Reducing small bowel toxicity in locally advanced cervical cancer treatment

de Boer, P.

Citation for published version (APA):
de Boer, P. (2019). Reducing small bowel toxicity in locally advanced cervical cancer treatment.
Prospective validation of craniocaudal tumour size on MR imaging compared to histoPATHology in patients with uterine cervical cancer: the MPAC study

Peter de Boer, Anje M. Spijkerboer, Maaike C.G. Bleeker, Luc R.C.W. van Lonkhuijzen, Melanie A. Monraats, Aart J. Nederveen, Marc J. van de Vijver, Gemma G. Kenter, Arjan Bel, Coen R.N. Rasch, Jaap Stoker, Lukas J.A. Stalpers

Submitted to Radiotherapy & Oncology on January 18th, 2019
ABSTRACT

**Purpose:** To determine the accuracy of MRI in detecting craniocaudal tumour extension, compared to histopathology, of the hysterectomy specimen in patients with early-stage uterine cervical cancer. Three complementary methods were investigated.

**Materials and methods:** Thirty-four patients with early-stage cervical cancer had pre-operative MRI, followed by radical hysterectomy or trachelectomy. 1) craniocaudal tumour extension was measured on MRI by two radiologists and compared to microscopy by a pathologist, 2) to compensate for changes in uterine shape between pre-operative MRI and the surgical specimen, craniocaudal tumour extensions were directly compared and appreciated as being a part of a 3-dimensional tumour by a radiation oncologist and resident, and 3) tumour size on MRI was compared macroscopically after digital non-rigid registration of the uterus, uterine cavity and tumour of both modalities.

**Results:** The craniocaudal tumour extension measured on histopathology minus MRI gives: 1) on average +3 mm difference when measured by a radiologist compared to the microscopic extension (range -13 to +15 mm), 2) -0.2 mm (range -11 to +6.0 mm) when evaluated on MRI by a radiation oncologist compared to the macroscopic tumour; 3) after non-rigid organ registration, a margin of 10 mm around the tumour on MRI would be needed to cover 95% of the tumour in 90% of the patients.

**Conclusions:** Results indicate that microscopic tumour extension in craniocaudal direction is within a margin of 10 mm around the visible tumour on MRI. The major source of measurement uncertainty is post-surgical change of organ shape.
Introduction

Assessment of tumour extension in patients with uterine cervical cancer plays a crucial role in both surgical decision-making and radiotherapy treatment planning. In women with locoregionally advanced disease (FIGO stage IIB-IVA), radiation oncologists depend increasingly on MRI to determine and delineate tumour extension for external beam radiotherapy (EBRT) and brachytherapy.[58,59,134,180,181] With the use of MRI, adequate brachytherapy coverage became feasible and excellent local control is achieved with less toxicity[61,88,182,183].

With excellent local control, the focus of EBRT improvements lies on reducing acute and late small bowel toxicity, particularly by reducing margins[184–186]. For instance, substantially more small bowel can be spared if treatment plans can be adjusted for tumour shrinkage with online MRI-guided radiotherapy plus online replanning[111,173]. Furthermore, assessment of tumour invasion on MRI is important in order to minimise margins around the gross tumour volume[186].

Both gynaecological surgery and radiotherapy depend on the accuracy of MRI. In previous retrospective studies, the accuracy of MRI compared to histopathology was determined using either non-rigid registration, macroscopy data, or microscopy data[107,124,125,159]. A major limitation of these studies was that microscopy compared to MRI measurements did not account for differences in uterine shape between MRI and histopathology. This implies that, in many cases, a comparison is not made of the same length in 3-dimensional (3D) space, thereby causing uncertainty regarding the actual tumour size and probably resulting in exaggerated margins. Moreover, those who did attempt to take into account a change in uterine shape between MRI and hysterectomy, did not include microscopy, which is the reference standard of choice.

Therefore, in a prospective cohort of women with early-stage uterine cervical cancer, the present study combined two methods of comparing craniocaudal tumour size measurement on pre-operative MRI with post-operative pathology data, that takes into account a change in uterine shape between MRI and hysterectomy. In addition, in the same cohort, tumour size measured on MRI was compared to microscopy.

Methods and Materials

Study design

This prospective study compared craniocaudal tumour extension on pre-operative MRI with histopathology of the surgical specimen in patients with early-stage cervical
cancer after a radical hysterectomy according to Wertheim-Okabayashi[50]. The study was approved by the institutional ethical board and all patients gave written informed consent before inclusion.

**Patients**

All cervical cancer patients had a diagnostic pre-treatment MRI. Patients were only included if they were eligible for radical hysterectomy for early-stage cervical cancer, i.e. FIGO stage IA1-IIA. Patients were excluded if there was a contra-indication for MRI. Patients were also excluded if all tumour was excised on for instance biopsy or conisation, which resulted in no tumour found in the hysterectomy specimen.

**MRI Procedure**

All patients were scanned on a 3.0T MR scanner using a phased-array sensitivity encoding (SENSE) torso or cardiac coil (Ingenia, Philips, Best, the Netherlands). Sagittal, axial oblique and coronal oblique T2 weighted Turbo Spin Echo (TSE) images were obtained (TE 80ms, TR 4298-5906ms, TSE factor 20, matrix size 240 x 240, resolution 0.6 x 0.7 x 3.0 mm). To avoid bowel movement artefacts, 20mg of butylscopolaminebromide (Buscopan, Boehringer, Ingelheim, Germany) was administered intramuscularly.

Additionally, a DWI-scan with axial single-shot echo-planar imaging (EPI) sequence of the pelvic region was obtained (TE 58 ms, TR 5004 ms, matrix size 240 x 240, resolution 2.0 x 2.0 x 4.0 mm, EPI factor 51, sensitivity encoding (SENSE) factor 3, b-values 0, 100, 500, and 1000 s/mm², number of averages of 1, 1, 2, 5 respectively).

For evaluation, first, a case report form with instructions was developed in conjunction with two radiologists and the first author. The MR images were independently evaluated by two radiologists with 22 and 2 years of experience, respectively, in assessing MRI of the pelvic region. To minimise bias, a dummy-run was done on an MRI of a patient not included in this study. The maximum craniocaudal tumour extension along or parallel to the uterine cavity was measured on the T2 weighted sagittal images and DWI was evaluated in case of any doubt. To facilitate optimal comparison, the radiologists were instructed to measure the craniocaudal tumour extension that would have been visible for the pathologist after exposing the uterine cavity with an anterior median incision. In this way, they aimed to make measurements in the same way as the pathologist (Figure 6.1). The radiologists were blinded for each other’s measurement and for the histological tumour measurements by the pathologist.
If image quality was found to be unacceptable according to the clinical standards of the radiologists, this was recorded. Reproducibility of measuring the craniocaudal extension between the two radiologists was expressed by the intraclass correlation coefficient (ICC)[160].

Figure 6.1 Example of a 54-year-old patient with FIGO stage IB squamous cell carcinoma of the cervix which was measured as 14 mm on the macroscopic pathology photograph (A). The uterine internal canal is opened by a ventral incision. On MRI (B) the tumour was 13 mm in craniocaudal direction when measurement took place in the same 3D direction. This example shows why, in this study, appreciating tumour as a 3D structure is one of the crucial conditions for correct comparison. For instance, as shown on MRI, in the dorsal part of the cervix the tumour is even larger than the internal tumour extension, whereas this is not seen on the pathology image (arrow).

Histopathology

After orientation of the cervical surgical specimen, the uterine cervix and corpus were incised ventrally to expose both the endocervical channel (and uterine cavity)(Figure 6.1A). Digital photos were made at straight angles. The surgical specimen was processed according to the regular protocols used at the Department of Pathology. The maximum craniocaudal extension was measured both on macroscopy (before fixation) and verified by microscopy (after fixation). All data on both the macroscopic and microscopic craniocaudal extensions were reviewed by a gynaecopathologist blinded for the MRI-data.
Comparison between MRI and (histo)pathology

Three complementary methods were used to minimise uncertainties in measurements due to differences in uterine shape between MRI and histopathology:

1)  **MRI vs. histopathology**

The average craniocaudal tumour extension on MRI independently measured by two radiologists was compared with both the macro- and microscopic tumour extension as measured by the pathologist. The agreement of measured craniocaudal tumour extension between MRI and macroscopic pathology was visualised in a Bland-Altman plot. Furthermore, descriptive statistics and Pearson's correlations were calculated.

2)  **3D-comparison**

This method is similar to conventional radiation oncology practice: Typically, when defining a target for radiotherapy the physician's goal is to delineate the 3D shape of the target volume, while interpreting data from 2D images. Analogous to this, after interpretation of the 2D images knowing they are a part of a 3D shape, we measured craniocaudal tumour extension of the delineated tumour on MRI-target in the dissection plane of the surgical specimen to minimise 3D inaccuracies. These measurements were made by a resident in radiation oncology (PB) and a radiation oncologist (LS). The same statistical analyses were applied as described in 1) above (Figure 6.1).

3)  **Non-rigid registration of the uterus**

Macroscopic photographs of the uterus with the exposed uterine cavity were matched to the corresponding pre-operatively acquired T2-weighted sagittal MRI slice by digital non-rigid registration of the organs by a PhD student (physics) according to the description of a recently published paper [125]. In this way the uterus, uterine cavity and tumour were delineated both on photographs and MRI. Next, delineated parts of the photograph were simultaneously registered to the sagittal MRI slice using a three-step multi-image registration strategy. These three steps in non-rigid registration were developed to minimise registration errors by respecting internal structures and boundaries of the uterus and the uterine cavity. Results of the registration were evaluated with the Dice Similarity Coefficient (DSC) and the surface distance error (SDE). Further details can be found in the referred paper [125].
Results

Patients
Between May 2013 and October 2016, 36 women with early-stage uterine cervical cancer were included who had pre-operative MRI and radical hysterectomy according to Wertheim-Okabayashi. Two patients had no tumour in the surgical specimen after hysterectomy due to conisation or large loop excision of the transformation zone and were excluded from the study (Figure 6.A1). For the remaining 34 patients, baseline characteristics at the time of inclusion are presented in Table 6.1.

Figure 6.A1. Flow diagram of patient inclusion.

- 36 women with early-stage uterine cervical cancer who had an MRI and radical hysterectomy.
- Exclusion of 2 patients with no tumour in the surgical specimen due to conisation or LLETZ.
- 34 included patients:
  - 6 patients were treated elsewhere of which one macroscopic photograph could be retrieved.
  - 26 patients of whom craniocaudal extension on MRI could be compared to photographs by experts.
  - 20 patients of whom craniocaudal extension on MRI could be compared to microscopy by experts.
  - 27 patients of whom craniocaudal extension on MRI could be ‘3D’ compared to photographs by radiation oncologist.
  - 10 patients of whom craniocaudal extension on MRI could be matched to macroscopic photographs by digital non-rigid registration according to van de Schoot et al.[118].
Table 6.1  Baseline characteristics of the patients (n=34).

| Description                                                                 | Value          |
|-----------------------------------------------------------------------------|----------------|
| Median age (range) on MRI: in years                                         | 45 (24-67)     |
| FIGO stage                                                                  |                |
| IA2                                                                         | 1              |
| IB1                                                                         | 29             |
| IB2                                                                         | 4              |
| Histopathological subtype                                                  |                |
| SCC                                                                         | 20             |
| AC                                                                          | 9              |
| ASC                                                                         | 2              |
| Other                                                                       | 3              |
| Lymphovascular space invasion                                              |                |
| SCC                                                                         | 9              |
| AC                                                                          | 2              |
| ASC                                                                         | 1              |
| Mucinous carcinoma                                                          | 1              |
| Treatment before MRI                                                        |                |
| LLETZ                                                                       | 8              |
| Conisation                                                                  | 6              |
| Median period (range) between MRI and hysterectomy: in days                 | 30 (3–94)      |
| Predominant tumour location in relation to the internal ostium              |                |
| Central                                                                    | 19             |
| Ventral                                                                    | 2              |
| Dorsal                                                                     | 6              |
| Left lateral                                                                | 2              |
| Right lateral                                                               | 5              |
| Type of lesion                                                              |                |
| Exophytic                                                                   | 21             |
| Endophytic                                                                  | 13             |

FIGO = International Federation of Gynaecology and Obstetrics; SCC = squamous cell carcinoma; AC = adenocarcinoma; ASC = adenosquamous carcinoma; LLETZ = large loop excision of the transformation zone

Evaluation of MR images
All MR-examinations were found to be of ‘acceptable’ quality by both radiologists. They found a mean craniocaudal tumour size of 18 mm (range 0-42, SD 11 mm) and 17 mm (range 0-41, SD 11 mm), respectively. For each patient, when the average of both radiologists was calculated, a tumour size of 18 mm (0-42, SD 11 mm) was found. The ICC showed a value of 0.93 (95% confidence interval [CI] 0.85-0.96) which is classified as an ‘almost perfect’ agreement between the two radiologists.

During 3D-comparison by the radiation oncologist in conjunction with the resident, mean tumour extension was 21 mm (range 0-47, SD 11 mm). The ICC between the average measurements per patient of the two radiologists and measurements during 3D-comparison was 0.93 (95% CI 0.76-0.97) and is classified as ‘almost perfect’.
Histopathology

Of the 34 included patients, six were treated elsewhere for trachelectomy or a second opinion and could not be processed at the department of Pathology (Figure 6.A1). Of the remaining 28 patients, in 26 of them macroscopic photographs were available for assessment of the craniocaudal extension. Additionally, one macroscopic photograph could be retrieved from a patient that was treated elsewhere, which could be used for the 3D-comparison method. In 20 patients, the microscopic craniocaudal extension could be measured accurately. In 14 cases the craniocaudal microscopic extension was estimated but could not be reconstructed with full certainty; therefore, these patients were excluded from analysis.

On macroscopy (n=26) and microscopy (n=20), craniocaudal tumour extension was on average 21 mm (range 0-50, SD 14 mm) and 17 mm (range 3-42, SD 8 mm), respectively. In a paired comparison of 18 cases, the median macroscopic craniocaudal extension was 4 mm smaller (range -7 to 22, SD 7 mm, test for normality borderline negative) compared to the microscopy measurement (median 14 vs. 18 mm). Three outliers showed an underestimation of macroscopic photographs of 15-22 mm; however, in these cases, MRI showed a smaller underestimation of -3.5 to 15 mm. In four cases the microscopic tumour extension was in fact 1-7 mm smaller than estimated on macroscopy. In 2 of 20 cases in which the microscopy was evaluated, no photograph was taken and therefore no comparison could be made.

On 3D-comparison by the radiation oncologist and the resident radiation oncology, craniocaudal tumour extension was found to be on average 20 mm (range 0-44, SD 11 mm). It should be noted that, with this method, 3D-reproducibility between macroscopic photographs and MRI was found to be crucial; therefore, in some cases only a part of the tumour was measured that could be well recognised on both modalities (Figure 6.1)

Comparison between MRI and histopathology

1) MRI vs. histopathology

The craniocaudal tumour extension measured by radiologists on MRI was on average 3 mm smaller (range -25 to 20 mm, SD 10.0 mm) compared to all 26 macroscopic measurements by the pathologist (18 vs. 21 mm, respectively, p=0.135) and both measurements showed a good correlation (r=0.72, p<0.001) (Figure 6.2).

The difference in craniocaudal extension between all 20 microscopy cases and MRI was 3 mm (range -12 to 15, SD 7.6 mm), this difference was not significant (14 vs. 17 mm, respectively, p=0.098) and both measurements showed a good correlation (r=0.73, p<0.001). Craniocaudal extension on MRI compared to microscopy showed a
smaller 95% CI than compared to macroscopic photographs (-12 to 18 mm vs. -17 to 23 mm, respectively) (Figure 6.2 and 6.3).

From a radiation oncologist point of view, there were four outliers where tumour extension would be underestimated on MRI by ≥10 mm and would, therefore, potentially not be recognised as tumour in radiotherapy target volumes. One patient had a conisation before MRI and hysterectomy: no tumour was recognised on MRI or on the macroscopic photographs, but microscopy revealed a tumour with a craniocaudal extension of 15 mm (Figure 6.A2). In the other three patients, the craniocaudal plane of the tumour was altered between MRI and histopathology; therefore, these underestimations were probably caused by measuring tumour in different directions (Figure 6.4, 6.A3, 6.A4).

2) 3D-comparison

Paired comparison of craniocaudal tumour extension on MRI and macroscopic photographs (both performed in consensus by radiation oncologist and a resident radiation oncology) could be performed in 27 patients. There was a minor difference (mean -0.2 mm; range -11 to 6.0, SD 3.4 mm) between craniocaudal tumour extension on photographs minus MRI (19.9 vs. 20.1 mm, respectively, Pearson’s correlation 0.95, p<0.001) (Figure 6.5). Intraclass correlation coefficient of tumour extension on macroscopy between the pathologist and radiation oncologist was 0.91 (95% CI 0.79-0.96) and is classified as ‘almost perfect’ (see also Table 6.A1).

3) Non-rigid registration of the uterus

In 10 patients, the minimal requirements were met for an adequate non-rigid registration [125]. We found a median DSC and SDE of 0.99 and 1.75 mm (whole uterus) and 0.82 and 5.24 mm (uterine cavity), respectively. An average SDE of 0.74 mm (range 0.36-0.89 mm) around tumour was found. A margin was applied to the tumour on MRI to cover the non-rigid registered tumour on microscopy. For 95% coverage of the tumour in at least 90% of the patients, a margin of 10 mm was needed.
Figure 6.2 Bland-Altman plot (A) for craniocaudal extension measured on MRI and macroscopic photographs evaluated by the pathologist. The dotted lines show a 95% confidence interval (-17 to 23 mm) which is larger than on microscopy but shows the same underestimation of MRI (mean 3 mm). The waterfall plot (B) visualises the difference per patient and shows seven outliers where MRI underestimates tumour size by >10 mm.
Figure 6.3 Bland-Altman plot (A) for craniocaudal extension measured on MRI and histopathology (microscopy). The dotted lines show the 95% confidence interval (-12 to 18 mm). Note that MRI slightly underestimates histopathology on average by 3 mm. The waterfall plot (B) visualises the difference per patient and shows four outliers where MRI underestimates tumour size by >10 mm.
Figure 6.4 38-year-old women with a FIGO stage IB1 adenocarcinoma of the cervix. Measurement of the cranio-caudal tumour extension on the sagittal MRI slice shows a tumour of 8.4 mm (A; upper image), however, on histopathology the measurement is 23 mm (B). In this case the pathologist accidently exposed the uterine cavity from the dorsal side, whereas the protocol recommends exposure by opening the ventral side of the uterus. The photograph (B) is a two-dimensional representation of the three-dimensional uterus which also alters shape after surgery and positioning on the pathologist’s table. Just as a normal cervix, the cervix in this patient felt weak and free to be bent and, therefore, would accommodate to the flat shape of the table (red arrow); therefore, the distance according to A (lower image) was probably measured which was 23.1 mm.

Figure 6.A2 A 43-year-old women with a FIGO stage IB1 squamous cell cancer of the cervix that underwent conisation before MRI and hysterectomy. No tumour was recognised on MRI (A), or on macroscopic pathology photographs (B); however, after conisation, a scar was clearly visible (B, black arrow). However, microscopy revealed a residual tumour with a cranio-caudal tumour extension of 15 mm.
A 44-year-old women with a FIGO stage IB1 adenocarcinoma of the cervix. On MRI the tumour was measured in 'craniocaudal direction' as being 9 mm and on histopathology as 22 mm. This discrepancy is probably due (for a large part) to positional changes between MRI and histopathology. The cervix was most likely bent according to the hard and flat table it was lying on and, therefore, an anterior flexion occurred changing the measurements on histopathology.

A 46-year-old women with FIGO stage IB1 squamous cell carcinoma of the cervix. Craniocaudal tumour extension was 8 mm on MRI (A; upper image) and 18 mm on microscopy, which is visualised in the macroscopic photograph (B). Again, the cervix seems to be bent differently on macroscopy (red arrow) compared to the position on MRI and, therefore, the craniocaudal direction is oriented in another plane of the tumour. This could explain the difference between MRI and histopathology. In A (lower image), the distance that was probably measured is visualised on histopathology when taking into this potential bending.
### Table 6.A1 Craniocaudal tumour extension according to the 3 methods

| Method                     | Modality                        | # available specimens | Average tumour size (mm) | Range (mm) | SD (mm) | Pearson's correlation |
|----------------------------|---------------------------------|-----------------------|--------------------------|------------|---------|-----------------------|
| MRI vs. histopathology     | MRI radiologist 1               | 34                    | 18                       | 0-42       | 11      |                       |
|                            | MRI radiologist 2               | 34                    | 17                       | 0-41       | 11      |                       |
|                            | Mean radiologist 1 and 2         | 34                    | 18                       | 0-42       | 11      |                       |
|                            | Macroscopy pathologist          | 26                    | 21                       | 0-50       | 14      |                       |
|                            | Microscopy pathologist          | 20c                   | 17                       | 3-42       | 8       |                       |
|                            | Microscopy – macroscopy         | 18                    | 4 (18-14)                | -7-22      | 7       |                       |
|                            | (difference)b                   |                       |                          |            |         |                       |
|                            | Macroscopy - mean MRI 1+2       | 26                    | 3 (21-18)                | -25-20     | 10      | 0.72 (p<0.001)        |
|                            | (difference)b                   |                       |                          |            |         |                       |
|                            | Microscopy - mean MRI 1+2       | 20                    | 3 (17-14)                | -12-15     | 7.6     | 0.73 (p<0.001)        |
|                            | (difference)b                   |                       |                          |            |         |                       |
| 3D comparison              | MRI radiation oncologist        | 34                    | 21                       | 0-47       | 11      |                       |
|                            | Macroscopic photographs         | 27                    | 20                       | 0-44       | 11      |                       |
|                            | radiation oncologist            |                       |                          |            |         |                       |
|                            | Macroscopy – MRI (difference)b  | 27                    | -0.2 (19.9-20.1)         | -11-6.0    | 3.4     | 0.95 (p<0.001)        |
| Non-rigid registration     | Macroscopy – MRI (difference)b  | 10                    | For 95% coverage of the tumour in at least 90% of the patients, a margin of 10 mm was needed | | | |

*a* Intraclass correlation coefficient for agreement between both radiologists = 0.93.

*b* The difference analysis here were paired comparisons.

*c* Intraclass correlation coefficient between the average measurements per patient of the two radiologists and measurements during 3D comparison = 0.93.

*Abbreviations:* SD: standard deviation;
Figure 6.5 Bland-Altman plot (A) for craniocaudal extension measured at 3D comparison on MRI and macroscopic photographs, which differs (on average) by only 0.2 mm. The dotted lines show the 95% confidence interval (-7 to 7 mm). Special effort was made to measure three-dimensionally the same tumour distance in exactly the same orientation. The waterfall plot (B) visualises the difference per patient.
Discussion

Craniocaudal tumour extension in early-stage cervical cancer measured on MRI gives a small underestimation of (on average) 3 mm of the length measured on the microscopy specimen, but may be as high as 10 mm, which we therefore recommend as a minimal clinical safety margin for radiotherapy target delineation.

In three patients, underestimation of tumour extension exceeded 10 mm; which was probably due to the change in uterine shape between MRI and post-hysterectomy (Figure 6.4, 6.A3, 6.A4). In a fourth patient, conisation probably caused the difference in tumour size (Figure 6.A2), since tumour measurement on MRI after conisation might be less reliable [187].

Only a few studies have compared cervical cancer tumour size measured on MRI with histopathology. For example, Sanuki et al. (2013) compared cervical tumour length in hysterectomy specimens with 1.5T MRI in 31 patients and found that MRI underestimates tumour length along the uterine cavity by 5 mm (19 on MRI vs. 24 mm on microscopy); their data suggests that a 10-mm margin around tumour delineated on MRI would be sufficient to include microscopic extension in 95% of the patients [107]. However cases where tumour was hard to recognise due to resemblance to surrounding tissue were excluded. Remarkably, Sanuki et al. did not report problems in 3D shape alternations between MRI and histopathology. Bourgioti et al. (2014) confirmed these results in 21 patients with tumours of 1-4 cm in maximum diameter, who had MRI before trachelectomy whereby no more than 10-mm underestimation of tumour size was found compared to microscopy [188]. Also de Boer et al. found in a retrospective series of 21 patients an underestimation 10 mm by MRI compared to microscopy and report similar spatial uncertainties due to difference of uterine between both modalities [124].

A limitation of the present study and of previous studies, is that comparison of tumour size measured by the radiologist and by the pathologist strongly depends on the spatial orientation of the tumour; this may result in spurious differences. For example, in the case shown in Figure 4, the craniocaudal tumour extension was 8 mm on MRI but, due to anterior flexion of the radial cervical extension after hysterectomy, the craniocaudal tumour extension became 23 mm on pathological macroscopy. By evaluating Figure 4, underestimation of tumour size by the radiologist seems unlikely causing such large difference. In general, post-surgical organ deformation is most misleading for microscopic measurements in which the spatial orientation of the pathological section is usually lost. It seems remarkable that other authors did not encounter/report this problem. However, this was recognised in studies on prostate cancer [153, 164, 189], head and neck cancer [172] and lung cancer [190], in which imaging and pathology were compared.
We aimed to minimise these measurement uncertainties due to alterations of the shape of the cervix and uterus by directly comparing MRI and macroscopic specimen measurements, interpreting both modalities as being part of a 3D-shape, and found (on average) almost no difference between measuring tumour size on the macroscopic specimen and MRI (mean difference -0.2 mm, range -11 to 6 mm, SD 3.4 mm)(Figure 5). The 3D-comparison method did not include microscopic data since it was not possible to repeat microscopy slicing in a different 3D-direction; this is a limitation of this method. On macroscopy, our pathologists found a median underestimation of tumour extension of 4 mm when compared to microscopy. Xie et al. (2015) compared macroscopy with microscopy in 318 hysterectomy specimens of women with cervical squamous cell carcinoma (SCC) and found that with a 5-mm margin around GTV, 99% of all microscopic invasion towards the uterine body would be covered[191].

In the third method, we followed the methodology for non-rigid registration as described by van de Schoot et al. and found similar results[125]. Here, we found it difficult to perfectly align a 2D sagittal MR-slice with a photograph of a 3D-object where depth is not visible. This limitation was reported earlier[125], particularly also because the exact plane in which the incision is made to expose the uterus is unknown. This limitation can be tackled by embedding the whole fresh hysterectomy specimen and systematically slicing the embedded organ into sections, thereby enabling direct 3D-comparison with MRI in multiple sections[125,153]. Moreover, embedding followed by systematic slicing of the uterus into whole-mount sections would eliminate most of the limitations in all three described methods.

With brachytherapy delineation and trachelectomy decision-making, physicians rely heavily on what they see on T2-weighted MRI when physical examination under anaesthesia does not provide the answer[57,58,192,193]. Our results, and those of other recent studies, indicate that a 10-mm margin would be sufficient to cover invisible microscopic extent for primary cervical tumour delineation on MRI[186]. Furthermore, it seems that microscopic tumour extension is smaller in SCC and in the case of no LVSI[191,192]. If data from 3D-pathology sections could be compared to multiple MRI-slices of cervical tumours, the difference between MRI and pathology caused by a change in uterine shape by surgery might be reduced.

**Conclusions**

In these patients with early-stage cervical cancer, MRI gives (on average) a 3-mm underestimation of the pathological craniocaudal tumour extension. However, in 10% of these patients, the underestimation may be as high as 10 mm; nevertheless, in these latter cases the tumour was limited within the uterine cervix. In these outlying
patients, measurement uncertainty is mostly caused by changes in the shape of the uterus between the *in situ* situation as captured on MRI and the pathological specimen.