (e.g. 95% isodose)
Irradiated Volume: Tissue volume receiving significant dose

![Fig 3.15](image)

**Organs at risk volumes**
Organs at Risk (OARs): Normal tissues whose radiation sensitivity may significantly influence treatment planning and/or prescribed dose.
Planning Organ at Risk Volume (PRV): Analogous to PTV for OAR. PRV = OAR + IM + SM

3.5.2 Delivery

Radiation delivery techniques are beyond the scope of this thesis. Nevertheless, technical improvements towards high-precision RT place increasing demands on imaging, so understanding the principles and abilities of the various techniques is important for image
guidance. Radiotherapy has substantially developed in the past decades, from conventional two-dimensional techniques to three-dimensional conformal RT and further to advanced modulating techniques. Furthermore, the geometrical accuracy of MR images is critical to high-precision RT.

The term conformal means generating dose distributions that conform to tumour targets. Modulating techniques can achieve complex dose distributions, delivering lethal dose to tumour while sparing nearby normal tissue. There are several types of modulation of the radiation delivery, intensity-modulated radiation therapy (IMRT), dynamic conformal arc (DCA) and volumetric-modulated arc therapy (VMAT), as well as combination of these techniques: Tomotherapy and intensity-modulated arc therapy (IMAT). Notwithstanding the pros and cons of each technique, the results are relatively consistent. These techniques provide a variety of treatment applications such as simultaneous treatment of multiple lesions, dose painting, simultaneous integrated boost (SIB), and stereotactic radiotherapy or radiosurgery [30].

**Dose painting**
The term *dose painting* describes the process of modelling the dose distribution to target volumes and OARs. The procedure can be based on anatomic and biologic image information. Based on anatomy it is referred to as *dose painting by contours* and prescribes global dose within the contoured volumes. Based on biologic image information it is referred to as *dose painting by number* and prescribes variable inhomogeneous dose distribution inside the target according to the intensity of the voxels [31]. Dose painting by contours utilizes mainly morphologic images, whereas dose painting by numbers utilizes mainly functional imaging [32]. Image guided dose painting can accomplish focal dose escalation to radiation resistant areas as well as dose de-escalation to sensitive OARs.

**Focal treatment**
Stereotactic radiosurgery and stereotactic body radiotherapy are characterized by accurate 3D target localisation and definition, sharp dose fall-offs and extremely high dose fraction exceeding the level of tissue destruction, so the demands on image guidance is approaching those of surgery. High-dose-rate (HDR) brachytherapy involves placing a source of high-energy radiation directly into the tumour. Similar to radiosurgery, the delivered radiation doses are high and the dose fall-offs are sharp.

3.5.3 Adaptive radiotherapy

The term adaptive means individually tailored time-varying treatment adjustments, usually according to treatment response. As for dose painting, the re-planning could be based on anatomic as well as biologic image information. The delivery can be performed with external conformal techniques as well as internal HDR brachytherapy.
3.6 Prostate cancer

3.6.1 Background

*Incidence*
Prostate cancer is now recognised as one of the most important medical problems facing the male population [33]. In Norway, it is the most frequently diagnosed cancer with 4919 new cases in 2012 (Kreftregisteret.no). There were 1006 deaths from prostate cancer in 2012, representing the second leading cause of cancer death among men.

*Diagnosis*
Digital rectal examination (DRE), serum concentration of prostate specific antigen (PSA), and transrectal ultrasonography (TRUS) guided biopsies are the main diagnostic tools. Tumour detection and characterization by MR are promising and encouraged by the European Society of Uroradiology (ESUR), which also has proposed a scoring system for the likelihood of significant cancer: PI-RADS [34]. Hence, MR is increasingly used to guide biopsies, mainly by fusion with ultrasound, but also by direct (in-bore) guidance [35]. Whilst transrectal access currently is the standard approach, transperineal biopsies may access anterior tumours better and can be performed as a sterile procedure, reducing the risk of infection. The definite diagnosis is verified at histopathology, in prostate biopsies or in operative specimens.

3.6.2 Histopathology

*Grading*
The majority of prostate cancers are acinar adenocarcinomas. Unusual subtypes as ductal (endometroid) and mucinous (colloid) account for less than 1%. The aggressiveness is graded according to a system described by Donald F. Gleason in 1966. Rare cancer types as signet ring cell carcinoma, small cell carcinoma and sarcomas are not assigned a Gleason grade. The Gleason grading system is based on the cellular architecture and classifies the glandular differentiation into five patterns. Patterns 1-2 are not reported as cancer. Pattern 3 is regarded as cancer with a fairly good prognosis and consists of homogenous well-formed glands. Pattern 4 is highly malignant and consists of ill-defined glands with poorly formed glandular lumina, often accompanied by fused glands. Pattern 5 is very highly malignant and consists of solid nests, occasionally cribriform, and typically with necrosis (comedo). The Gleason system reports the aggressiveness as a score, defined as a sum of the most and the second most predominant patterns, i.e. Gleason 3+3=6, 3+4=7, 4+3=7, 4+4=8. Pattern 5 is reported as tertiary grade if present. The ESUR Guidelines 2013 refers to the consensus on Gleason grading from 2005 [36], later the grading system was updated in 2010 [37] (Fig 3.16). This update improves the premise for MR-histopathologic correlation, and thus for MR-guided clinical patient management, because the hardly detectable pattern 3 with favourable prognosis is now clearly separated from the better detectable pattern 4 with worse prognosis.
Fig 3.16 Schematic representations of Gleason grading systems. The most important difference between 2005 and 2010 is patterns 3 and 4. In the modified system, most cribriform patterns and poorly defined glands are included in pattern 4 [38]. Reprinted with permission.

**Growth pattern**

The tissue composition and the growth pattern of prostate cancer are of particular concern to imaging, due to intermixture of benign and malignant tissue, which is difficult to discriminate. In 2008 a group of researchers [39], including internationally acknowledged experts in uropathology and prostate MR imaging, performed thorough matching of whole-mount histopathology and MR of the prostate. They introduced the terms *sparse* and *dense* tumours and showed that sparse tumours were not significantly different from normal peripheral zone neither at T2W-images nor at ADC-maps, whereas dense tumours were clearly depicted. Outside the prostate, perineural invasion is the major mechanism of spread [40, 41]. Inside the prostate, the significance of perineural infiltration is debated, however Aumayr et al found that the intraprostatic number of infiltrated nerves (NIN) correlated with extraprostatic NIN, which in turn correlates with Gleason grade [42]. The propagation of tumour along nerves penetrating the prostatic capsule lead to a cribriform dissemination of tumour cells into periprostatic fatty tissue. As a consequence, nests of tumour are admixed with benign tissue [41].

### 3.6.3 Classification

Prostate cancer classification is based on tumour extent (TNM) and tumour aggressiveness (Gleason score and PSA-level).

**TNM (7th edition of AJCC/UICC )**

- **T1** Clinically inapparent tumor neither palpable nor visible by imaging
- **T2** Tumor confined within prostate
T2a, Tumor involves one-half of one lobe or less
T2b, Tumor involves more than one half of one lobe but not both lobes
T2c, Tumor involves both lobes

T3 Tumor extends through the prostate capsule.
T3a, Extracapsular extension* (unilateral or bilateral)
T3b, Tumor invades seminal vesicle(s)

T4 Tumor is fixed or invades adjacent structures other than seminal vesicles

*Pathologists generally use extraprostatic extension (EPE) to describe all cases of tumour growth beyond the confines of the prostate.

Risk stratification
The management of prostate cancer is mainly risk-based, divided into three main groups originally described by D’Amico [43].

- **Low-risk prostate cancer**: cT1-T2a, Gleason score < 6 and PSA < 10 ng/mL
- **Intermediate-risk, localized prostate cancer**: cT2b-T2c or Gleason score = 7 or PSA 10-20 ng/mL
- **High-risk localized and locally advanced prostate cancer**: cT3a or Gleason score 8-10 or PSA > 20 ng/mL

Clinical T-staging is according to EAU guidelines performed using DRE and TRUS. The distinction between intraprostatic (T1-T2) and extraprostatic (T3) disease is crucial for the treatment decisions. Despite that standard clinical assessment with DRE regularly underestimates the tumour extent, MR staging is not widely used in clinical work-up. MR is recommended only for selected cases where more precise staging directly affects the treatment decisions, preferably using an endorectal coil [33]. Notwithstanding these EAU guidelines, MR staging is already recommended by ESUR, which also have agreed on technical minimal requirements [44], not requiring the use of an endorectal coil.

Nomograms, based on PSA level, clinical stage and Gleason score, is used to predict the pT-stage of disease [EAU Guidelines 2014]. Well-known nomograms are Partin Tables, provided by Johns Hopkins Hospital and CAPRA score, provided by the University of California, San Francisco (UCSF). Furthermore, The Memorial Sloan Kettering Cancer Center provides several nomograms to predict treatment outcome.

3.6.4 Treatment

Treatment options
The treatment options for prostate cancer are diverse, spanning from active surveillance and focal treatment, to prostatectomy and RT combined with hormonal therapy, and eventually palliative chemotherapy or watchful waiting. For low-risk, localized prostate cancer it remains unclear whether there is a survival benefit of treatment over active
surveillance. For intermediate-risk, localized prostate cancer curative treatment is recommended: Radical prostatectomy or RT (external or internal) are considered equal with respect to oncologic outcome, but the profile of adverse effects are different. For high-risk prostate cancer there is no consensus regarding the optimal treatment, and management decisions should be made after all treatments have been discussed by a multidisciplinary team consisting of urologist, radiation oncologist and radiologist [33].

Locally advanced prostate cancer (LAPC) means that tumour extends beyond the prostate and into extraprostatic tissue: periprostatic fat (T3a), seminal vesicles (T3b) or other organs (T4), usually the bladder. Hence, surgical treatment has traditionally been discouraged due to high risk of positive surgical margins and the need for subsequent (adjuvant) RT to avoid local recurrence. External RT combined with hormonal therapy (ADT) is the preferred treatment, although no trials have shown a clear advantage over surgery. In recent years, there has been renewed interest in surgery for LAPC and some studies indicate that surgery has a place in the treatment of non-organ confined disease [33]. A new Scandinavian multicenter study (SPCG15) is designed to investigate the outcome of surgery as compared to the outcome of RT and ADT for LAPC.

*Surgical dissection planes*

The nerves responsible for erection, ejaculation and urinary continence derive from the inferior hypogastric plexus. The dense neural network runs within a fibro-fatty sagittally orientated space between the bladder and the rectum. Fibers of the pelvic plexus enclose the bladder neck, the prostate, the seminal vesicles and the vas deferentia like a meshwork. The nerves are accompanied by vascular structures, referred to as the neurovascular bundle (NVB). These nerves are located very close to the neighbouring structures. The minimal distances have been reported as direct contact (0 mm) to the seminal vesicles and the prostate base, 4 mm to the bladder neck, and 2 mm to the levator ani [45].

Fascias enclosing the NVB are the landmarks for the dissection planes in prostatectomy (Fig 3.17). The prostatic fascia covering the prostate delineates the NVB medially and the levator ani fascia delineates the NVB laterally. Posteriorly, the Denonvilliers’ fascia closely covers the prostate and the seminal vesicles. *Intrafascial* dissection follows a plane along the prostate capsule staying medially to the prostatic fascia so that no fascia remains on the prostate. This allows a complete preservation of the NVB. *Interfascial* dissection also follows a plane along the prostate capsule, but laterally to the prostatic fascia, leaving the fascia attached to the prostate. This allows a wider tissue buffer on the prostate, probably resulting in an oncologically safer approach. However, depending on anatomic variations, the NVB might be more prone to partial resection with this interfascial technique. *Extrafascial* dissection extends laterally to the levator ani fascia and posteriorly to the Denonvilliers’ fascia, leaving the NVB on the prostate. This approach results in a complete resection of the posterolateral aspect of the NVB and probably complete erectile dysfunction. It is the most oncologically safe dissection [45].
3.7 Rectal cancer

3.7.1 Background

**Incidence**
Anorectal- and rectosigmoid cancer is the second leading cause of death from pelvic malignancies. In 2012, there were 1304 new cases and 398 deaths in Norway (Kreftregisteret.no).

**Diagnosis**
The diagnosis of rectal cancer requires histological confirmation from biopsies, primarily by digital examination and rectoscopy.

3.7.2 Histopathology

Most rectal tumours are adenocinomas, however small cell carcinoma, squamous cell carcinoma, gastrointestinal stromal tumour (GIST), carcinoid, melanoma, lymphoma and sarcomas also occur in the rectum, and require different treatment strategies.

Mucin production is an occasional feature of adenocinomas and is divided into two groups: Primary mucinous adenocarcinoma and signet ring cell adenocarcinoma depending on whether the mucin is located extracellularly or intracellularly, respectively. Primary mucinous adenocarcinoma is known as an uncommon histological subtype that affects several organ sites. In the colorectum, mucinous adenocarcinoma is defined if at least 50% of the tumour’s volume is composed of extracellular mucin [48]. At MR, mucin was found in up to 20% of rectal cancer patients and was an independent marker of poor prognosis.
and poor response to preoperative CRT [49]. The clinicopathologic significance of presence of mucin following neoadjuvant treatment is not unambiguous. Colloid response, i.e. acellular mucin pools was first described by Dworak [21] and is probably associated with RT since it was found in rectal cancer but not in colon cancer [50]. The recommendation by the CAP is to regard acellular mucin as a type of treatment response and not as residual tumour since its presence was not associated with local recurrence [51]. However, mucin pools following CRT is more frequent in primary mucinous carcinoma and signet ring cell carcinoma [52] so their presence warrants a meticulous search for residual vital tumour cells [50]. Regardless of clinicopathologic significance, the presence of mucin is of particular importance to imaging: (I) For tumour characterization, because mucin is reliably recognized at MR, but rarely preserved in tissue biopsies [49]. (II) For MR interpretation, because both mucin and oedema appear bright on T2W. (III) For assessment of treatment response, because extracellular mucin pools, despite successful sterilization, do not necessarily shrink.

Primary signet ring cell adenocarcinoma is distinct from primary mucinous adenocarcinoma, with specific molecular alterations, different clinical behaviour [48] and worse prognosis. Characterized by extensive tissue infiltration and dissemination of cancer cells, the appearance at MR is typical: It results in an oedematous, colitis like impression where all tissue structures of the bowel wall are thickened and abnormally separated (Fig 3.18). Although a typical appearance, the diagnosis is hard to realize if the reader is not aware of the distinctive growth pattern. As a result of the extensive admixture within normal tissue, tumour delineation is very challenging at MR.

**Fig 3.18** Illustration of the growth pattern of primary signet ring cell carcinoma **Left:** T2W image with typical appearance. The layers of the rectum wall are separated by disseminated tumour infiltration. Tumour cells propagate into the mesorectal fat in all directions. **Right:** corresponding H&E section. Small mucin deposits are present within the submucosa (white arrows), within the inner circular muscle layer (yellow arrow), between the muscle layers (green arrow), within the outer longitudinal muscle layer (red arrow) and within the mesorectal fat (grey arrow). In addition, solid deposits (with variable sizes) are present scattered throughout the section. The blue arrows show particularly small and peripheral tumour deposits. (Image courtesy Turid Vetrhus [53]).
3.7.3 Classification

TNM [AJCC/UICC]
The 5th edition of the AJCC classification was used for radiologic as well as histopathologic assessment of the rectal cancer material. In the current 7th edition, some minor changes have been made to the definition of T3 and T4.

5th EDITION Primary Tumor (T)
TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
Tis Carcinoma in situ: intraepithelial or invasion of lamina propria
T1 Tumor invades submucosa
T2 Tumor invades muscularis propria
T3 Tumor invades through the muscularis propria into submucosa, or into non-peritonealized pericolic or perirectal tissue
T4 Tumor directly invades other organs or structures, and/or perforates visceral peritoneum

7th EDITION Primary Tumor (T) (Fig 3.19)
TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
Tis Carcinoma in situ: intraepithelial or invasion of lamina propria
T1 Tumor invades submucosa
T2 Tumor invades muscularis propria
T3 Tumor invades through the muscularis propria into pericolicorectal tissues
T4a Tumor penetrates to the surface of the visceral peritoneum
T4b Tumor directly invades or is adherent to other organs or structures

Fig 3.19 Illustration of T-stages of rectal cancer in AJCC/UICC 7th edition
Definition of LARC
The most common definition of LARC is T3-4 and/or N+. The appropriate definition of LARC is debated; with respect to treatment and prognosis it is suggested not to include T3-tumours with minor extramural tumour extent and unthreatened margin [54-56].

Risk stratification
The purpose of preoperative assessment is two-fold, namely prognostic staging and mapping the anatomy in order to define the appropriate surgical dissection plane. Risk stratification enables customised approach to treatment, minimizing overtreatment, while allowing aggressive treatment for high-risk patients.

Most established markers of tumour aggressiveness and poor prognosis originate from histopathology of surgical specimens (not biopsies). MR provides preoperative information, and has therefore become a cornerstone in the work-up of rectal cancer as reflected in current guidelines [56-58].

The most important features for treatment stratification provided by MR are: the local tumour extent and localisation with respect to the sphincter and the peritoneal reflection, the assessment of whether MRF is threatened or involved, T- and N-staging, quantification of extramural tumour extent and presence of extramural venous invasion (EMVI). Engelen et al [59] have recently shown that tailored treatment of primary rectal cancer based on preoperative MR imaging leads to a high rate of complete resections (95.6%) and a low rate of local recurrence (2.2%).

3.7.4 Treatment
In the two past decades there have been major advances in the work-up and treatment of rectal cancer. Improvements in preoperative staging, surgical technique, CRT and histopathologic assessment have led to substantial improvements in the outcome for these patients. Variations in opinion as well as practice within and between countries are reflected in consensus conferences [56, 58, 60, 61]. The various treatment regimens for rectal cancer, especially for the heterogeneous T3-group are complex, and their details are beyond the scope of this thesis.

Treatment strategy is mainly based on TNM-classification according to AJCC and UICC. T1 tumours can be treated both with total mesorectal excision (TME) and trans-anal endoscopic microsurgery (TEM). T2 tumours are adequately treated with TME without preoperative RT. The treatment of T3 tumours is founded on the combination of RT and TME surgery. In more advanced stages, either in terms of threatened surgical margin or presence of local lymph node metastasis it is advocated to use concurrent CRT. Patients at high risk of systemic disease are likely to receive systemic chemotherapy: neoadjuvant, adjuvant or both. Locally very advanced and initially unresectable T4 tumours should be re-evaluated for resectability using en-bloc extended TME-surgery (ETME) resection after completion of neoadjuvant treatment.
Surgery
Surgery remains the cornerstone in curative treatment of rectal cancer. Radical rectal cancer surgery consists of two main procedures: low anterior resection (LAR) and abdominoperineal resection (APR). Whereas LAR preserves the anal sphincters and their function, APR removes the anal sphincters en bloc and a definitive colostomy is necessary. In 1982, Heald et al [62] recognized tumour foci in the mesorectum several centimetres from the primary tumour and proposed total excision of the mesorectum. In 1986, Quirke demonstrated that lateral tumour spread caused local recurrences due to inadequate surgical resection [63]. Since then, TME is established as the method of choice. Introduction of standardised surgery and TME has resulted in low local recurrence rates (2-5%) [59, 64].

TME: The idea behind TME is to remove the primary tumour together with its routes of vascular and lymphogenic spread. The technique consists of sharp dissection on the plane between visceral and parietal fascia layers, i.e. the holy plane of TME (Fig 3.20 and 3.21).

**Fig 3.20** Illustration of the TME plane, as depicted in Heald's original publication [62]. Reprinted with permission.

TME is the standard surgical method for T2 and T3 rectal cancers. The outcome of TME correlates strongly with the distance from tumour to the circumferential resection margin (CRM). Involved surgical margin, defined as tumour within 1 mm from the CRM [63] is a strong predictor of local recurrence and poor prognosis. Maughan et al [65] found that there was a steady decrease in survival as this distance narrows, with the most pronounced fall at 1 mm or less.
Fig 3.21 The mesorectal fascia (MRF) visualized by red dotted lines at high-resolution T2W MR. In the lower part (A) MRF is adjacent to the levator ani. In the middle part (B) MRF clearly separates mesorectum from the pelvic sidewalls. In the upper part MRF proceeds anteriorly as the mesosigmoidal fascia (C).

Since there is a well-established link between CRM involvement and outcome, a clear radial resection margin of at least 1 mm at histopathology is the goal of TME-surgery. MR demonstrates the distance from the most lateral extent of a mesorectal tumour deposit to the mesorectal fascia (Fig 3.22 A). Provided that the surgeon manages to stay in the TME-plane, this distance equals the resection margin at histopathology [3, 66]. The extramural depth (EMD) of tumour invasion beyond the muscularis propria and into the mesorectal fat is prognostic and measurable at MR (Fig 3.22 B). A sub-classification system of T3 tumours based on MR was proposed in the MERCURY study [54].

Fig 3.22 Illustration of prognostic distances measured with MR. A) The radial distance from tumour to the mesorectal fascia (red arrows) predicts the circumferential resection margin. The distance is measured where it is the shortest: from the primary tumour (T3), from a lymph node metastasis (N) or from an extramural venous invasion (EMVI). B) Extramural depth (EMD) of tumour invasion beyond the muscularis propria and into the mesorectal fat.

**Threatened margins:** Patients with T3 tumours are likely to receive neoadjuvant chemoradiotherapy if the CRM is threatened at MR. The appropriate cut-off value for sufficient distance from tumour to MRF is however debated. The MERCURY-study [66] demonstrated that 1 mm is safe, both at histopathology and at MR. In this study all
components of the interdependent multidisciplinary chain were quality-assured; the participating radiologist, pathologist and surgeons were trained, imaging and grossing techniques were standardized and the results were structurally reported. In other studies, the reported results did not have the same precision. In 2001, Beets-Tan et al [67] found that 5 mm at MR was needed to ensure good prognosis. In 2002, Nagtegaal [68] showed that ≤ 2 mm margin was a strong predictor of local recurrence. Follow-up data from the Norwegian Gastro Intestinal Cancer Group (NGICG) in 2009 [69] and from the Memorial Sloan-Kettering Cancer Center [70] in 2013 also concluded that a margin of 2 mm or less indicated a poorer prognosis. The quality of surgery is also important. Quirke (2009) found that only in 52% of surgical specimens from TME, the plane of surgery was mesorectal, whereas the plane was intramesorectal in 34% and in the muscularis propria plane in 13%.

**Low rectal cancer**: The management of low rectal cancer is challenging. Low rectal cancers have poorer outcome [71]. Generally, the lower the site, the worse is the prognosis. The need for oncological safe wide excision, extralevator abdominoperineal excision (ELAP) [71-73] must be balanced against sphincter conservation and function preservation. The definition of low tumours varies [56]. Based on anatomical T2W MR guidance, definitions, appropriate plane of surgery (mesorectal and extralevator) and a staging system is proposed and currently investigated [71-73] (Fig 3.23).

**TABLE 1. The low rectal anatomical staging system**

<table>
<thead>
<tr>
<th>Tumor level</th>
<th>Maximal tumor extent</th>
<th>Plane of surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above the level of the anal sphincter</td>
<td>Confined to muscle coat</td>
<td>Mesorectal</td>
</tr>
<tr>
<td></td>
<td>Beyond muscle coat into mesorectum only</td>
<td>Mesorectal</td>
</tr>
<tr>
<td></td>
<td>Extending to &lt;1 mm of levator muscle/mesorectal fascia</td>
<td>ELAPE</td>
</tr>
<tr>
<td></td>
<td>Extending into or beyond levator muscle</td>
<td>ELAPE</td>
</tr>
<tr>
<td>At the anal sphincter</td>
<td>Submucosal layer/part thickness of muscularis propria</td>
<td>Mesorectal</td>
</tr>
<tr>
<td></td>
<td>Full thickness of muscularis propria</td>
<td>ELAPE</td>
</tr>
<tr>
<td></td>
<td>Into intersphincteric plane</td>
<td>ELAPE</td>
</tr>
<tr>
<td></td>
<td>Into external sphincter</td>
<td>ELAPE</td>
</tr>
</tbody>
</table>

ELAPE = extralevator abdominoperineal excision.

**Fig 3.23** Illustration of surgical dissection planes of low rectal cancer. Left: Coronal T2W MR. Mesorectal plane of surgery (red line). Extralevator plane of surgery (yellow line)[71]. Right: MR criteria for selecting plane of surgery [73]. Reprinted with permission.

**Beyond TME**: Primary rectal cancers that breach the MRF and infiltrate into the surrounding tissues (T4b-TNM7) require en bloc resection, beyond the TME plane, of the involved organs or structures to achieve R0 resection [61][van der Velde EJSO 2013]. This also applies to locally recurrent rectal cancer, as the TME-plane is lost after the primary surgery. Smith et al recently reported that multivisceral resection for LARC had good oncological outcome when clear resection margin was achieved. Therefore, T4b (and recurrent) rectal cancers require an individualized approach. In a consensus statement
from the Beyond TME Collaborative all participants agreed that high-resolution T2W MR was the imaging technique of choice [74]. Furthermore, they stated that close collaboration between surgeon, radiologist and pathologist is of particular importance to ensure that the margins most likely to be involved are sampled adequately; international guidelines should be developed to guide specimen dissection and reporting [74].

Wait and see: Rectal cancer surgery leads to substantial morbidity [61]. When apparent downstaging or complete tumour response is achieved by the neoadjuvant oncological treatment, it is debated whether less extensive surgery or even deferral of surgery (wait and see) could be sufficient.

Radiotherapy
Radiotherapy is an integral part of the treatment of LARC. RT with concurrent fluoropyrimidine-based chemotherapy improves local control. The German rectal cancer study demonstrated that long-course RT was superior when administered preoperatively as compared to postoperatively [8, 75]. Both local recurrence and toxicity were lower and quality of life was better. Only a few studies have directly compared short-course RT and long-course CRT; long-course appears to be preferable, particularly in low tumours with threatened margins [55, 76].

Chemotherapy
In contemporary management of LARC, chemotherapy may be used at different time-points during the total treatment course: neoadjuvant as induction therapy, concomitant with RT as sensitizing therapy and adjuvant as systemic therapy. Standard therapy for LARC is preoperative concurrent CRT. The combined use of fluoropyrimidine-based chemotherapy with RT improves local control, but has no significant effect on survival [77-79]. Some centres have begun to offer neoadjuvant chemotherapy as initial treatment for patients with high-risk LARC to target micrometastases while treating the primary tumour [80]. Intensification of systemic therapy by neoadjuvant chemotherapy before standard treatment is feasible in poor-risk rectal cancer, with acceptable safety and promising long-term outcomes [Chua 2010]. Disseminated metastatic disease is the major cause of death in rectal cancer so adjuvant systemic treatment strategies are considered, especially for patients with positive margins or lymph node involvement [55, 56]. Introduction of neoadjuvant systemic chemotherapy to poor-risk patients leads to an extensive total treatment package of NACT+CRT+TME + adjuvant chemotherapy and may result in local overtreatment [55, 59]. Researchers at Memorial Sloan-Kettering Cancer Centre hypothesize that RT could be selectively omitted for patients who responds to NACT, supported by their recent published results from a pilot study [80]. European colleagues also support this opinion [81]. Schrag [55] advocate to administer systemic chemotherapy earlier in the treatment course to prevent disseminated disease.
4 Hypothesis and aims

4.1 Hypothesis

MR provides accurate information of the presence and the extent of tumour:

- MR can safely be relied upon for detailed planning of surgery
- MR provides reliable assessment of chemoradiotherapy response
- MR guidance can improve radiation planning during treatment
- MR guidance improves histopathology of large specimens

4.2 Aims

The aims of the thesis are to explore the potentials and explain the limitations of MR guidance in multidisciplinary cancer management.

The ambition is to understand the limitations given by MR technology and by tumour biology at a sufficient basic level to generalize the findings, and to point out in which direction MR could be improved.

The goal is to combine insight of MR technology with clinical needs and demands in order to find new applications of MR guidance that can lead to new treatment strategies.
5 Materials and methods

5.1 Materials

Paper 1 (SIB), paper 3 (MR-guided histopathology) and paper 4 (MR Volumetry) are based on patient cohorts of rectal cancer. Paper 2 (Detection of EPE) is based on a patient cohort of prostate cancer.

5.1.1 Prostate cancer patient cohort

The patient material was recruited between December 2007 and January 2010, when the MR examination protocol was fairly consistent. The majority of patients with primary prostate cancer, who were referred to the Norwegian Radium Hospital for treatment, were examined with MR. In all MR examinations, TNM-staging was prospectively performed and recorded. The surgical patients were prospectively enrolled in the institutions DaVinci database, from which the patient characteristics were obtained. The regional ethics committee approved the study and waived the need for informed consent.

Patients considered for radical prostatectomy had no evidence of skeletal metastases, neither at skeletal scintigraphy (PSA>10 ng/ml) nor at MR. Presence of suspicious local lymph nodes at MR was not an exclusion criterion; extended lymph node dissection was performed in high-risk [43] patients and whenever metastases were suspected at MR. Patients preoperatively treated with RT or androgen deprivation were excluded. Clinical data, including T-stage (cT) were deduced from routine DRE and TRUS performed at referral hospitals.

All patients who were referred to preoperative MR were consecutively included in the study material. However, not all patients who underwent surgery were referred to MR. Some patients, mostly low-risk patients were operated on without MR. No patients who underwent MR were excluded, regardless of suboptimal image quality. The percentage of high-risk patients referred to surgical treatment was high because the hospital is a third line referral centre for prostate cancer treatment and there was a renewed interest in surgical treatment of high-risk prostate cancer, often as an integrated part of multimodal management. Being a referral hospital, the time interval between diagnosis and treatment was relatively long; the median number of days between biopsy and MR was 89 days (range; 14-1621 days).

5.1.2 Rectal cancer patient cohorts

The rectal cancer patients were recruited from two partially overlapping (n=10) patient cohorts (Fig 5.1).
The Norwegian Radium Hospital is a tertiary referral cancer centre for multimodal treatment of primary LARC and locally recurrent rectal cancer. Since September 1990 data from the multimodality or rectal cancer treatment have been prospectively entered into a database (approved by the ethical committee at the University of Oslo), maintained by senior consultant surgeon Stein Gunnar Larsen. The material in paper 3 consists of 268 consecutive registered patients from January 2002 until April 2007. All patients had primary rectal adenocarcinoma located 0-15 cm from the anal verge and were preoperatively staged at MR, 28 had T1-2 tumours and did not receive neoadjuvant treatment, 99 had T3 tumours and 26 patients had tumours that involved the peritoneal reflection but no other neighbouring structures. The remaining 115 had tumours that invaded other organs or adjacent structures. The rate of T4 tumours was high (43%) because T4 tumours were systematically referred to The Norwegian Radium Hospital.

Seven patients did not receive RT or the treatment was not completed. Another 8 patients did not have MR after neoadjuvant RT and 4 had MR of suboptimal quality. Three patients were surgically explored and found non-resectable due to abdominal carcinomatosis or extensive liver metastases. One patient was excluded due to previous history of pelvic malignancy. Thus, a total of 92 consecutive patients with T4b (TNM7) rectal cancer were included in the study.

Pelvic RT was given as 2-Gray (Gy) fractions to a total dose of 50 Gy. The median time to surgery from RT/CRT completion was 56 days (range, 36-119).

Until December 2003, CRT was not routinely used, however 7 patients received chemotherapy (5-fluorouracil/leucovorin) as part of a randomized multicentre trial (Nordic
LARCS-A Trial [82]). 44 patients received 5-fluorouracil/leucovorin and 9 patients received oxaliplatin/5-fluorouracil/leucovorin. In total, 53 patients received CRT and 39 patients received RT.

The patient materials in papers 1 and 4 originate from the Locally Advanced Rectal Cancer – Radiation Response Prediction (LARC-RRP; ClinicalTrials.gov Identifier: NCT00278694) study A. The eligibility criteria were histologically confirmed rectal adenocarcinoma that was either T4, T3 with predicted CRM \( \leq 3 \) mm, or any T-stage with lymph node involvement within \( \leq 3 \) mm of the predicted CRM as assessed by MR.

Treatment was given every second week for 2 cycles before start of RT: oxaliplatin (85 mg/m\(^2\)) day 1, 5-fluorouracil (500 mg/m\(^2\)) day 1 and 2, calciumfolinate (60 mg/m\(^2\)) day 1 and 2 (the Nordic FLOX regimen). Following the second FLOX cycle: start of RT (2 Gy x 23 followed by boost 2 Gy x 2) for 5 weeks. During the RT: oxaliplatin (50 mg/m\(^2\)) once weekly and capecitabine (825 mg/m\(^2\) x 2) from Sunday evening through Friday morning.

MR was performed prior to treatment, after NACT + 3 fractions of RT (6Gy), and prior to surgery (Fig 5.2).

**Study material interrelationships**

The two rectal cancer patient cohorts (Larsen et al. and LARC-RRP) had partially overlapping inclusion periods (Fig 5.3). Furthermore, the patient populations in paper 1 (SIB), paper 3 (MR-guided histopathology), and paper 4 (volumetry) are to some extent also overlapping (Fig 5.1).

The MR-guided histopathology study material consisted of 52 patients from the original 92 patients in the study from Larsen et al [4] of whom 10 also were included in the LARC-RRP study. Four of these 10 patients were subjected to re-assessment.

The SIB study material consisted of 10 patients from the LARC-RRP study population, of whom 9 were included both in paper 3 (MR-guided histopathology) and in paper 4 (volumetry), but none were subjected to re-assessment.

The volumetry study material (paper 4) consisted of 69 patients from the LARC-RRP study population, of whom 9 patients also were included in paper 3, four of these patients were subjected to MR-guided histopathology (paper 4).
Fig 5.3 Time-diagram of the inclusion periods of the two partially overlapping rectal cancer patient cohorts (Larsen et al. and LARC-RRP) and the patients included in paper 1 (SIB), paper 3 (MR-guided histopathology), and paper 4 (volumetry).

5.2 Methods

5.2.1 MR of prostate cancer

There were no widely accepted or recommended technical requirements for MR staging of prostate cancer at the time of the study (paper 2). The examinations were performed on a 1.5T scanner with phased-array coils without the use of an endorectal coil. The T2W morphologic images were based on transversal 2D sequences with slightly variable resolution, but mainly 0.6 x 0.6 x 3.0 mm³. In addition, a 3D isotropic sequence with a voxel size of 1 mm³ covering the whole pelvis was performed. Coronal T1W were used to detect intraprostatic haemorrhage. DW was performed both quantitatively and qualitatively. For ADC calculation, the number and strength of the diffusion weightings varied with b-values spanning from 0 to 1500. The resolution was usually 2 x 2 x 4 mm³. A heavily DW sequence with a b-value of 2000 and a resolution of 3 x 3 x 6 mm³ was utilized to assess the location and extent of the tumour. DCE was not performed. Peristalsis was suppressed by intravenous administration of 1 ml (20 mg) butyl scopolamine (Buscopan®) and intramuscular administration of 1 mg glucagon. A table with all sequence parameters are included in paper 2.

Two radiologists interpreted the examinations independently without consensus double-reading. T-status was interpreted prospectively. In a few cases where the T-status was not recorded, the T-status was retrospectively recorded by using the prospective free-text interpretation, thus not re-assessed. No patients were excluded regardless of image quality so the results reflect the performance of MR in clinical routine.

5.2.2 MR of rectal cancer

After Brown et al [1] in 1999 showed that MR reliably could predict free surgical margins, MR has gradually gained international acceptance as the best method for local staging of primary rectal cancer. The inclusion of patients started in 2002 when the institution was participating in the MERCURY study [3], so the MR protocol and data registration was in
acquaintance with the requirements of MERCURY [54]. Following the MERCURY multicenter study, MR was established as the preferred method for treatment stratification of rectal cancer and subsequently included in the national guidelines in Norway. In 2005, Brown participated in a national symposium in Oslo, where, based on the protocol in the MERCURY project, the recommended minimum national technical requirements were established. Accordingly, the study protocol was unchanged during the 5 years of study inclusion.

The examinations were performed on a 1.5T scanner with a pelvic phased-array coil. The extent of tumour was assessed using morphologic T2W in the sagittal, transversal, and coronal planes with 0.8 x 0.8 x 4 mm\(^3\) resolution. A high-resolution (0.6 x 0.6 x 3 mm\(^3\)) sequence was acquired perpendicularly to the long axis of the tumour. T1W transversal images were performed, but no functional sequences. Peristalsis was suppressed by intravenous or intramuscular administration of 1 mg glucagon.

5.2.3 MR-guided histopathology

Cross sections from standard TME specimens commonly fit into large sized glasses providing genuine whole-mounts, whereas large specimens from extended TME usually must be split up into several smaller tissue blocks.

*Matching MR and histopathology*

For correlation of imaging and histopathology, it is crucial that the acquired tissue planes are comparable. The high-resolution MR images as well as the histopathological grossing were performed perpendicularly to the longitudinal axis of the rectum and consecutively numbered in the anal-oral direction. The radiologist and the pathologist jointly performed the matching and the renewed evaluations. First, the primary extent of the tumour was determined at pretreatment MR, providing the anatomic overview. Since downstaging was the main objective, it was assessed whether areas of initial extramesorectal involvement were adequately sampled; if not, histopathology was stated as inconclusive. Next, all sections covering the actual anatomic area were identified and carefully matched with the MR images: crosswise and longitudinally. Histopathology that only consisted of standard sized glasses, generally lacking anatomic landmarks necessary for orientation and matching, was stated inconclusive.

Direct matching to axial MR images was not feasible in anus and the distal two centimetres of the rectum, because the sectioning was performed in the sagittal plane in order to assess involvement of the recto-anal transition. The diagnosis of ypT4 was then determined by the presence of tumour cells within or peripherally to skeletal muscle cells, originating from either the external sphincter or the levator ani muscle.

*Detection of remaining tumour cells*

Particular attention was given to areas peripheral to the mesorectum, wherever MR showed fibrosis or mucin. All extramesorectal fibrosis and mucin were meticulously examined for remaining tumour cells. If inconclusive findings, or in cases of abundant fibrosis and mucin, deeper sections of the specimen tissue block were prepared. Presence
of apparent viable tumour cells was interpreted as residual tumour without considering the clonogenic ability of the cells. The finding of tumour cells located closer than 1 mm to adjacent extramesorectal structures or organs was defined as ypT4 [63]. When tumour advances through the MRF plane, the integrity of the fascia is often lost. Subsequently, if good tumour response to CRT is accomplished, the fascia is replaced by thick fibrosis. As the fascia represents the staging criterion distinguishing between γT3 and γT4, accurate and unambiguous assessment of γT is prevented. When tumour islets were detected within such fibrosis, an experienced gastrointestinal specialist surgeon was consulted and a pragmatic approach was taken: if standard TME surgery would have been sufficient to remove all tumour-containing fibrosis, stage ypT3 was recorded. Conversely, if extended TME would have been necessary to obtain complete removal, stage ypT4 was recorded. In low rectal cancers, ypT4 was interpreted whenever tumour cells were intermixed with skeletal muscle cells, originating from either levator ani muscle or external anal sphincter.

The radiologist and the pathologist that jointly compared the MR images and the H&E stained whole-mount sections, both had longstanding experience in assessment of LARC, and were both participants in the MERCURY study [3]. They had knowledge of the initial, prospectively recorded TNM and TRG assessments. Except for one case, they were not aware of local recurrences.

Additional immunohistochemistry was performed in a few cases with particularly small tumour islets among fibroblasts and vasculature: Cytokeratin AE1/AE3 to indicate the epithelial origin of residual tumour cells as distinguished from clusters of radiation-induced proliferation of fibroblasts and CD31 to indicate endothelial origin, because ring formation of cross-sectioned capillaries with glandular elements, can be misinterpreted as adenocarinoma.

_Tumour detectability at MR_
A former study [4], on which paper 3 is based, concluded that MR could not discriminate tumour within fibrosis. However, in that study the primary objective of MR was to guide the extent of the surgical resection needed to remove all tumour deposits. Consequently, any extramural fibrosis or mucin was interpreted as ypT4, based on the presumption that MR cannot safely exclude remaining tumour. The ability of MR as a method to predict complete response and detect remaining tumour, was thus not tested. Therefore, an additional objective when doing the comparison of MR and histopathology, was to investigate to what extent residual tumour cells are detectable at MR. In all cases it was determined whether residual tumour at histopathology was visible at the matched T2W MR image, referring to the tumour regression grade [22].

_Re-assessment of MR_
Precise assessment of the primary T-stage is a prerequisite for true down-staging rate and essential for patient stratification. In the process of exploiting MR-guided histopathology as a method, also the specificity of MR to prove stage T4 was investigated. Presence of extramesorectal mucin, or localized fibrosis in the same areas where pretreatment MR indicated extramural tumour, was interpreted as indicative of true pretreatment mT4. Accordingly, primary stage T3 was assumed if no signs of extramural reactive tissue were found.
5.2.4 MR volumetry

The volumetry was based on T2W morphologic images using PACS as well as the Oncentra Masterplan (Nucleotron, Columbia, United States) radiation planning system. Tumour was contoured on transversal T2W images while the system showed the resulting tumour delineation in sagittal and coronal reconstructions. DW images were available in a number of patients. Although the image quality was generally low, not adequate for tumour delineation, DW was used as an integrated part of the image interpretation when available. The volume was calculated by adding the delineated area of tumour in every image multiplied with the slice thickness and intersection gap.
6 Results

6.1 Summary of papers

6.1.1 Paper 1

*MR-guided simultaneous integrated boost in preoperative radiotherapy of locally advanced rectal cancer following neoadjuvant chemotherapy*

The goal of RT is maximal tumour cell eradication with minimal normal tissue toxicity. The LARC-RRP study included NACT prior to CRT, but dose planning was performed based on pretreatment MR. The aim of paper 1 was to evaluate if a SIB strategy using pre- and post-chemotherapy volumes could allow increased tumour dose without increased dose to OARs. Ten LARC patients were included. Pre- and postchemotherapy MR tumour images were co-registered with CT images for IMRT planning. Three planning target volumes were defined: PTVrisk, PTVpre_chemo and PTVpost_chemo. For SIB, prescribed mean doses to the PTVs were 46, 50 and 58 Gy, respectively, given in 25 fractions. The new three-volume SIB strategy was compared to a conventional two-volume SIB plan, in which PTVpost_chemo was ignored, using dose-volume histograms (DVHs) and the generalized equivalent uniform dose (gEUD). All patients showed tumour shrinkage following NACT. For the SIB, population-based mean doses to PTVrisk, PTVpre_chemo and PTVpost_chemo were $46.8 \pm 0.3$, $50.6 \pm 0.4$ and $58.1 \pm 0.4$ Gy, respectively. DVHs and gEUDs for PTVrisk, PTVpre_chemo, bladder and intestine revealed minimal differences between the two SIB strategies. In conclusion, following tumour volume reduction (NACT), an adaptive SIB strategy allows for increased tumour dose without increasing the dose to OARs.

6.1.2 Paper 2

*Routine pelvic MRI using phased-array coil for detection of extraprostatic tumour extension: accuracy and clinical significance*

MR had been used in the multidisciplinary management of rectal cancer and uterine cancers for several years. In our institution, MR staging of prostate cancer was increasingly implemented from 2005. The aim of paper 2 was to determine the accuracy and assess the clinical significance of the widely applicable MR protocol that was used to detect LAPC. Between December 2007 and January 2010, 209 patients consecutively treated with RALP were prospectively staged by MR. Conventional clinical tumour stage and MR stage were compared with histopathological tumour stage. Overstaged and understaged cases at MR were reviewed. 134 patients (64.6 %) had locally advanced disease. Sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy for the detection of locally advanced disease were 25.9, 95.9, 92.1, 41.2 and 50.5% , and 56.3, 82.2, 85.4, 50.4 and 65.4% for clinical staging and MR, respectively. Among patients understaged at MR, the resection margin was free in 64.4% of the cases (38/59). In conclusion, the detection of locally advanced disease improved substantially when MR was added to routine clinical staging, but overall accuracy was limited. Strict MR criteria for EPE ensured
minimal overstaging, but induced understaging. Nevertheless, the majority of the understaged patients achieved free margin. The results imply that to assess the clinical significance of MR staging, the extent of EPE and the dissection plane must be considered.

6.1.3 Paper 3

*Magnetic resonance-guided histopathology for improved accuracy of tumor response evaluation of neoadjuvant treatment in organ-infiltrating rectal cancer*

In a preceding study of organ-infiltrating LARC (T4), the assessments of downstaging after RT/CRT by MR and histopathology were very different. The aim of paper 3 was to explain the discrepant assessments and find the true downstaging rate. 92 consecutive patients who had received RT/CRT with subsequent extended TME were included in the preceding study. Independent interpretations of MR and histopathology were concordant in 40 cases. The remaining 52 cases were subjected to the procedure *MR-guided histopathology* where histologic sections were jointly re-assessed for residual tumour particularly in areas peripheral to the mesorectum, using MR images as guidance for where to inspect. After RT/CRT, 67.5% of the cases were found to remain ypT4 even though half of the study population had good response (complete response or microscopic tumour residuals). The false-negative rate of conventional histopathology for detection of ypT4 was 41.1%. Small, scattered tumour islets within fibrosis were undetectable at MR. In conclusion, good treatment response led to substantial tumour fragmentation and conventional evaluation of treatment response to neoadjuvant RT/CRT overestimated downstaging.

6.1.4 Paper 4

*MRI volumetry for prediction of tumor response to neoadjuvant chemotherapy followed by chemoradiotherapy in locally advanced rectal cancer*

Neoadjuvant systemic chemotherapy prior to CRT was introduced to target distant micrometastases in LARC. The total treatment package of NACT+CRT+surgery and adjuvant chemotherapy may lead to over-treatment. The aim of paper 4 was to investigate whether MR volumetry could predict response to the complete treatment course already after NACT. 69 prospectively enrolled patients were included. Tumour volumes were contoured in T2W MR images obtained pretreatment ($V_{PRE}$), after NACT ($V_{NACT}$), and after NACT+CRT ($V_{CRT}$). $V_{PRE}$ and tumour volume changes relative to $V_{PRE}$, $\Delta V_{NACT}$ and $\Delta V_{CRT}$, were calculated and correlated to histologic TRG. $V_{PRE} < 10.5$ cm$^3$ and $\Delta V_{NACT} > -78.2$% yielded 100% specificity for prediction of good histologic tumour response (TRG1-2) to NACT+CRT, although sensitivity was limited ($V_{PRE}$; 36.7%, $\Delta V_{NACT}$; 32.7%). 57% of TRG1-2 cases were identified through small $V_{PRE}$ (18 patients) or $\Delta V_{NACT}$ (16 patients). There was no significant difference in $\Delta V_{CRT}$ for tumours achieving good and poor histologic tumour response (TRG3-5). In conclusion, MR-assessed small pretreatment tumour volume or large volume regression to NACT predicted good histologic tumour response to the total treatment course (NACT+CRT). The combination of small pretreatment volume and large volume regression identified more good responders.
6.2 Non-published results

6.2.1 Re-assessment of pretreatment mrT-stage in LARC

During the procedure of MR-guided histopathology, the pretreatment mrT-status was also re-assessed (Fig 6.1). After completed RT/CRT, the separate assessments by MR and histopathology were concordant in 40 out of 92 cases: 32 were yT4 and 8 were yT3 (paper 3). These 40 cases were not included in the procedure of MR-guided histopathology. Of the 52 cases submitted to MR-guided histopathology, 6 were re-interpreted as pretreatment T3, 6 patients remained undetermined due to inadequate pathology (n=5) and inadequate surgery (n=1). Assuming that tumour progression during RT/CRT was unlikely, T4 after treatment (yT4) was considered to have been T4 also prior to treatment.

Pretreatment T-status could be re-assessed by MR-guided histopathology in 78 out of 92 patients. 72 were interpreted as pretreatment T4 and 6 as pretreatment T3. Consequently 72 out of 78 patients (92%) were correctly identified as T4 at pretreatment MR. Since the study design did not included false-negatives (true T4 misinterpreted as T3 at MR), sensitivity could not be calculated.

![Flow-chart illustrating the re-assessment of pretreatment T-stage.](image)
6.2.2 Detectability of residual tumour at MR

The detectability of residual tumour cells following neoadjuvant treatment was assessed during the procedure of MR-guided histopathology. Areas containing residual tumour deposits at histopathology were identified at the corresponding MR image and the visibility were assessed with reference to the TRG classification:

Only TRG4 and TRG5 were reliably visible at MR. Scattered tumour islets throughout fibrosis (TRG2) were not visible; the signal strength on T2W MR was very low, dominated by abundant fibrosis. Consequently, T2W MR could not differentiate near-complete regression (TRG2) from complete regression (TRG1). TRG3 occupied an intermediate position. In some cases of TRG3, the signal intensity at T2W MR was slightly higher than fibrosis. TRG3 was more frequently observed in patients with only radiation as neoadjuvant treatment.

6.2.3 Causes of misinterpretation of ypT-stage at histopathology in LARC

The procedure of MR-guided histopathology revealed several causes of misinterpretation by the primary histopathologic assessment. Inadequate sampling, either due to inadequate histopathology (n=8) or due to inadequate surgery (n=1) was the cause in the indeterminate cases, ypTX (n=9). In the 23 cases that were re-interpreted as stage ypT4, two causes stood out: It was difficult for the pathologist to orientate in sections from large specimens (n=9) and differentiation between stage ypT3 and ypT4 was difficult when the MRF had been replaced by fibrosis (n=8). The orientation difficulties were mainly due to lack of anatomic overview. Large specimens from ETME-resections were cut into several sections and many sections lacked an anatomic landmark. Therefore, residues of fibrously thickened fascia were difficult to recognize as actually being the fascia and tumour extension across several sections was difficult to follow (Fig 6.2). Moreover, the search for sparse tumour cells was hampered by the orientation difficulties as the pathologist could hardly identify areas to focus on, i.e. areas that most likely contained tumour cells or were critical for the differentiation of ypT3 and ypT4 (n=4).

Specimens containing large mucin pools disintegrated during the grossing process, which impeded sampling and spatial orientation (n=2). In several of the re-interpreted cases, more than one cause was present. A general observation was that pronounced treatment response aggravated the sampling and interpretation difficulties.
Fig 6.2 Illustration of spatial orientation difficulties. Tumour (T) appears as a mesorectal deposit at histopathology (D), but the serial MR images (A) demonstrate how the primary tumour extends continuously through mesorectum and into the pelvic sidewall. The fibrous streaks (D, black arrows) running through the tumour is actually the mesorectal fascia (B, white arrows). At initial histopathology tumour was interpreted as a mesorectal lymph node metastasis, but MR-guided histopathology (A-C) proved yT4.
7 Discussion

7.1 Introduction

Customized and multimodal treatment increases the need for instruments to individually guide the therapy: for initial planning and for adjustment during the treatment course.

Image guidance requires two-way communication to be properly utilized. The radiologist needs to know the demands, challenges and consequences of the treatment that is guided. Likewise, the surgeon and the oncologist need to take into account the uncertainties of imaging. The potentials and limitations of MR should be considered based on the consequences for the treatment; the sensitivity and specificity of a method is not a goal in its own right. The implications may be different depending on the clinical question. Sensitivity is particularly important when overlooking leads to severe under-treatment, whereas specificity is important when an equivocal finding may lead to over-treatment and substantial morbidity.

This thesis seeks to discuss the potentials and limitations of MR guidance in the context of clinical implications, with emphasis on: (I) to what extent established MR-methods could be relied upon, (II) the technical requirements of the MR sequences, and (III) how to develop MR guidance to improve the basis for clinical decisions. The transferability of the findings, to other tumour entities and treatment options, is discussed based on whether the limiting factors are methodological or tumour-biological.

7.2 Guidance of surgery

MR precisely depicts the extent of the primary tumour and is widely used to select the appropriate surgical dissection plane, to decide whether neoadjuvant treatment is needed or if oncologic treatment alone is the best option.

7.2.1 Surgery of primary tumour

The patient materials are appropriate to assess MR guidance of surgery because they mainly consist of locally advanced tumours where individually planned surgery is essential to achieve free surgical margin.

The material in paper 2 consisted of 135 out of 209 tumours that had advanced beyond the confine of the prostate. The recruitment of the patients was not optimal, because not all cT3 cancers were submitted to MR before surgery, and some patients with low risk profile [43] had surgery without MR. To assess the clinical impact of MR guidance for achieving R0, not recruiting advanced tumours may have underestimated the impact, whereas not including organ-confined (T2) tumours may have overestimated the impact. Considered the low clinical detection rate (25%) of non-organ confined disease, preoperative MR
seems justified to recognize patients that are at risk of positive margin if standard dissection plane is used. The material in paper 3 consisted of MR-assessed LARC where standard dissection plane would have lead to involved margins, accordingly neoadjuvant CRT was delivered. The procedure of MR-guided histopathology showed that 72 out of 78 patients (92%) was correctly identified as having stage T4 disease at pretreatment MR.

Methodologically, T-classification was chosen as the endpoint for MR because it correlates with the tumour extent and therefore is decisive for surgical dissection plane. R-status was chosen as endpoint for surgery because it is well established in the AJCC/UICC classification system and by the surgical community, and it is predictive of outcome. Using R-status as reference for MR guidance is problematic since R-status depends on the actual and not the planned dissection plane. Ideally, the patients should be randomized into two groups, with and without MR guidance. However, considering the risk of under-treatment, randomisation would be unethical. Oppositely, if surgery is performed with unnecessarily wide margins, free margins can be achieved without image guidance. In addition to R-status, morbidity and functional loss should therefore be considered. This is particularly important in prostate cancer surgery where the surgical techniques span from strict nerve-sparing dissection to wide extrafascial or en bloc-resection. Accordingly, a serious shortcoming of paper 2 was that neither dissection plane nor morbidity was recorded. The conclusion of paper 2 acknowledges this shortcoming, stating that the clinical significance of preoperative MR guidance hardly can be appreciated using only the standard evaluation parameters of T-stage and R-status.

The MR method used in this thesis to assess the primary tumour extent of LARC was an established technical standard, often referred to as high-resolution T2W MR. Since less than 1 mm margin at histopathology is associated with high risk of local recurrence [63], the goal is to predict if a free resection margin $\geq$ 1 mm is achievable with TME-surgery. In 1999 Blomqvist et al [83] demonstrated that 1 mm distance at MR of resected specimens corresponded to 1 mm at cross-sectional histopathology. Later, the MERCURY-study confirmed that 1 mm at preoperative MR predicted free surgical margin [66]. Recently, follow-up data from the MERCURY study [84] showed that a distance from tumour to MRF $\leq$ 1 mm assessed at MR was predictive for local recurrence, DFS and OS. The high-resolution T2W MR had a pixel size of approximately 0.6 mm or smaller. According to the Nyquist sampling theorem (cf. 3.2.3), this is technically adequate to discriminate a distance of approximately 1 mm. The slices are angled perpendicularly to the curvature of the mesorectum in order to minimize the slice partial volume effect. Thus, for measuring the distance from the tumour to the MRF, the pixel size is a critical image parameter. The finding that 92% was correctly staged as mT4 (cf. 6.2.1), supports the relationship between technical prerequisites and staging performance.

It was difficult to choose MR imaging parameters to explore T-staging of prostate cancer; at the start of the study MR was only regarded as a promising method. Technical consensus recommendations [34, 44] were reached as late as two-three years after the end of the study (paper 2). Varying staging accuracies were reported in the literature, (<50% up to 90%), probably reflecting a wide range of technical standards and risk profiles in the patient materials. Conventional clinical staging using DRE and TRUS is rather insensitive to detect non-organ confined disease. Consequently, a widely applicable MR
protocol that could be performed at most hospitals offering radical prostatectomy was chosen, i.e. 1.5T system, phased-array coils and standard protocols for staging pelvic malignancies. At the start of the study, 3D SE sequences with variable flip angle had become available. For T2W morphologic imaging, a combination of 2D and 3D techniques with comparable voxel volumes were chosen. The 2D technique had anisotropic voxels of 0.6 x 0.6 x 3 mm³, whereas the 3D technique had isotropic voxels of 1.0 x 1.0 x 1.0 mm³ both with a voxel volume = 1 mm³.

The small pixels of anisotropic voxels enable detection of small structures provided that the tissue structures do not vary in the slice thickness direction. Since the prostate is almost spherical, partial volume effect substantially influences the tissue resolution when anisotropic voxels are used, except where the scan plane is orthogonal to the prostate curvature. Acquiring anisotropic images in three orthogonal planes could have improved the staging performance. Due to limitations in total scan time, only a transversal anisotropic scan was performed. In return a 3D sequence was included. The major disadvantage of 3D compared to 2D sequences, is inferior in-plane resolution (larger pixels). According to the Nyquist sampling theorem, a pixel size of 1 mm limits the reliable detection of EPE to 2 mm extent. The included diffusion sequences had even lower resolution (2 x 2 x 4 mm³). DCE was not included, but would probably not have improved the detection of focal EPE (paper 2) as the in-plane resolution usually is larger than 1 mm. All these technical limitations are taken into account in the design of the ongoing Funcprost study (cf. 9.3).

The results for MR guidance of surgery are clearly better for TME than for RALP, both for T- and R-status. 92% of LARCs were correctly staged as T4 and consequently had neoadjuvant treatment and individually MR-guided ETME. If MR had not recognized primary tumour extent closer than 1 mm to the resection plane, R0-resection would have been impossible. This precise depiction of tumour extent is in accordance with the MERCURY experiences [3, 54, 84]. Furthermore, in a study comprising a wide range of tumour stages and treatment strategies, Engelen et al [59] demonstrated that tailored treatment of primary rectal cancer based on preoperative MR imaging leads to a high rate (95.6%) of complete resections, and that the cause of R1-resections (4.4%) was not due to underestimation of tumour extent at MR.

For prostate cancer, MR correctly identified 64% of T3 and T4, whereas clinical assessment using DRE and TRUS only indentified 26%. Routine MR staging of prostate cancer therefore improves the premise for achieving free resection margin. Interpretation of resection status following MR guidance of RALP in paper 2 can hardly be assessed, as it depends on radial extent of EPE, dissection plane, and whether the surgeon changed the surgical strategy according to MR findings. None of these factors were consistently reported.

There may be several explanations for the superior results of MR guidance for TME than for RALP. In the LARC material (paper 3), only specificity was assessed because non-recognized T4 tumours (false-negatives) would not have been included in the study. However, considering the precise T3/T4 differentiation with low rate of overstaging (8%), it is unlikely that substantial understaging has occurred. Another, and more likely explanation is that the critical margins for selecting the appropriate surgical dissection
plane in RALP are narrower than what can be visualized by the applied image resolution. A third explanation may be the presence of tumour satellites, intermixed with periprostatic fat, smaller than the voxel size. The difference between sensitivity (56%) and specificity (82%) for T3-detection, suggests small amounts of extraprostatic tumour, either as focal EPE (<1 mm radial extent) or as small satellites [41] along periprostatic nerves.

7.2.2 Surgery following neoadjuvant treatment

The patient material is appropriate to assess MR guidance of surgery following neoadjuvant treatment because; (I) standard surgical treatment (TME) would have been inadequate and (II) a change in surgical dissection plane can be considered due to generally good treatment response.

The aim of the study by Larsen et al [4] was to investigate whether it would have been safe to perform TME following neoadjuvant treatment of T4-LARC and whether this could be predicted by MR. MR-guided histopathology revealed that following successful CRT, tumour predominantly fragmented into small clusters of residual tumour cells with low degree of downstaging. If the surgery had been changed from ETME to TME, less than one out of three patients would have achieved complete tumour removal. The residual tumour clusters are too small to be detected by MR, thus MR cannot safely be used to guide a change of surgery. Due to the fragmentation of tumour after CRT, R0 as response parameter may be misleading as remaining tumour cells may not be present at the resection margin.

The study design does not enable assessment of whether the microscopic tumour residuals are clonogenic. It remains to be proven if small residual extramesorectal tumour cells actually lead to local recurrence when standard TME is performed after neoadjuvant treatment regardless of initial tumour extent. However, recent follow-up data from the German CAO/ARO/AIO-94 trial [8] showed an incremental increase of local recurrences throughout 11-year follow-up, indicating that residual tumour cells are clonogenic.

The MR method used was the established standard for preoperative assessment of tumour extent. The resolution (voxel size ≈1 mm$^3$) is probably close to the optimal trade-off between SNR and scan time at 1.5T for imaging the pelvis, considering that comparable resolutions are recommended also for staging prostate cancer [34] and gynaecologic cancers [85-87]. The procedure of MR-guided histopathology revealed however, that this resolution is far from adequate to depict the microscopic tumour residuals in good responders, i.e. TRG1 [20, 22]. The voxel size of (≈1 mm$^3$) is in the order of 10 million times larger than a single tumour cell (HeLa, an immortal cell line derived from cervical cancer). Consequently, substantial number of tumour cells clustered together is necessary to determine the signal of a voxel. Increasing the magnetic field strength to 3T enables somewhat higher resolution, but in practice no more than twice the resolution compared to 1.5T [34]. Heavily DW sequences seem a better option to depict residual tumour because they will produce black background images (cf. 3.2.3), however microscopic tumour residuals would probably not be technically depictable: Currently, a typical resolution of heavily DW images is 2 x 2 x 4 mm = 16 mm$^3$ voxel size [34], at higher field
strength the gain in SNR seems counteracted by stronger susceptibility effect, so the feasible resolution is probably similar [88].

7.3 Guidance of oncology

Oncologic treatment of pelvic malignancies involves chemotherapy in addition to RT as essentially the most important modality. The primary tumour extent is important in the decision of oncologic treatment. Compared to surgery, the precision required for treatment planning is generally lower; therefore the ability to visualize the primary tumour extent is mainly discussed in the chapter 7.2 Guidance of surgery.

7.3.1 Evaluation of treatment response

The second objective for MR guidance of oncology is the assessment of treatment response. The most common response parameters are RECIST [16] (volumetry, downsizing), TRG [20, 21], and to some extent TNM (downstaging) [26].

For assessment of chemotherapy, RECIST is mostly used. Without subsequent surgery, no short-term reference standard is available. Thus, short-term endpoints that are predictive for the long-term endpoints (LR, DFS, OAS) are warranted. Following surgery, MR-findings can be correlated to histopathology with TRG and TNM (downstaging). In LARC, downstaging is most relevant for surgical strategy planning, whereas TRG is the reference standard for chemoradiotherapy.

The material is well suited to assess the treatment response: The tumour size was variable and the treatment response was heterogeneous, leading to large differences in downsizing and a wide range of TRGs. Oppositely, consistent changes would have made it difficult to assess the significance of MR guidance.

The methods used to assess treatment responses were beyond the standard methods, because the purpose was to explore the potentials of MR guidance.

In paper 4, volumetry was explored to assess the response to NACT alone. The methodological advantage of volumetry is measuring the volume compared to only measuring the longest diameter as in RECIST. Volumetry is not dependent of spherical tumour shapes and thus more sensitive to small volume changes [18, 89], but is more time-consuming. However, considering the impact the result may have on the subsequent multidisciplinary treatment, the extra time seems justified: Good responders may be adequately treated with less extensive regimens, in addition to the important issues related to cutting costs and reducing morbidity, whereas poor responders may need intensified treatment to improve the outcome. Furthermore, introduction of computer assisted semi-automated volumetry would speed up volumetric assessment, and may reduce the inter-interpreter uncertainty through standardized measurements.
In paper 3, the response was explored by a novel method – MR-guided histopathology. The method itself is discussed in the chapter 7.4 Guidance of pathology. The retrospective design is essential to assess the methodological limitations of MR and histopathology as interpreter uncertainty is reduced to a minimum. The joint reviewing of MR and histopathology addressed false-positives as well as false-negatives. Tumour islets at histopathology were searched for at MR and tumour suspect areas at MR were identified at histopathology. Pretreatment and posttreatment MR were used to guide the pathologist to where to look for residual tumour cells in order to account for false-negative findings at histopathology. This is important as non-recognized residual tumour by the reference standard leads to (I) overestimation of the ability of MR to prove complete response because false-negative findings at MR is categorized as true-negatives and (II) underestimation of the reliability of MR to prove residual tumour because true-positive findings at MR is categorized as false-positives. The surgical strategy to remove all potentially tumour-containing tissue, i.e. ETME based on pretreatment extent, prevented that residual tumour cells were left behind, undetectable at histopathology of the specimen.

T2W volumetry of chemotherapy (paper 4) showed that small pretreatment tumour volumes as well as substantial volume reduction are predictive for good response. Good response was defined as TRG1+TRG2 because the procedure of MR-guided histopathology had revealed that neither MR nor histopathology could reliably differentiate TRG1 from TRG2.

The cut-off value of ≈ 70% volume reduction to predict TRG1-2 is in accordance with the findings from two other independent research groups [90, 91], indicating that MR volumetry is a robust method to assess treatment response.

The optimal timepoint for volumetric assessment is a balance between sensitivity and specificity, and depends on the degree of shrinkage. If performed too early, small volume changes may lead to under-estimation of response. If performed too late, generally large volume reductions impede differentiation. In paper 4, the early response assessment (after NACT only) showed significant difference in volume reduction between good and poor responders, but the late assessment (after NACT+ CRT) showed no difference, probably because extensive fibrosis had time to occur so that all tumours were substantially smaller regardless of TRG. Treatment induced oedema may have led to overestimation of tumour volume and thus underestimation of shrinkage at T2W sequences. DW differentiates oedema from residual solid tumour; if a (co-registered) high-quality heavily DW sequence had been used to delineate the residual tumour volume, the measured volume changes would probably have been larger, hence improving the sensitivity of volumetry to indentify good responders.

The choice of timepoint must also consider when in a treatment course it is most appropriate to adjust the treatment. The optimal timepoint is therefore probably specific for the chemotherapeutic agent and the cancer type.

In paper 3, MR-guided histopathology led to only minor changes in TRG, whereas T-classification (downstaging) was significantly revised. Detection of residual tumour
peripherally to the MRF resulted in upstaging to T4, but did not change the TRG as long as the TRG (ratio between residual tumour and treatment-induced fibrosis) was the same as within the mesorectum. Therefore, MR cannot predict downstaging or complete response when treatment causes extensive tumour fragmentation into scattered, microscopic residual tumour cells (TRG2). However, MR reliably predicts TRG provided that TRG1 and TRG2 are combined to classify good response. The observations of Patel et al [92, 93] also indicate that MR predicts TRG. Abandoning the distinction between TRG1 and TRG2 should be considered even for histopathology, because it is impossible to exclude residual tumour using a technique based on sampling.

7.3.2 Guidance of radiotherapy planning

MR is used to delineate GTV and OARs at many institutions. This use of MR guidance is based on many of the same considerations that are discussed in chapter 7.2 Guidance of surgery. Although not as critical as for surgery, accurate tumour delineation is increasingly important in modern high-precision conformal RT: small changes in volumes may have substantial effect on the delivered dose and rather modest changes in dose (both to the tumour and normal tissue) can significantly influence the clinical outcome. MR guidance of RT planning differs from surgical planning in two respects: (I) CTV adds a margin to GTV to account for microscopic spread, which is a major limitation for image guidance of close surgical dissection planes (cf. 3.6 Prostate cancer). (II) Geometric distortion in MR images is a major concern to the delivery of RT; MR images are therefore generally co-registered with CT to ensure geometric precision. Furthermore, CT contains tissue density information that is used to calculate the dose distribution.

MR may also guide intraoperative and adjuvant RT (following surgery) based on primary tumour extent and the pathways of spread. MR guidance is also applied to adaptive RT strategies. In treatment of cervical cancer, MR-guided boost to residual tumour has been used for years [94, 95]. Boost strategies are recently also applied in the treatment of rectal cancer [96], also for re-planning during CRT [97]. Paper 1 exploits adaptive re-planning during NACT.

The material in paper 1 is well suited to assess MR guidance of radiation re-planning because the tumours were generally large and treatment induced significant downsizing.

The methods used in paper 1 were standard high-resolution T2W MR sequences for staging of rectal cancer and standard RT planning system for GTV-delineation. Re-imaging and re-planning were performed only once during the total course of 25 fractions. Ideally, re-planning should be performed for each fraction to establish optimal timepoint(s). To delineate tumour at T2W is relevant [94, 95], but probably not optimal. In the presence of oedema, delineating residual tumour on heavily DW images should improve the precision (Fig 3.8). The feasibility design is an important limitation of the study. The impact of changing geometry and dosimetry during treatment, in terms of normal-tissue toxicity or cancer control was calculated, not performed. Encouragingly, recent data from a study exploiting MR-guided adaptive boost in LARC demonstrated good response (CR 36%) and acceptable toxicity [97].
The results in paper 1 showed that the dose to PTV could be increased from 50 Gy to 58 Gy without increasing the dose to the OARs, as tumour shrunk during NACT. To my knowledge, no other study has explored adaptive boost RT based on treatment response of chemotherapy alone in LARC. Since CRT induces more shrinkage than NACT, the impact of adapting geometry and dosimetry during CRT is likely to be even more pronounced. The volumetry data from paper 4 support this assumption; despite considerable tumour shrinkage after completed NACT+CRT, not all were good responders, and probably they would have benefitted from higher dose to the remaining tumour.

The findings from the procedure of MR-guided histopathology raise the question of whether it is safe to adapt GTV to residual tumour as visualized at MR. Considering the extensive fragmentation of tumour, there is a risk that small tumour foci are not included in the adapted GTV. Given two essential conditions, this may nevertheless be appropriate: (I) There is a dose-volume relationship between tumour volume or cell density and tumouricidal dose [98, 99] and (II) the adaptive boost strategy is not used to de-escalate the scheduled dose to the pretreatment extent of tumour. This reasoning is supported by follow-up data from treatment of cervical cancer, where introduction of MR-guided adaptive boost led to improved local control and reduced morbidity [95].

**Considerations for dose painting**

The observation that higher radiation dose is needed to eradicate larger tumour volumes and higher cell densities [98, 99] imply that heavily DW sequences may be particularly well suited to guide treatment-adaptive boost strategies. Provided adequately weighted, the signal intensity of DW sequences correlates with cell density and thus probably reflects the dose needed for eradication (dose painting by numbers). The major disadvantage of DW is the inherited geometric distortion. This limitation can be outweighed if DW is co-registered and co-interpreted with morphologic T2W images (Fig. 7.1). First pass DCE also possesses the properties of a black background sequence (cf. 3.2.3), but it is probably not as well suited to guide treatment-adaptive radiotherapy as the signal intensity reflects vascularisation. The angiogenesis may be inhibited by the chemo- and radiotherapy, leading to insensitive depiction of residual tumour, and treatment induced inflammation of the surrounding tissue may mask tumour or be misinterpreted as tumour.

Tumour hypoxia is associated with decreased radiosensitivity and is therefore important for dose painting. It is not well understood how, or if, imaging can reflect tumour hypoxia in vivo. Logically, hypoxic tumours are expected to appear with low signal intensity at perfusion imaging. If so, hypoxic tumours might be difficult to detect at DCE. However, if tumour is depicted at another sequence, lack of contrast enhancement on DCE could be used to recognize that the tumour is hypoxic.

Heavily DW sequences are probably sensitive to hypoxic tumour because the visibility of tumour cells at DW relies on intact cell membranes and not on vascularisation. The extreme condition of hypoxia, acute infarction or coagulative necrosis, appears with extremely high signal intensity at DW and may thus be integrated in the adapted GTV if DW is used for dose painting. However, over-radiating necrotic tissue seems a minor problem. Considering that treatment induces fibrosis intermixed with residual tumour (cf.
the TRG definition), ADC is not well suited to guided adaptive boost strategies since both tumour and fibrosis appears with low signal.

The PET technology has the potential to be very useful for dose painting. It is co-registered with CT and thus not subjected to geometrical distortions, but the spatial resolution is low compared to MR (Fig 7.1). PET has the properties to act as black background sequence (cf. 3.2.3) if the tracer possesses high specificity for tumour. For unspecific tracers, activity of the surrounding tissues will reduce the conspicuity of sparse residual tumour. Hypoxia is challenging to visualize also for PET. Hypoxia may be measured with tracers such as $^{15}$O of $^{18}$F-Fluoromisonidazole (F-MISO), but no tracers are available in clinical routine. Hypoxia-induced low metabolism may cause the widely used metabolism tracer FDG to be insensitive. However, the most dangerous tumours are probably those that maintain metabolic activity despite hypoxia. If so, combined imaging of perfusion (DCE) and metabolism (FDG-PET) may be particularly appropriate to identify aggressive tumours and to guide adaptive RT strategies FDG-PET may be (Fig 7.1).

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**Fig 7.1** Illustration of combining functional black background sequences with morphologic images. Transversal images of the lower, posterior pelvis from a 68y male treated with low anterior resection for pT2N0 rectal cancer. Postoperative long-course CRT due to peroperative tumour perforation. Biopsy proven residual/recurrent cancer within presacral fibrosis 16 months after surgery and RT. Functional source images in the upper row, colour coded and fused with morphologic images in the lower row. T2W showed several areas of medium signal strength, suspicious of tumour within fibrosis (white arrows). All three functional imaging techniques clearly detected tumour and distinguished tumour from oedema within the thick fibrosis. DW specifically and precisely delineated the tumour. First pass DCE also precisely delineated the tumour, but did not clearly distinguish tumour from the well vascularised bowel wall. FDG-PET specifically depicted the tumour, but with lower resolution than DW and DCE.
7.4 Guidance of histopathology

Throughout the procedure of MR-guided histopathology we realized that imaging could assist and improve histopathology. Sampling is the Achilles heel of histopathology; even though the level of detail is high, the accuracy relies completely on representative and diagnostic samples. The samples are selected from unstained tissue blocks of (large) surgical specimens where tumour location and extent are difficult to recognize. Extensive treatment response aggravates the sampling difficulties. Histopathologic sections from large specimens may lack an anatomical reference structure such as rectal lumen, leading to difficulties with orientation at microscopic assessment. Guidance from MR correlation may therefore improve sampling as well as interpretation. A search in the PubMed database with the keywords MRI, pathology, histopathology, guidance, and sectioning did not result in references that explore MR guidance of histopathology.

The material used, organ-infiltrating rectal cancer is well suited to prove benefit of MR guidance as large specimens from en bloc resections (ETME) and many cases with extensive treatment response caused handling and sampling difficulties.

The method MR-guided histopathology is new and described in the separate methodology section. The MR sequences and the histopathologic procedure underlying MR-guided histopathology were standard methods used in the MERCURY study [3, 54]. Whereas correlating MR to histopathology is standard [1], MR-guided histopathology is the other way around; areas of primary extramesorectal involvement were identified at MR and then sought out at the corresponding histopathologic section, in search for residual tumour cells. The method was therefore particularly adapted to assess downstaging, not equally optimal to re-assess TRG or CR; The strategy to closely scrutiny all areas of initial extramesorectal involvement minimized false-negative ypT4, but was not equally appropriate to address false-negative ypCR (pT0, TRG1). The aim of the procedure was not to find the true rate of CR. Deeper sections and immunohistochemistry would probably reveal more residual tumour cells. By examining additional deeper sections from patients initially diagnosed as pCR, Park et al revealed residual cancer cells in 7 out of 88 patients (8%) [100]. False-positive interpretation of small tumour islets at histopathology may occur if endothelial cells or proliferating fibroblasts are misinterpreted as tumour cells. In a case with particular sparse and small tumour residuals, immunohistochemistry staining was performed to indicate epithelial (AE1AE3) and endothelial (CD31) origin (Fig 7.2).

Fig 7.2 Illustration of particularly small and scattered residual tumour cells (A and B). B shows AE1AE3 staining of epithelial tumour cells and no staining of the surrounding fibroblasts. C shows CD31 staining (brown) of endothelial small vessels. The arrows point at the similar ring-structure of residual adenocarcinoma.
Immunohistochemistry confirmed the H&E-based interpretation of the pathologist, but was not systematically performed so false-positives may have occurred. The strategies to minimize false-positive histopathologic interpretation were: The pathologist was experienced and trained in the MERCURY-project and immunohistochemistry spot-checks were performed. For transparency, all 52 cases were carefully documented and published as a supplementary download: pre- and post treatment MR images, (macro tissue blocks), and histopathologic sections with close-ups accompanied by figure legends explaining the findings and the interpretation (Fig 7.3).

Fig 7.3 An example of how MR-guided histopathology was documented

The results from MR-guided histopathology showed that about half of the cases were up-staged from ≤ ypT3 to ypT4. In one out of five (20%) cases the sampling was inadequate to prove downstaging. Park et al found that re-assessment of histopathology slices did not improve detection of scarce residual tumour, but deeper sections improved the detection rate, however only by 8% [100]. Therefore, it is likely that the substantially larger upstaging rate by MR-guided histopathology (50%) predominantly was accomplished by the anatomical overview provided by pretreatment and post-treatment MR, and not by the renewed and meticulous search for residual tumour per se. Therefore MR guidance can improve sampling as well as interpretation of histopathology.

The transferability of the results depends probably on the extent of primary tumour, the degree of treatment response, and the choice of response parameter. Handling and sampling difficulties are not likely to be as pronounced if the tumour or the specimen is
small, or if there is abundant residual tumour. The difficulties of handling and sampling are presumably not specific for the tumour entity, but an infiltrating growth pattern, as for adenocarcinoma, and fragmentation during treatment aggravates the problems. The choice of response parameter influenced the impact of MR guidance; the differentiation between ypT3 and ypT4 MR guidance substantially improved the accuracy of histopathology, but TRG remained unchanged except for two cases.

The procedure of MR-guided histopathology is obviously too resource-demanding to be implemented into clinical routine. However, if applied prior to handling and sampling of the specimens, image guidance of histopathology is less resource-demanding and many of the shortcomings of histopathology could be counteracted. Following the experiences with MR-guided histopathology we adopted the MR-guiding to the grossing process of new patients in a three-level design. (I) In most cases the cross sectioning was adequately planned by jointly viewing pre- and posttreatment MR. (II) In cases of large tumours, the radiologist sketched the tumour extent. (III) In cases of particularly large and extensive tumours the grossing was performed (Fig.7.4).

![MR-images in PACS](image1)

![Surgical specimen](image2)

![Sketch drawing form](image3)

![Appropriate section](image4)

![Whole mount from the appropriate area](image5)

**Fig 7.4** Illustration of each steps in MR guidance of the grossing and sampling procedure.
This illustrates that MR guidance of histopathology could be applied with a wide range of complexity, also at minor costs. Eventually, the grossing was planned by phone and ultimately the pathologist self-viewed the MR images. In an ongoing study of prostate cancer aggressiveness (cf. 9.3), the pathologist uses a sketch intended for guidance of surgery to improve biobanking. Following MR guidance the detection rate for index tumour increased from 80% using five biopsies to 96% using three biopsies (Fig 7.5).

![Image](image_url)

**Fig 7.5** Illustration of image guidance of biobanking. Based on mpMR, the tumour was sketched (A). Guided by the sketch, the biopsy was taken from the unfixed specimen (B). Frozen section of the biopsy confirmed tumour (C). H&E section with a punch biopsy hole (arrow) at the site of the tumour (D).

**TRG**

To implement TRG in histopathologic reports of neoadjuvant treatment of gastrointestinal carcinoma is highly recommended [26, 101, 102]. Large studies [25, 92, 103] have demonstrated the prognostic significance of TRG on LR as well as OS. The histopathologic classification of tumour regression is however not standardized; several regression grading systems exist (cf. 3.4.4) with variable and worrying low inter-observer agreement [24, 104].

The challenges for the pathologist are mainly two-fold. (I) The assessment of response is based on the relationship between residual tumour and regressive changes, in particular fibrosis and mucin. However, significant fibrosis may be seen as desmoplastic response to cancer or may be caused by radiation per se [24, 104]. Mucin may be present as remnants of aggressive mucinous primary tumour [49] or as colloid response to RT [51, 101]. (II) Heterogeneous treatment response may result in variable TRG throughout the different specimen sections. In the Mandard [20] and Dworak [21] systems the worst area dictates
the grade, but in a recent report from an international study group of 18 gastropathologists [104], it was strongly felt that a cumulative score of all sections more truly reflected the tumour regression. It is however not known whether a predominantly good response or a focus of poor response (worst grade) best reflects the clinical outcome. Maybe TRG could be best reported as a score, combining an overall (cumulative) grade and a worst grade, analogous to Gleason score in prostate cancer?

MR could probably to some extent guide the pathologist in the assessment of TRG. MR shows if fibrosis or mucin is present prior to treatment and can demonstrate heterogeneous treatment response, and thereby guide histopathologic sampling of areas of worst grade. Upon detecting suspected areas of worst grade on MR, two TRG-gradings were re-assessed by the procedure of MR-guided histopathology. The overall impression however, was that TRG, unlike downstaging, was relatively independent of spatial guidance by MR.

As TRG proves a strong predictor of LR and OS, a new challenge arises for MR because clinicians advocate that the degree of response to neoadjuvant treatment could be used to alter the subsequent treatment [8, 55, 105]. If so, the assessment of tumour regression must be based on imaging. Paper 4 shows that volumetry correlates to TRG, but MR also has the potential to estimate the histopathologic TRG. The international study group on rectal cancer regression grading [106] argued that a new regression grading system should be designed that reproducibly distinguishes good responders (complete response/minimal residual disease) from poor responders and with an in-between group. Such a classification would greatly improve the premise for MR to correlate with histopathology; the procedure of MR-guided histopathology revealed that TRG1 and TRG2 appeared as complete response at MR, whereas TRG4 and TRG5 were recognized as residual tumour. TRG3 constituted an in-between group. Ergo, each separate grade in the translated 5-point mrTRG [27] from the system of Dworak [21], could not be discriminated. By dichotomizing the 5-point scale in good (TRG1-3) and poor (TRG4-5) responders, Patel et al demonstrated that mrTRG correlated to pTRG [93] and that mrTRG predicted 5-year OS [92].

7.5 Treatment response parameters

The observations from MR-guided histopathology imply that tumour responds to CRT by fragmentation, not only by shrinkage. The extent to which tumour fragments or shrinks will probably influence the performance of the different treatment response parameters (Fig 7.6.). It is also important if the assessment is performed at histopathology or at imaging. RECIST is only used for imaging, because a pretreatment measure is required. Downstaging is for the same reason mainly used for imaging, but may be used in histopathologic assessment if the pretreatment tumour extent can be assessed (Larsen et al [4] and Paper 3). TRG is mainly used in histopathology, but Taylor et al has proposed a TRG as seen on MRI [27].

Tumour shrinkage is correctly reflected by RECIST because it is based on the size on the tumour. However, tumour shrinkage may not necessarily be reflected by downstaging because this parameter is categorical. Substantial shrinkage may not lead to a lower
tumour stage so the treatment response may be underestimated. Tumour shrinkage may be difficult to interpret by histopathologic TRG, particularly if the treatment-induced changes are subtle. Despite substantial shrinkage, solid residual tumour may be interpreted as poor treatment response (c.f. 7.4 TRG).

Tumour fragmentation, provided adequate sampling, is correctly reflected by histopathologic TRG, but the treatment response may be overestimated by imaging-based parameters if the tumour residuals are too small to be detected. Accordingly, MR-guided histopathology led to significant re-assessment of downstaging, but only two TRGs were changed.

The combination of shrinkage and fragmentation (Fig 7.6) is challenging for histopathologic TRG because there is no consensus on how heterogenous treatment response should be reflected in TRG [106].

These considerations of treatment response parameters are probably transferable to other tumour entities than rectal cancer.

<table>
<thead>
<tr>
<th>Mechanisms of treatment response</th>
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<td>Pretreatment tumour</td>
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- **RECIST**
  - *Response Evaluation Criteria In Solid Tumors*
  - Pretreatment tumour: correct
  - Shrinkage: overestimated
  - Fragmentation: overestimated

- **TRG**
  - *Tumor Regression Grade*
  - Pretreatment tumour: correct or underestimated
  - Shrinkage: correct
  - Fragmentation: undetermined

- **Downstaging**
  - *T-classification*
  - Pretreatment tumour: correct or underestimated
  - Shrinkage: overestimated
  - Fragmentation: overestimated or underestimated

*Fig 7.6 Illustration of how treatment response may be assessed by different parameters, depending on the mechanism of treatment response.*
8 Conclusion

8.1 Exploration of the potentials

8.1.1 MR guidance of surgery

MR guidance of surgical treatment relies on the ability to visualize the extent of the tumour. The precision is given by the smallest amount of tumour, or tumour-free distance, that reliably can be detected, which in turn depends on the voxel size. This applies to primary surgery as well as surgery after neoadjuvant treatment.

MR guidance can accurately guide primary surgery of LARC because tumour is predominantly solid and the required distance to the MRF ($\geq$1 mm) is at least twice the pixel size (cf. Nyquist sampling theorem). Following neoadjuvant treatment, residual tumour islets may be too small to be detected; MR may thus not adequately guide surgery.

MR is reasonably accurate to guide primary surgery of prostate cancer. The difference in accuracy compared to LARC, may be explained by the relevant distances being less than twice the pixel size: Radial extent of EPE is $<1$ mm or dissection plane closer than 1 mm to the prostate. Moreover, microscopic tumour satellites within periprostatic fat may contribute to inaccurate prediction of tumour extent.

For surgical guidance in general, the specificity is principally high, so the surgical dissection planning should account for the tumour extension actually visualized at MR. Oppositely, the sensitivity is not equally high, so surgical planning should not unconditionally trust a negative MR. The likelihood of sub-voxel sized tumour deposits should be taken into account, based on tumoural growth pattern, path of spread, surgical dissection plane and image resolution. Given these considerations, the conclusions are probably transferable to MR guidance of surgery of other (pelvic) malignancies and transferable to guidance of biopsy and focal ablative treatments, as they likewise rely on the ability to detect and delineate tumour, respectively.

8.1.2 MR guidance of oncology

MR guidance of oncologic treatment relies on the ability to demonstrate treatment response. MR can accurately differentiate good from poor response (RECIST, TRG), but cannot exclude residual tumour ($yT0, CR, TRG1$).

MR volumetry of NACT in LARC did predict good response to the full treatment (NACT+CRT) with high specificity, but limited sensitivity; large volume regression proved good response, but small volume change did not equal poor response, especially for small tumours. The procedure of MR-guided histopathology indicated that MR differentiates good from poor response also by means of predicted TRG; no residual tumour at MR corresponded to TRG1-2, and detectable residual tumour corresponded to TRG4-5.
The clinical impact, equivalent to guidance of surgery, is that positive findings are reliable, whereas the implications of negative findings are conditional: MR volumetry predicts good response because it is based on a significant change (positive finding), whereas poor response is based on insignificant change (negative finding) and therefore does not exclude good response. MR assessed TRG predicts poor response (TRG4-5) because it is based on detected residual tumour (positive finding), whereas no detectable residual tumour (negative finding) does not differentiate the degree of good response (TRG1-2). However, good response can reliably be predicted using TRG if compared to pretreatment MR, because that involves a change (positive finding).

To what extent the results from T2W volumetry of NACT in LARC are transferable to other cancer types and chemotherapy regimens, remains undetermined. The principles probably apply, but appropriate cut-off values as well as optimal timepoint need to be established.

T2W volumetry can improve RT by guiding adaptive boost strategies. The premise for guidance, especially for dose painting, can probably be improved by co-registration of T2W and heavily DW, particularly when tumour disintegrates into areas with variable tumour cell densities.

8.1.3  MR guidance of histopathology

MR guidance can improve histopathologic assessment given two conditions: Firstly, if the surgical specimen is larger than the glass size and (residual) tumour is difficult to localize macroscopically. Secondly, if the response parameter critically relies on sampling: pT depends on where residual tumour is located, whereas TRG requires only a representative sample, except for complete response (TRG1, pT0).

8.2  Explanation of the limitations

The limitations of MR guidance can largely be explained by the partial volume effect caused by composite tissues within a voxel and tissue movements across voxels.

As the voxel signal is a sum, tissue with high signal strength will outshine low-signal tissue. At T2W both water and fat have high signal intensity while tumour has medium. Consequently, a voxel that contains fat or water in addition to tumour may appear brighter than the radiologists interpret as tumour. This partial volume effect in T2W probably explains the limited ability to guide prostatectomy because the extent of focal EPE was too small to determine the signal of a voxel dominated by periprostatic fat. The partial volume effect probably also explains the limited sensitivity of T2W volumetry, because treatment-induced oedema, generally brightens all voxels, and thereby masks small volume changes. Likewise, adaptive boost strategies probably could be guided even more precisely, if the partial volume effect in T2W was accounted for, e.g. by including a heavily DW sequence. The general observation that MR guidance has high specificity but limited sensitivity seems explained by the partial volume effect: High sensitivity when solid tumour dominates the
content of the voxel, but low sensitivity when tumour is admixed with surrounding tissue within the voxel.

The procedure of MR-guided histopathology discovered that considerable amounts of residual tumour within fibrosis (TRG3) were difficult to detect at T2W. This observation is difficult to explain by the partial volume effect that rather should favour the detection of medium grey tumour against black fibrosis. An explanation for the limited ability to discern residual tumour from fibrosis at T2W may be found in the nature of digital imaging (tone mapping cf. 3.2.3). Compared to the high signals of fat and water, fibrosis appears very black on T2W, although the signal intensity of fibrosis actually was approximately 10-fold the noise floor in the used sequences. Thus, the difference in signal intensity between fibrosis and residual tumour was relatively small, and their absolute values were small compared to the high values from fat and water. SE T2W images generally have high dynamic range; if interpreted with a global window/level setting, the tone range will be highly compressed and the pixel values of fibrosis and residual tumour may be tone mapped to similar brightness levels. If a user selective (locally adaptive) window/level is applied to increase the tone difference between fibrosis and tumour, these low brightness levels might not hold enough information to allow discrimination, but dissolve in image posterization and exaggerated noise (i.e. insufficient CNR).

The explanations of the limiting factors of T2W, partial volume effect and tone mapping, imply that the ability to detect residual tumour would improve if tumour had high signal intensity, whereas the surrounding tissue had negligible signal, black background (cf. 3.2.3). Then, the visibility would largely depend on the SNR, not the voxel size, so even sub-voxel amounts of tumour might be detectable (Fig 3.7 B). The partial volume effect would then display tumour somewhat larger than it actually is, but that is rather an advantage.

Heavily DW has black background signal characteristics: Fat is suppressed because DW is echo-planar based, signal from the fast diffusion water pool is lost due to heavy DW, most solid tissue have negligible signal due to rapid T2*-relaxation and only water in the slow diffusion pool (adherent to cellular membranes) give rise to signal. Therefore, clusters of residual tumour appear bright against a black background. Heavily DW complements T2W because the limiting factors of T2W, the partial volume effects and the tone mapping, turn advantageous. First pass DCE may also produce black background images. However, DCE reflects vascularisation so treatment-induced inflammation may limit the ability to discriminate residual tumour.

Motion across voxels generally causes partial volume effect in SE T2W due to substantial signal averaging. To counteract this effect, phase encoding directions were carefully chosen to reduce the impact of respiration, and antiperistaltic drugs were administered. However, small pulsatile tissue movements, particularly in rich vascularised tissue, may have contributed to partial volume effects.

The explanations of the limitations are founded on tumour biology down to the cellular level as well as fundamental technical considerations and are therefore probably transferable to other tumour entities and treatments.
8.3 MR in the management of pelvic malignancies

MR proves a central role in the multi-disciplinary team. MR guidance can improve surgery, oncology and histopathology in the management of advanced pelvic malignancies.

In order to benefit from image guidance it is important to account for the limitations given by tumour biology, particularly the presences of intermixed tumour satellites, and the image resolution.
9 Perspectives

9.1 Technological perspectives

9.1.1 Field strength

Clinical high-field MR (>3T) currently emerges in brain imaging. Higher field strength enables improved SNR and image resolution. At present, inhomogeneous RF-transmit limits the use on humans, particularly outside the brain. The fast expanding field of high-field brain imaging is likely to drive developments in RF-transmit technology and enable high field also for body imaging.

T2* relaxation (susceptibility contrast) and magnetization transfer effects increase with the field strength so these mechanisms will probably be more utilized.

9.1.2 Contrast agents

Several new probes for MR-based molecular and cellular imaging are emerging. They will probably lead to a wide range of new applications and opportunities (NMR in biomedicine, special Issue: Novel Probes for MRI-based Molecular and Cellular Imaging, July 2013, Volume 26, Issue 7, Pages i–ii, 725–884)

9.1.3 Multishot techniques – beyond the limitation of the voxel

Intravoxel tissue mixture is a fundamental limitation of MR guidance. One strategy to counteract this limitation is to remove (suppress) a specific (often background) tissue (Fig 9.1 left). Another strategy is to separate tissue components within a voxel (Fig 9.1 right). A widely used technique that utilizes multishot to decompose the voxel is the Dixon technique [107], in which two (or more) echo times (in phase and opposed phase) are used to separate the signal from fat and water. In addition to the acquired images, the Dixon technique calculates a fat image and a water image.
Fig 9.1 Illustration of techniques that counteract the limitation of the voxel. **Tissue suppression:** When the background tissue 1 is suppressed, the admixed tissue 2 is tone-mapped to a higher brightness value and becomes detectable. **Voxel decomposition:** Multiple measures of the same voxel (multishot) are used to discriminate different tissues within the voxel, displayed as separate images.

In DW imaging, multishot datasets (wide range of b-values) can be used to discriminate components with known characteristics such as microperfusion (IVIM), and the fast and the slow diffusion water pools.

If the repetition time and the echo time are varied in multiple, otherwise identical images, the T1- and T2 relaxation times (and proton density) can be calculated. Then, each voxel is represented by three values (PD/T1/T2). This provides more specific tissue characterization that can reduce the impact of the partial volume effect and facilitate computer-based segmentation. Another advantage is that different weightings can be displayed from the same sequence, analogous to adjusting the window/level setting. Such type of multishot morphologic imaging is known as relaxometry, MR synthesis, synthetic MRI etc.

By slightly changing the position of the pixel of otherwise identical images, higher resolution can be reconstructed. Such *super-resolution* technique is well known from the field of photography.

Geometrical precision can also be improved by multishot techniques. By altering the gradient polarity of otherwise identical images, image distortion can be calculated and corrected. By acquiring multiple identical images, in-plane motion can be calculated and corrected, if applied to 3D-volumes, possibly also through-plane motion.
9.1.4 Computer-aided detection (CAD)

The increasingly multiparametric information load is approaching the limit of what humans effectively can process. At present, modern pelvic imaging consists of T1W, T2W, ADC, DW, wash-in and wash-out information, probably also CT and PET. Computers are well suited to process such interconnected information [108]. One option could be computer-aided coregistration of multiparametric information visualized as new calculated risk-images, optionally overlaid onto morphology. Another option would be computer-aided recognition and segmentation of given multiparametric characteristics. This enables (semi-) automated volume calculation of lesions and predefined radiation planning volumes etc.

9.2 Clinical perspectives

MR-guided cancer management is probably evolving in two directions: by contour and by risk.

Increased spatial resolution (contour) will be needed as therapy becomes more precise; the use of robotic assistance increases in surgery, more conformal techniques are applied in RT, and focal ablative therapies emerge. Higher magnetic field strength will improve image resolution and progress in motion correction techniques will improve the actual tissue resolution. Geometric distortion correction techniques and automated (non-rigid) fusion with CT-images will ensure geometric precision.

Tumour characterisation (risk) will become increasingly important. Functional imaging techniques, particularly MR and PET, will provide ever more information that is important when deciding on, or changing treatment: i.e. risk of loco-regional spread, metastatic potential, resistance to therapy (e.g. hypoxia) or sensitivity to chemotherapy (e.g. high angiogenesis). Despite improved detection of small amounts of tumour, microscopic tumour will probably remain undetectable by imaging. Thus, the presence of microscopic spread, micrometastases and microscopic residual tumour, must be based on risk-assessment, not detection.

Genetic profiling is likely to become important for risk stratification. Furthermore, the choice of chemotherapy agent is likely to be based on presence of specific genetic translocations. Genetics require representative tissue samples. In order to guide the biopsies, an important future role for (MR) imaging will be to identify high-risk areas within the primary tumour as well as residual resistant foci during and after treatment.

Cancer is not always curable; then cancer control becomes the strategy. In addition to identification of new metastases, imaging will become important to identify areas of transformed cancer, to choose and guide targeted treatments, and to (early) assess the response of (very expensive) treatments.

In research, imaging may provide information of the mechanism of action of new chemotherapy agents. In dose escalation studies, imaging will be used to determine the level of maximal response, which may be substantially below the level of toxicity.
9.3 Further studies

In an ongoing prospective study (Funcprost: ClinicalTrials.gov Identifier: NCT01464216) MR-guided management of prostate cancer is further explored, by contour and by risk. The image resolution of T2W is improved to 0.5 x 0.5 x 3 mm³ and acquired in three orthogonal planes, in order to reliably detect EPE with 1 mm radial extent. Whole-mount histopathologic assessment prospectively records the distinction between focal EPE (<1 mm radial extent) and established EPE. The surgical dissection plane is also recorded. The aggressiveness of the tumour is explored using DCE and multiple b-value DW in all patients, and FACBC PET/CT in a subcohort. The study hypothesis is that hypoxia indicates tumour aggressiveness. Pimonidazole is given as a hypoxia marker, and immunostained in whole-mounts sections. The extension of the index tumour and hypoxic areas are contoured on the MR and PET-images by correlation to whole-mount histopathology. Short-term (Gleason score, TNM) and long-term (LR, DFS, OS) endpoints are used.

In another prospective study (ClinicalTrials.gov Identifier: NCT01276483), early and late treatment responses to imatinib in GIST-patients are assessed by comparison of multiple b-value DW and FDG-PET/CT (Revheim et al, c.f. other publications by the candidate).

Currently, serial MR imaging (T1W, T2W and DW) is used to assess treatment response in a phase I/II study exploring safety/toxicity of a new anti-tumoural vaccine in oligo-metastatic prostate cancer patients (ClinicalTrials.gov Identifier: NCT01784913).

In the management of patients with cervical cancer, a new imaging study is about to begin. Multiparametric MR and FDG-PET/CT will be performed in the stratification of patients to surgery or RT, and as follow-up. Adaptive RT based on T2W+DW is planned. Short-term endpoint is pTN-classification in cases of surgical treatment. For oncologic treatment and risk-assessment, long-term endpoints (LR, DFS, OS) and treatment morbidity are used.

A new multiparametric MR study that utilizes vessel architectural imaging (Emblem et al, Nat Med. 2013 Sep;19(9):1178-83) is planned in the follow-up on patients receiving stereotactic radiotherapy for brain metastases. The aim is to differentiate radionecrosis from recurrent and residual tumour.

Participation in the new SPCG 15 study is planned. Multiparametric MR will be performed prior to treatment. The aim is to compare the outcome of oncological and surgical treatment of locally advanced prostate cancer (T3).
References


11 Papers