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HDR Brachytherapy as monotherapy for low risk prostate cancer: dosimetric and clinical evaluation

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Perfil em Radiações em Diagnóstico e Terapia

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2016

Resumo

Na Europa, o cancro da próstata é um dos tumores malignos mais comum nos homens. A idade média no diagnóstico é de 65 anos, raramente sendo este tumor diagnosticado antes dos 50 anos. Em Portugal, segundo a Direção Geral de Saúde, a taxa de incidência de cancro da próstata é aproximadamente 82 casos por 100 000 habitantes. Na Holanda, 11 158 novos casos de cancro da próstata são diagnosticados por ano.

A radioterapia tem vindo a ocupar um lugar de elevada importância no que diz respeito a tratamento de foro oncológico. Novas técnicas de radioterapia com intuito curativo do cancro da próstata têm surgido ao longo dos anos. No entanto, nos últimos anos, a braquiterapia de alta taxa (HDR) tem vindo a ocupar um lugar importante no tratamento de tumores malignos na próstata. Inicialmente, esta terapia foi combinada com a radioterapia externa convencional, funcionando como reforço de radiação na próstata, vulgarmente conhecido pelo termo inglês *"boost"*. Este tratamento combinado é administrado em pacientes de intermédio-alto risco de cancro de próstata. Ao longo dos anos, HDR braquiterapia tem vindo a ser bastante utilizada como terapia única em pacientes de baixo risco, evitando deste modo procedimento mais radicais como a prostatectomia radical.

A braquiterapia de alta taxa é caracterizada por uma distribuição de dose bem conformada na próstata e por ter *"dose fall-off"* acentuado. Assim, esta técnica salvaguarda os órgãos de risco (bexiga e reto) de uma forma mais eficaz do que outras técnicas de radioterapia, tais como a radioterapia de intensidade modelada (IMRT) ou radioterapia conformada tridimensional (3D RT). No *ErasmusMC – Cancer Institute* (Roterdão, Holanda) este tratamento é executado em 4 sessões de 9.5Gy (38Gy) cada, separadas de um intervalo mínimo de 6 horas. O doente é internado por 2 dias, e tem alta médica no final do segundo dia. O procedimento clinico da implementação das agulhas é guiada por ultrassons e é indolor, uma vez que o paciente está sob o efeito de anestesia epidural. Antes de cada sessão de tratamento, uma imagem de raios-x lateral é adquirida por forma a verificar possíveis desvios das agulhas na direção caudal. Os desvios superiores 3 mm são corrigidos de modo a evitar o risco de subdosagem na próstata e/ou sobredosagem nos órgãos de risco.

A braquiterapia da alta taxa como terapia única é conhecida por ter uma incidência de toxicidades agudas nos tecidos reduzida e por ter um controlo bioquímico bastante elevado. Contudo, existem alguns efeitos secundários após o tratamento, tais como a retenção urinária aguda (AUR) e o sangramento retal (RB). Estes efeitos secundários, embora transitórios, provocam um acréscimo de ansiedade e desconforto no paciente afetando as suas rotinas diárias sendo importante investigar as possíveis causas.

Nesta tese, o principal objetivo é investigar quais são os fatores associados a estes dois efeitos secundários de forma a minimizá-los e a melhorar a qualidade de vida dos pacientes após o tratamento. Para cada um dos efeitos secundários, ferramentas estatísticas apropriadas, tais como Mann-Whitney teste, Chi-Square teste e Regressão Logística uni-variável e multivariável, foram usadas para comparar parâmetros dosimétricos (dose-volume histograms - DVH) e clínicos (idade, IPSS - International Prostate Symptom Score, volume da próstata, etc.) entre o grupo de casos (14 AUR e 15 RB) e o grupo de pacientes considerado como controlo (28 no-AUR e 30 no-RB).

Dos vários parâmetros clínicos e dosimétricos em estudo, apenas o fluxo urinário medido antes do tratamento (Baseline urinary flow - Qmax) inferior 10 ml/s e 25% do volume da bexiga recebendo doses (Bexiga

D25) superiores a 30-40% da dose prescrita foram os principais fatores associados a um elevado risco de desenvolvimento de retenção urinária aguda com a necessidade de argália após o tratamento. Este resultado foi confirmado quando se analisaram estas variáveis novamente, na base de dados completa dos pacientes tratados com HDR braquiterapia (210 pacientes). Outro parâmetro, uretra membranosa D0.5cc $\geq$ 55% da dose prescrita, mostrou-se estar estatisticamente associado a um aumento do risco de desenvolvimento de retenção urinária aguda após o tratamento. No entanto, este resultado necessita de ser confirmado em estudos futuros. Para além disso, por forma a confirmar os valores limite de dose para os quais o risco de desenvolvimento de AUR é elevado, foram utilizadas as curvas ROC (Receiver Operating Characteristic Curve). Este método provou que Qmax $<$ 10 ml/s e bexiga D25 $\geq$ 30-40% estimam bem o risco associado ao desenvolvimento de retenção urinária aguda com uma área abaixo da curva ROC superior a 0.7.

No que diz respeito ao segundo efeito secundário, RB, os resultados são inconclusivos, quer em termos de parâmetros dosimétricos quer em termos de variáveis clínicas. Embora alguns parâmetros dosimétricos se tenham mostrado estar estatisticamente relacionados com o desenvolvimento de sangramento retal, estes não têm significado clinico relevante. PTV volume $\geq$ 55 cc e Hipertensão mostraram-se estar estatisticamente associados ao risco de RB mas essa relação não é fidedigna, uma vez que PTV volume $\geq$ 55 cc não se mostrou estar estatisticamente associado ao RB na base de dados de 210 pacientes e não existem dados que indiquem que os pacientes hipertensos estão a ser corretamente medicados e/ou que seguem o tratamento prescrito.

Em suma, este estudo é o primeiro estudo retrospetivo sobre HDR braquiterapia como terapia única com resultados bastante promissores. Os resultados sugerem que se deve limitar a dose entregue a 25% do volume da bexiga, a 30%-40% da dose prescrita, e que Qmax deve ser incluído na lista de critérios de seleção de pacientes para o tratamento. Este projeto, sugere ainda que se deve ter em conta a dose recebida em 0.5cc de volume da uretra membranosa, mas este resultado está sujeito a futuras investigações.

Palavras-chave: HDR braquiterapia, cancro da próstata, retenção urinária aguda, sangramento retal, avaliação dosimétrica e clínica

Abstract

Prostate cancer is the most common cancer in Europe for males. In Portugal, it is estimated that prostate cancer has an incidence of 82 cases per 100 000 inhabitants. In the Netherlands, 11 158 new cases of prostate cancer are diagnosed each year. For the past years, High-Dose Rate Brachytherapy (HDR BT) as monotherapy has been playing an important role in treatment of prostate cancer. This type of radiotherapy has excellent results because of its highly conformal dose distribution within the prostate with a rapid dose fall-off outside, sparing the organs at risk. Even so, side effects, such as acute urinary retention (AUR) and rectal bleeding (RB), occur after treatment.

In this thesis, predictive factors for AUR and RB were investigated in order to find and/or improve new treatment constraints to avoid and/or minimize the occurrence of these side effects, consequently, improving patient's quality of life after treatment. In two investigations, dose-volume histograms (DVH) and clinical parameters were compared, between cases (14 AUR and 15 RB) and controls (28 no-AUR and 30 no-RB). In both projects, appropriate statistical tools, such as Chi-Square test, Mann-Whitney test and Univariate and Multivariate Logistic Regression, were used.

In AUR project, baseline urinary flow (Qmax) $<$ 10 ml/s and 25% of bladder volume receiving doses (bladder D25) $\geq$30-40% of prescribed dose were the most important risk factors for AUR. These two parameters were afterwards confirmed as risk factors for AUR in a large dataset of 210 patients and also through the Receiver Operating Characteristic Curve (ROC). Another dosimetric parameter, urethra membranous D0.5cc $\geq$ 55% of PD, was statistically associated with the increased risk of AUR. However, this result needs to be confirmed in future studies.

In RB project, either in terms of DVH or clinical parameters, the results were inconclusive. Some DVH parameters of cranial rectum were statistically correlated with RB but without clinical relevance. PTV volume $\geq$ 55 cc and Hypertension were statistically significant but they did not show a clear relationship with RB.

In summary, this first HDR BT retrospective study suggests that bladder D25 and Qmax could be considered during selection and treatment patients to minimize AUR.

Keywords: HDR Brachytherapy, prostate cancer, acute uninary retention, rectal bleeding, dosimetry and clinical evaluation.

Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisor Dr. Inger-Karine Kolkman-Deurloo for the continuous support of my research, for her patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and writing of this report.

Besides my supervisor, I would like to thank to Dr.Shafak Aluwini for his insightful comments and encouragement.

I thank Professor Dr. Luis Peralta, my supervisor in Portugal, for all the advice and support during the past 9 months.

I thank my fellow labmates, specially Wendy Busser and Rik Bijman, in for the stimulating discussions, for all the help and for all the fun we have had in the last 9 months.

I thank all the colleagues from the medical physics department for welcoming me and for the daily lunches and free time activities that we all enjoyed together. And also, for the valuable discussions!

I must also acknowledge the Reitoria da Universidade de Lisboa for the financial support, under the ERASMUS program, that made this internship possible.

Last but not the least, I would like to thank my family: my parents and to my boyfriend for supporting me spiritually throughout writing this report and supporting me in this new experience of my life: living abroad alone.

Dedication

I dedicate this thesis to my grandparents, Maria Fernanda and João Gomes, who have taken care of me since I was a small baby. Without their support, both spiritually and financially, it would not have been possible to do my degree and this project.

Esta tese é dedicada ao meus avós maternos, Maria Fernanda and João Gomes, que cuidaram de mim desde terra idade. Sem a sua ajuda, tanto esperitual como financeira, não teria sido possível frequentar o curso de Engenharia Biomédica e Biofísica e fazer a tese de mestrado fora do país. O meu sincero obrigado!

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List of Abbreviations

BT	Brachytherapy
PCa	Prostate Cancer
EBRT	External Beam Radiotherapy
IMRT	Intensity Modulated Radiotherapy
LDR BT	Low-dose rate Brachytherapy
HDR BT	High-dose rate Brachytherapy
PDR BT	Pulsed-dose rate Brachytherapy
RALs	Remote Afterloads Machines
GU	Genitourinary
GI	Gastrointestinal
PSA	Prostate-specific Antigen
IPSS	International Prostate Symptom Score
EORTC	European Organization for Research and Treatment of Cancer
RTOG	Radiation Therapy Oncology Group
WHO	WHO performance status classification
DVH	Dose-Volume Histograms
CAD	Patients with indwelling Bladder Catheter
AUR	Acute Urinary Retention
RB	Rectal Bleeding
DM	Diabetes Mellitus
LRB	Late Rectal Bleeding
OAR	Organs at risk
PTV	Planning target volume
GTV	Gross tumour volume
CTV	Clinical target volume
PUS	Prostatic Urethra Superior
PUM	Prostatic Urethra Mid
PUI	Prostatic Urethra Inferior
UM	Membranous Urethra
TRUS	Transrectal Ultrasound
UVA	Univariate Logistic Regression
MVA	Multivariate Logistic Regression
OR	Odd-ratio
CI	Confidence Interval
ROC	Receiver Operating Characteristic Curve
AUC	Area Under Curve
MCMC MI	Markov chain Monte Carlo Multiple Imputation
MRI	Magnetic resonance imaging
CT	Computed Tomography
IVD	*In vivo* dosimetry

Chapter 1

Brachytherapy Introduction

1.1 Historical Background

The beginning of radiation treatment started when Wilhelm Röntgen discovered the x-rays in November 1895, and shortly afterwards, Henri Becquerel accidentally exposed a photographic plate to uranium in 1896, identifying the phenomenon of emitted radiation. Some years later, Becquerel himself experienced the effects of radiation exposure by carrying a tube containing radium chloride in his vest pocket.

The first clinical applications belonged to Danlos and Bloch (1901) in Paris, and Abbé (1904) in New York. The basic principles of systematic use of radiation were established somewhat later after World War I in the Radium Hemmet in Stockholm, The Memorial Hospital in New York and the Radium Institute in Paris.

Nowadays, there are several types of radiation treatment but at that time only so called Brachytherapy existed. This way, the term Brachytherapy (BT) can be defined as a near therapy with radiation sources being placed directly on, in or through the area of interest that is to receive a high radiation dose, i.e. the target.

Therefore, Brachytherapy has been used from very early days of radiation discovery. Several types of sources have been used until now. One of the most important during the first two decades of the twentieth century was ^{226}Ra. At that time it was necessary to create a set of rules related to the arrangement of the radioactive sources (geometry patterns), definition of the source strength, spacing and treatment time in the treatments. These criteria were developed by three important institutions in Stockolm (1914), Paris (1919) and Manchester (1967) for intracavitary treatments.

Two other important moments in the history contributed to the development of this area: the discovery of artificial radioactivity and the development of remote afterloading devices, which provided improved radiation protection.

There are 3 different types of BT: LDR BT (low-dose rate), HDR BT (high-dose rate) and PDR (Pulsed-dose rate). The main difference between these 3 types is the method of delivery:

- **LDR BT**: continuously dose delivery using LDR source;
- **HDR BT**: dose is delivered in 1 or few fractions using HDR source;
- **PDR BT**: dose is delivered in many fractions separated 1 to few hours.

LDR BT was the technique that was implemented using ^{192}Ir, for temporary implants, and more recently ^{125}I, for permanent implants, as radioactive sources. During the last decades this kind of BT is mainly used for permanent implant technique for prostate cancer. Due to afterloading devices development, HDR BT started to grow and nowadays it is the most used technique to treat several kinds of tumours such as gynaecology and prostate cancer.

1.2 Technical and Physical Aspects of Brachytherapy

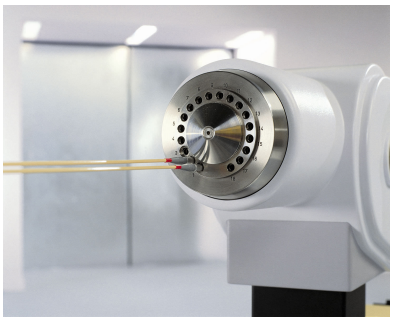
As I said before, in the firsts years of BT, the radioactive sources were manually implanted into the tumour, thereby subjecting the physician and other medical personnel to unwanted radiation exposure. At the middle of the last century, the Remote Afterloaders (RALs), which is a computer-driven system that transports the radioactive source from a shielded safe into the applicator placed in the patient and back to its safe, minimized the radiation exposure to personnel.

HDR BT, administering discrete fractions in a temporary implant, have become common in treatment of gynaecological, breast and prostate cancer. Nowadays, this type of radiotherapy is very useful because, as monotherapy or combined with External Beam Radiotherapy (EBRT), it allows a good growth control of tumour cells.

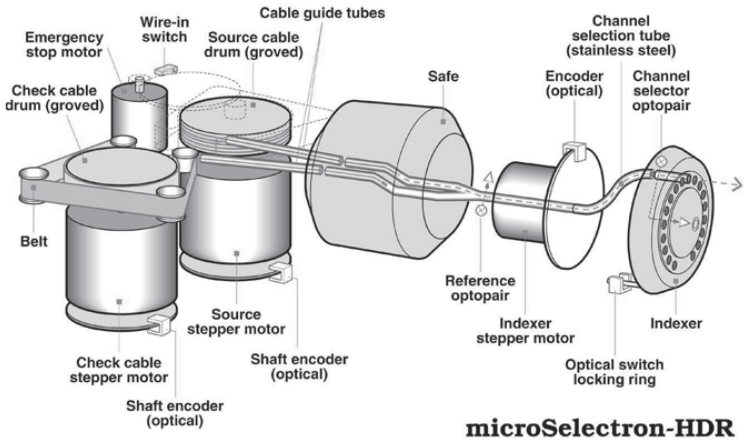
Components of an HDR RAL (see figure 1.1)

HDR RAL is built by different components which these will explained in following items.

- **Shielded safe and radioactive source:** a stepping source usually consist of ^{192}Ir with an activity up to 370 Bq to provide a dose rate up to 700cGy/min at 1 cm from the source. To house his highly radioactive source, a shielded safe made of tungsten or depleted uranium of sufficient thickness to provide enough radiation shielding is an integral part of the treatment unit.
- **Source drive mechanism and transfer tubes:** there are two different cables: check cable (without radioactive source inside) and source cables (with radioactive source). when the treatment starts, the check cable stepper motor drives the check cable to the programmed length plus a couple of millimetres to verify the integrity of the system. After this procedure, the source drives through the transfer tubes to the plastic needles to perform the treatment (with certain dwell positions and weights for each needle). After the procedure in the first needle, the source goes to the house shielding and drives again for the second needle. This procedure is repeated for all needles.
- **Indexer:** is the part of RAL that directs the check source cable from the exit of the safe to one of the exit ports from the unit called as channels. It uses one channel for each needle and the connections between the channels and needles are the transfer tubes. Most machines have up to 40 channels.
- **Treatment Control Station:** this part allows the user to select the dwell positions and dwell times to be used in each channel. Nowadays, the data from a treatment planning system is imported for the systems and radiation delivered according to the treatment plan.
- **Treatment Control Panel:** the treatment control station transfers the data to the treatment control panel after the treatment is started. Also, this panel has an interrupt button and an emergency button.



(a) A schematic figure of the head of RAL and transfer tubes (Elekta).

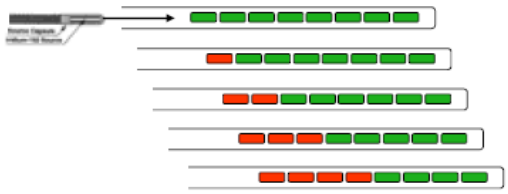


(b) A schematic figure of the head components of RAL (Elekta).

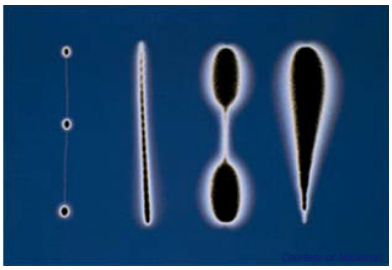
(c) Complete configuration of devices composing the remote afterloader machine. 1) Treatment Delivery Unit; 2) Treatment Control Station; 3) Treatment Control Panel. Courtesy of Nucletron.

Figure 1.1: Figures of remote afterloaders machines. Taken from [2, 3].

The currently available HDR RALs use stepping-source technology, consisting of a singles source at the end of a cable that moves the source in steps through the applicators placed in the treated volume. The important advantage of this stepping source is that the dose distribution can be modified by altering the source positions and the dwell times (i.e., the time spent at each source position). Figure 1.2 illustrates the dwell positions and the dose distribution due to a stepping single source.



(a) A schematic figure of source and your dwell positions.



(b) Autoradiograph image of dose distribution produced by a single source in a single catheter.

Figure 1.2: Dwell positions and dose distribution. Taken from [4].

The 3D dose distribution around a source is determined by the following factors:

- The **inverse square law:** the particle fluence around a point source in vacuum falls of with the square of the distance to the source.

- The **interaction** of emitted particles with the materials within the source itself and around it.

The dose distribution in tissue is mostly dependent on the type of source (e.g. energy), type of tissue and dwell times and positions. Figure 1.3 shows the difference in isodose curves produced by different kinds of sources in phantom.

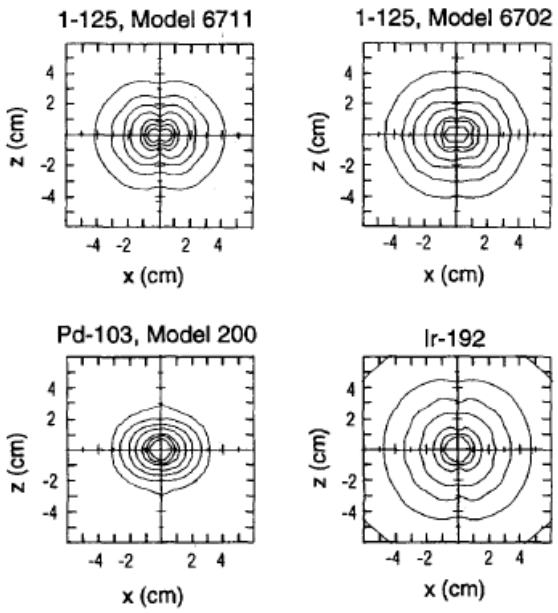


Figure 1.3: The isodose curves produced by different sources. Taken from [5].

Nowadays, the dose calculation formalism is recommended by Task Group 43 [6] of AAPM. It is the generally accepted method to express the dose distribution around brachytherapy sources.

1.3 Brachytherapy for Prostate Cancer

Interstitial BT with **permanent seeds** where the activity of source decays for almost zero in some months ($T_{1/2}$ = 59 days) or **temporary high-dose rate implants** where the source only stays inside the patient for some minutes has received a renewed interest in the last 20 years, mostly because the technological improvement of ultrasound image guidance and also because of the highly sophisticated precision of this new technique. The appearance of new radioisotopes also was a good contribution for the development of brachytherapy.

Prostate cancer has a different tumour behaviour, suggesting a low alpha/beta ratio [7, 8] which is smaller than that of rectum and bladder [9], makes it possible to apply hypofractionation using high fraction dose to treat PCa. The α/β ratio is the dose where cell killing due to the linear and quadratic components are equal. In general:

- High value of α/β ratio, the more linear the cell survival curve will be;
- Low value of α/β ratio (i.e. high beta relative to alpha), the more curved the cell survival curve.

So this way is explained why HDR brachytherapy is adequate for prostate cancer. Prostate is more sensitive to high doses and it has a good tissue response because the proliferation rate of cancer cells is low.

1.3.1 Target Definition in Prostate Brachytherapy

The target definition in Brachytherapy for prostate cancer is very similar to the target definition for other types of Radiotherapy. This definition is described below:

- **GTV** (Gross tumour volume): is the palpable, visible, or clinically demonstrable location and extent of malignant growth;
- **CTV** (Clinical target volume): is the volume that contains the GTV and includes subclinical malignant disease at a certain probability level. (In prostate cancer, this growths goes for capsule and for seminal vesicles); In this specific technique CTV volume is the whole prostate gland;
- **PTV** (Planning target volume): the CTV plus one margin includes whole tumour's movements. For BT the CTV is equal to PTV because there are no significant opportunities for set-up errors;
- **OAR** (Organs at Risk): urethra, rectum, penile bulb and bladder.

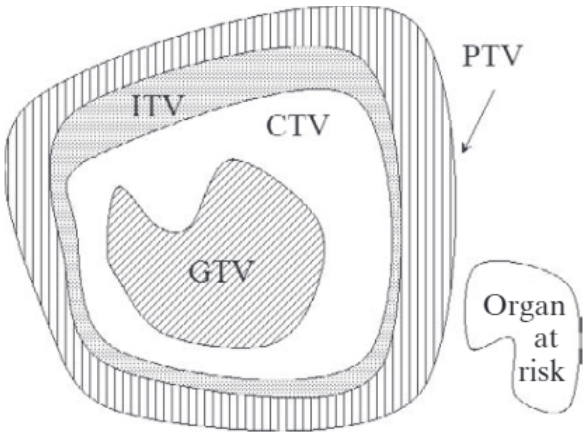


Figure 1.4: Graphical representation of the volumes of interest, as defined in ICRU Reports No. 50 and 62. Taken from *Radiation Oncology Physics*, chapter 7 [10].

1.3.2 EBRT + HDR BT

EBRT combined with HDR Brachytherapy is beyond the scope of this research topic but several studies reported that they had good results when combining these two techniques, because with HDR boost they manage to deliver high doses into the target volume sparing the organs at risk. The HDR boost also has a radiobiologic advantage gained by hypofractionation schema.

For this type of technique the GEC/ESTRO [11] recommendations have several dose prescriptions for EBRT as: 45Gy in 25 fractions over 5 weeks, 46Gy in 23 fraction over 4.5 weeks, 35.7Gy in 13 fraction over 2.5 weeks or 37.5 in 15 fractions over 3 weeks. Regarding to the HDR boost, the dose prescription is as: 15Gy in 3 fraction, 11-22Gy in 2 fraction or 12-15Gy in 1 fraction.

1.3.3 HDR BT as monotherapy

HDR BT as monotherapy is associated with low acute toxicity and high biochemical control rates. The schedules (planning aim) which have been used include: 34Gy in 4 fraction; 36-38 in 4 fractions; 31.5Gy in 3 fractions or 26Gy in 2 fractions [12].

In *Erasmus MC - Cancer Institute* the schedule of 38Gy in 4 fractions is used. It consists of a single implant followed by four factions of 9.5Gy delivered twice daily with a minimum of 6h apart.

1.3.4 Patient Selection for HDR BT in ErasmusMC

Patients diagnosed with low- and intermediate risk prostate cancer (PCa) can be treated with this technique. Low-risk patients are defined as patients with clinical stage T1c-T2a, GS 6 and PSA $\leq$ 10 ng/ml, whereas patients with PSA $\geq$ 10 ng/ml, T2b and/or GS 7, are defined as intermediate-risk PCa [13]. The general requirements are shown below:

- Patients with clinical stage II (T1b-T2b) disease;
- Gleason score $\leq$ 7;
- Pre-treatment PSA $\leq$ 16 ng/ml;
- IPSS score before treatment $\leq$ 18/35;
- Prostate Volume before treatment $\leq$ 50 cm^3(cc).

This criteria selection could vary between Institution/Hospital, these are the values used for my group of patients reported in the paper of **Alumini et al**. [13].

It's important to explain the meaning of the clinical stage II and the acronyms T1b-T2b. The next table and figure explain the clinical stage according to UICC TNM Classification of Prostate Tumours (2009). The entire classification of Prostate tumours is in *Appendix A*.

Table 1.1: Classification of Tumour Stage

T1b	Tumour incidental histological finding in more than 5% of tissue resected
T1c	Tumour identified by needle biopsy (e.g., because of elevated PSA)
T2a	Tumour confined within Prostate and involve one half of one lobe or less
T2b	Tumour confined within Prostate and involves more than one half of one lobe

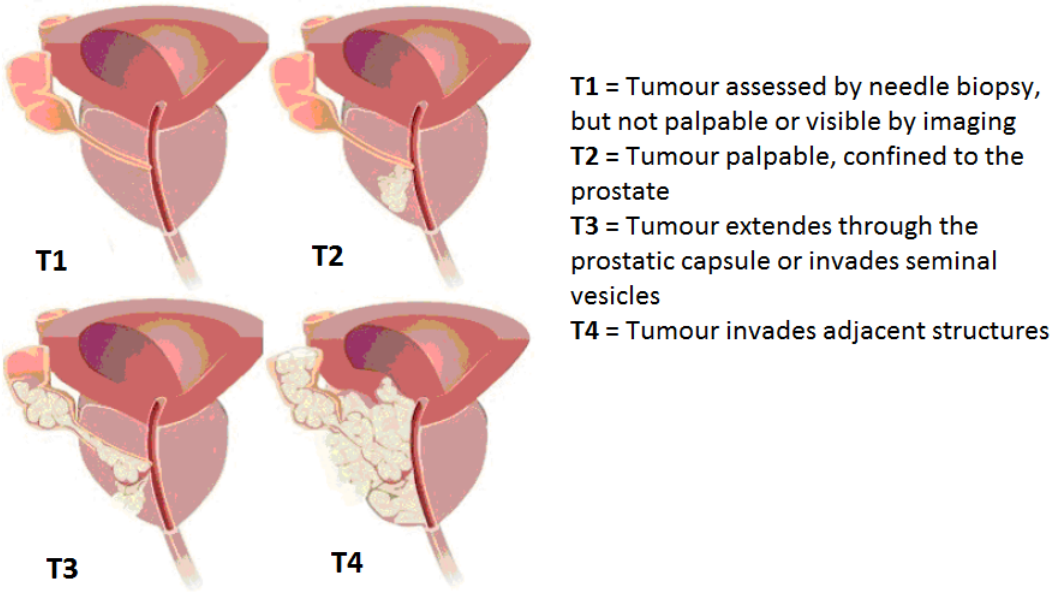


Figure 1.5: Tumour stage classification according to TMN criteria. Taken from [14].

Another important parameter that should be taken into account is the Gleason score. A system of grading prostate cancer tissue based on how it looks under a microscope. Gleason scores range from 2 to 10 and indicate how likely it is that a tumour will spread. A low Gleason score means the cancer tissue is similar

to normal prostate tissue and the tumour is less likely to spread; a high Gleason score means the cancer tissue is very different from normal and the tumour is more likely to spread.

The IPSS score is the International Prostate Symptom Score and it is used to evaluate the urinary function before and after treatment by questionnaires.

The last requirement is that the prostate size should be small, less than 50 cc. The reason for these constraints is: if the prostate gland is too large, the pelvic bones can shield the lateral parts of prostate gland. As you can see in figure 1.6, if the prostate gland is larger than 50 cc, there will be an inappropriate implantation of the needles and consequently a unsuitable dose coverage [15].

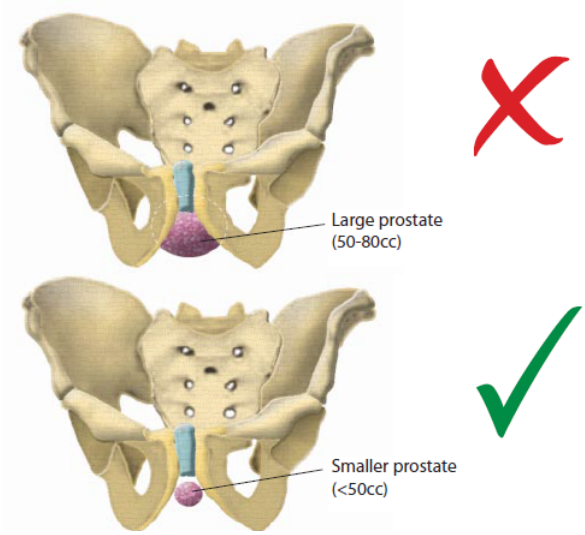
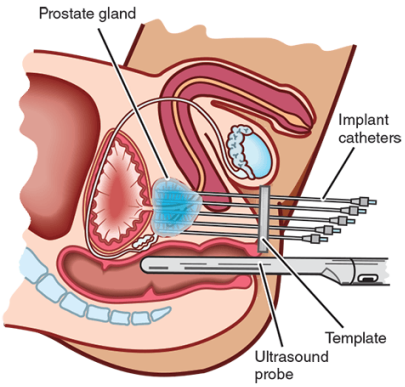


Figure 1.6: Illustration showing how the pelvic bones can shield parts of larger prostate glands. Taken from [15].

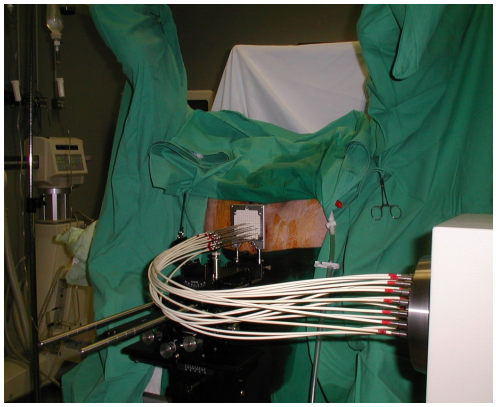
1.3.5 Clinical Procedure of HDR BT in ErasmusMC

The implantation of the needles is made under spinal anaesthesia and transrectal ultrasound guidance (TRUS). When the patient and TRUS probe are ready the implantation starts. The template is positioned at the perineum of the patient and the first step is the insertion of 4 markers, two more dorsal and two more caudal. These markers will be useful for displacement checks and, if necessary, correction of the needles between treatments and also for organ delineation.

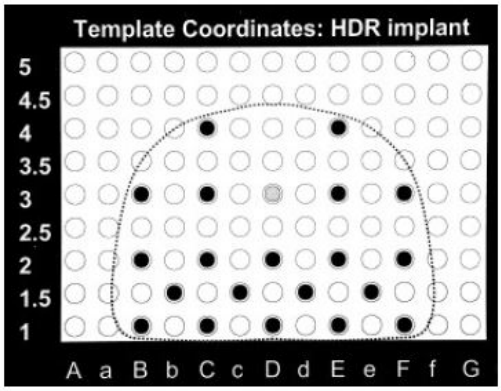
The second step is the prostate immobilization, this is done by implantation of two special needles where the tip has a kind of anchor. After these two steps, the needle implantation starts (the physician already chose the needle configuration and the number of needles based on US). Regarding the needles, first the metallic needles are implanted and then they are replaced by plastic needles. The physicians start with central needles and go for more ventral and dorsal needles. Figure 1.7 shows the schematic representation of this procedure.



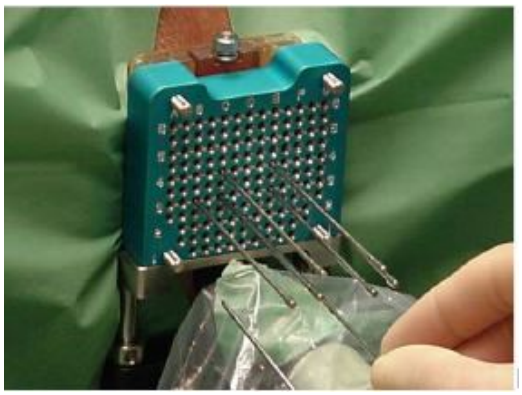
(a) Schematic HDR BT implantation.



(b) Image of treatment delivery.



(c) Schematic configuration of needles.



(d) Image of needles template fixation.

Figure 1.7: Needles implantation and treatment application. Taken from [16, 17, 18].

Following the implantation of the needles, the doctor checks whether the needles are close enough to the bladder neck to obtain a good prostate coverage because there is a small part in the end of the needle that the source can't drive through. This evaluation is done by *Cystoscopy*[1].

After this, a CT-scan is made. The images are imported in the treatment planning system where the physicians do the organ/target delineation and the technicians do the needle reconstruction. The next step is the dose distribution calculation. For more conformed dose distributions they use inverse planning. In inverse planning, you take into account all constraints, and you mathematically determine the optimum parameter values to provide the ideal answer.

In this way, the optimal dose distribution is obtained. After this step, the treatment is performed respecting the treatment protocol that was mentioned in subsection 1.3.3.

1.3.6 Side effects of Brachytherapy

In the weeks after brachytherapy treatment, normally, some secondary effects can occur due to treatment. These effects are divided to acute and late toxicities in 2 domains: Genitourinary (GU) or Gastrointestinal (GI) systems. The acute/early toxicities are defined as the symptoms occurring within 90 days after treatment and the late toxicities are classified as the complications occurring after 90 days. The complete classification can be seen in *Appendix B*.

The possible adverse events after treatment for prostate cancer include: dysuria, urinary incontinence,

[1]Diagnostic procedure that is used to look at the bladder, collect urine samples, and examine the prostate gland. Performed with an optic instrument known as a cystoscope, this instrument uses a lighted tip for guidance to aid in diagnosing urinary tract disease and prostate disease.

urinary retention, frequent voiding, hematuria, erectile impotence, diarrhoea, rectal pain and rectal bleeding.

The European Organization for Research and Treatment of Cancer (EORTC) and Radiation Therapy Oncology Group (RTOG) have a toxicity score based on questionnaires which have been used to assess the toxicities that I mentioned before. The patient has been followed during the months after treatment.

Urinary Retention

Urinary retention can be defined as the inability to spontaneously empty the bladder. For that reason, the patients have an indwelling catheter in their bladder. This catheter drains the urine from the bladder into a bag outside their body (see figure 1.8). According to the time when the symptoms start, the urinary retention will be classified as acute or late urinary retention. In HDR BT, acute urinary retention (AUR) is most common than late urinary retention.

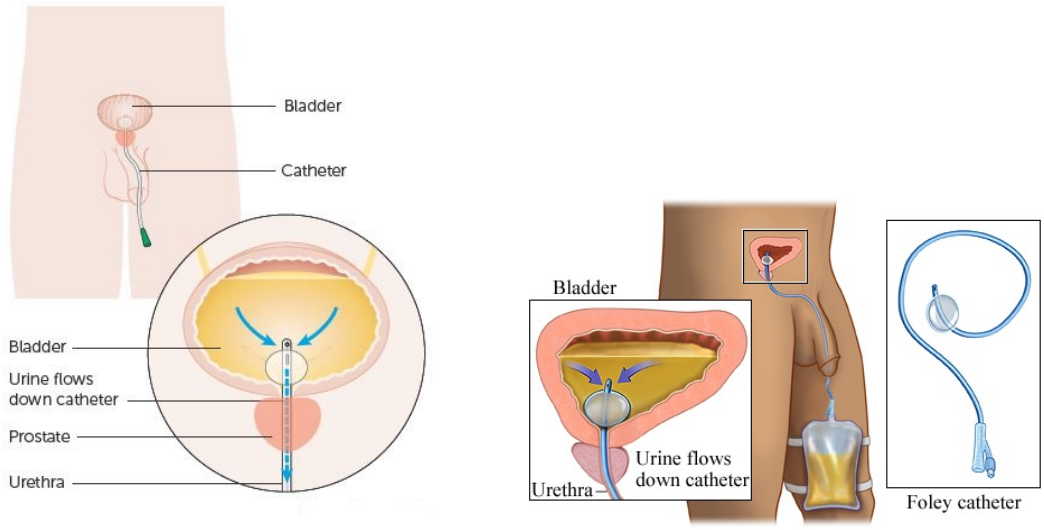


Figure 1.8: Schematic representation of indwelling bladder catheter. Taken from [19, 20].

Rectal Bleeding

Rectal bleeding refers to the passage of bright blood via rectum. The rectum is the final 15 cm of the colon (large intestine) where faeces accumulate before being expelled from the body via the anal canal. Rectal bleeding can be due to bleeding from anywhere in the lower gastrointestinal tract namely the colon, rectum or anus. As well as in urinary retention, this side effect can be classified as acute or late complication. The rectal bleeding associated to HDR BT is usually a late effect and depending on the severity, the patients could use pads/diaper or not.

Chapter 2

Statistical Approach

In the most scientific areas, we want to know what the relation is between two categorical variables and/or between continuous and categorical variables. First of all, the types of data need to be explained. Categorical data/parameters is usually defined as an independent or predicting variable that contains values indicating membership to one of several possible categories and it can be further categorized as either nominal, ordinal or dichotomous.

- *Nominal variables:* parameters that have two or more categories, but which do not have an intrinsic order, e.g. marital status (married, single, divorced, widowed);
- *Dichotomous variables:* nominal variables which have only two categories or levels and those are often assigned numerical values used as labels, e.g 0 = male 1 = female.
- *Ordinal variables:* variables that have two or more categories just like nominal variables only the categories can also be ordered or ranked. One example of that is when the answer of one question is: yes, very much; yes, quite; yes, a bit; no, not at all.

Continuous variables are not restricted to particular values, e.g. reaction time, age, weight, height, etc. Sometimes continuous data can be transformed in into categorical data, e.g. length $<$ x vs $\geq$ x. This chapter will provide a summarized view of the statistical approaches [based on [21, 22]], particularly, tests used for categorical and continuous variables and their assumptions.

2.1 Statistical tests

2.1.1 Chi-Square

Chi-square test (χ^2) is one of most used test to evaluate the association between two categorical variables. This statistical approach is based on the simple idea of comparing the frequencies observed in certain categories to the frequencies you might expect to achieve in those categories by chance. The chi-square test is always testing what is called the null hypothesis, which states that there is no significant difference between the expected and observed result. The value of this test is calculated according to the following equation:

$$\chi^2 = \Sigma \frac{(f_o - f_e)^2}{f_e} \qquad \text{(Eq.2.1)}$$

Where, f_o is the frequency of the observed data and f_e is the frequency of the expected values. In this specific research chi-square test of independence will be used with the following hypotheses:

H0: The two categorical variables are independent.

Vs.

H1: The two categorical variables are related.

Associated to χ^2 statistic value is the p-value or its significance value. If the significance value is small enough, usually p-value ≤ 0.05, the null hypothesis will be rejected and confidence is gained in the hypothesis that the two parameters are in some way related. This test has an important assumption related to expected frequencies. Expected counts are the projected frequencies in each cell if the null hypothesis is true. In large tables, the rule is that all expected counts should be greater than 1 and no more than 20% of expected counts should be less than 5. If this assumption is broken, the result is a huge reduction in test power. When looking at association between only two categorical variables, the solution for this problem is to use the Fisher's Exact test. If there are more than 2 categorical variables, there are other tests that can be used. However, that is beyond the scope of this sections.

2.1.2 T-student test and Mann-Whitney U-test

To analyse relations between continuous parameters, the t-student test can be used. The t-student test evaluates mean differences in variables of interest between two groups. The assumptions are:

1. The data is continuous;
2. The data follow the normal probability distribution;
3. The variances of two populations are equal;
4. The two samples are independent. There is no relationship between the individuals in one sample as compared to the other.

If the normal distribution is not met, the correspondent non-parametric test of t-student called Mann-Whitney U-test can be used. Therefore, one of the first steps before applying the statistical test is to evaluate the distribution of the data. To assess the normality the Kolmogorov-Smirnov test is performed. If the significance value of that test is lower than 0.05, we will conclude that there is a deviation from normality. Therefore, when the data breaks assumption 2 and the variance of two populations is unknown, the suitable test for this cases is the Mann-Whitney test.

The Mann-Whitney test uses the ranks of the values rather than the values themselves, so this test compares the median differences between two different groups. The statistic value of this test is calculated by:

$$U = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1 \qquad \text{(Eq.2.2)}$$

Where, n_1 and n_2 are the sample sizes of group 1 and 2 respectively, and R_1 is the sum of ranks for group 1. Therefore, the hypotheses in this test are:

H0: The median of certain variables is the same across the categories.

Vs.

H1: The median of certain variables is not the same across the categories.

Once again, when the significance value is lower than or equal to 0.05, the null hypothesis is rejected. Therefore, in that case, we can conclude that those variables have differences across the groups and because of that we gain confidence that there is in some way an association.

2.2 Logistic Regression

The linear logistic regression is commonly used for epidemiology, but it can also be used for other areas of medicine as, for instance, radiotherapy. This type of statistical approach is computed in order to describe the relationship between disease/toxicities and one or more explanatory variables which might influence the dependent variable. Logistic regression has some important features. One of them is that the dependent variable must be dichotomous, which means it should have values as 0 or 1, depending on disease status (e.g. alive or dead, case or control).

Another important characteristic is the output of this method. While linear regression predicts the value of dependent variable from one or more predictor variable, logistic regression can predict the probability of Y occurring given known value(s) of X. The logistic regression equation is:

$$P(Y) = \frac{1}{1 + e^{-(b_0 + b_1 X_1 + b_2 X_2 + ... + b_n X_n)}} \qquad \text{(Eq.2.3)}$$

Where b_0, b_1,. . ., b_n are the coefficient values estimation. In equation 2.3, you might notice that the equation within *e* (the base of natural logarithms) brackets is similar to the simple linear regression. The independent variables in logistic regression may be continuous or discrete, qualitative or quantitative. The output of the analysis provides important parameters such as the coefficient values estimation (b_0, b_1,..., b_n), the p-value, the odds ratio and 95% Confidence Interval. The coefficient value for each X variable is estimated using a technique called maximum-likelihood estimation. This method chooses the coefficients that make the observed values most likely to have occurred.

The p-value is a function of the observed sample results that is used for testing the statistical hypothesis as previously described. If the p-value is less than or equal to the chosen significance level (1% or 5%), the test suggests the Y variable is better explained when X_1, X_2,..., X_n are added than when only the intercept (b_0) is used. Usually, using only b_0 is the null hypothesis.

In the logistic regression, the statistical test used is the **Wald Test** and the associated **z-statistic** is calculated by:

$$z = \frac{b}{SE_b} \qquad \text{(Eq.2.4)}$$

Where b is the regression coefficient and SE_b is the standard error associated to the regression coefficient.

Another important parameter is the odds ratio, which is essential for a good interpretation of logistic regression. The odds ratio (OR) is a tool to quantify how strongly the presence or absence of a certain characteristic is associated with dependent variable Y. Even so, the OR is an indicator of the change in odds resulting from a unit change in the predictor. The OR of a certain feature is given by an exponential of this property (e.g. exp(b_1)) and it can be interpreted as:

- OR $>$ 1: if predictor increases, chance of Y increases;
- OR $<$ 1: if predictor increases, chance of Y decreases;
- OR $=$ 1: predictor does not affect chance of Y.

The confidence interval (CI) for odds ratio is another way to analyse the association between predictors and dependent variable. The basic idea is to construct a range of values within which it is expected that the population value falls. In particular, the CI provides the likelihood that it contains the true value of the issue we are trying to estimate. If the entire interval is above 1, we conclude a positive association, while an interval below 1 indicates negative association between the dependent variable and predictors. In other words, if the interval contains 1, we cannot conclude there is an association.

2.2.1 Univariate and Multivariate Method

Univariate analysis is the simplest form of statistical analysis. This method assumes that the response variable is influenced only by one factor/predictor. In other words, there is one dependent variable and one independent variable. The univariate linear logistic regression equation is given by:

$$P(Y) = \frac{1}{1 + e^{-(b_0 + b_1 X_1)}} \quad \text{(Eq.2.5)}$$

Multivariate analysis assumes that the dependent variable could be explained by more than one independent variable. In other words, the response variable is influenced by multiple factors. In this case, the model equation is given by Eq.2.3.

Usually, the univariate method is used for pre-selection of predictors to include in multivariate analysis. In this method, we do not only include the parameters with p-value ≤ 0.05 but we can use an less conservative attitude and accept parameters with p-value ≤ 0.2 or 0.3.

This strategy has advantages such as reducing problems of overfitting and stepwise selection. The predictors are eliminated at an early stage if they do not meet the univariate pre-selection threshold. The univariate method is pretty useful with large data sets because it reduces the number of predictors and, consequently, the complexity of the model.

In case of a small dataset with few cases versus controls, the logistic regression only allows to build a model with 1 covariate per 10 cases [24, 25]. In these cases, the multivariate analysis can be used to assess whether the variable of interest is affected by another variables or not. In other words, other parameters are added as confounders in the analysis of the variable of interest. Therefore, in order to assess if those parameters are confounding factors, adjusted OR and unadjusted OR can be compared. Basically, when adjusted OR changes substantially from unadjusted OR, we conclude that those confounder parameters affected the outcome and the variable is not an independent factor associated with the dependent variable.

2.3 Missing Values

In data analysis, one of the most common problems is missing values. This often occurs when the data is dependent upon responses to questionnaires, old data or medical files. There are different types of missing values and the way to deal with them can also be different. Missing values can be defined as, missing values completely at random (MCAR) or missing values at random (MAR). A database with missing values is MCAR if the subjects who have missing data are a random subset of the complete sample of subjects. In order words, imagine your data set as a large matrix in which the missing values do not follow a specific pattern. The typical example is when a questionnaire of a study subject is accidentally lost.

The data is classified as MAR when the missing values are associated with a certain patient feature/behaviour at the time of analysis. One example of this is when you want to assess a certain symptom score after treatment: if the patient is not feeling well, he is more likely to not answer the questionnaire.

There are several methods to minimize this problem, from traditional approaches until more modern techniques. Examples of traditional techniques are listwise/pairwise deletion or mean/median substitution. These techniques are the most simple and less time consuming, but could result in biased outcomes and it may reduce or increase the statistical power.

Listwise/Pairwise Deletion

Listwise or casewise deletion is the default option in most statistical software. The listwise deletion works this way: whenever the statistical software finds one missing value in one variable, it deletes all va-

riables for that subject. This technique is often used, but it results in 20%-50% loss of the data and it could be a problem when using a small population.

Pairwise deletion uses all available information. The procedure cannot include a particular variable when it has a missing value, but it can still use the case when analysing other variables with non-missing values.

Mean/Median Substitution

Another traditional approach to work with missing values, probably the most simple one, is the mean or median substitution. The mean imputation consists of replacing the missing values of a certain variable by the mean of all known values for that variable. This procedure is used when the variable with missing values is normally distributed. When the distribution of the parameter with missing values is skewed the median should be used rather than mean.

Some other variant of this method is the mean/median substitution for subgroups. In case of a variable with missing values divided by group, the median for each group can be calculated to replace the missing values of the correspondent group. This procedure results in a better estimate and preserves more variance than giving everyone with a missing value the overall median [26].

Single and Multiple Imputation

Several newer techniques have been developed for dealing with missing values and two new approaches will ne discussed here: Single Imputation using Expectation Maximization (EM) and Multiple Imputation. Both techniques can only be applied when the missing values are classified as MCAR.

Single imputation using EM creates a new data set that has no missing values. This method is based on the observed relationship among the variables and replaces the missing values by the maximum likelihood value.

Multiple imputation estimates the missing values using *n* iterations instead of only one. In SPSS tool this method has three options:

1. ***Markov chain Monte Carlo (MCMC) method:*** suitable for data with an arbitrary pattern of missing values. The method fits a univariate (single dependent variable) model using all other available variables in the model as predictors, then imputes missing values for the variable being fit. The method continues until the maximum number of iterations is reached, and the imputed values at the maximum iteration are saved to the imputed dataset.

2. ***Monotone method:*** noniterative method that can be used only when the data have a monotone pattern of missing values. A monotone pattern exists when you can order the variables such that, if a variable has a nonmissing value, all preceding variables also have nonmissing values. Here, the method to impute is equal to MCMC procedure only the univariate model is different.

3. ***Automatic method:*** SPSS chooses an imputation method based on scan of your data and uses the monotone method if the data show a monotone pattern of missing values; otherwise, MCMC is used. If you are certain of which method you want to use, you can specify it in SPSS software.

After imputation all statistical tests and logistic regression can be applied. Figure 2.1 illustrates the multiple imputation method.

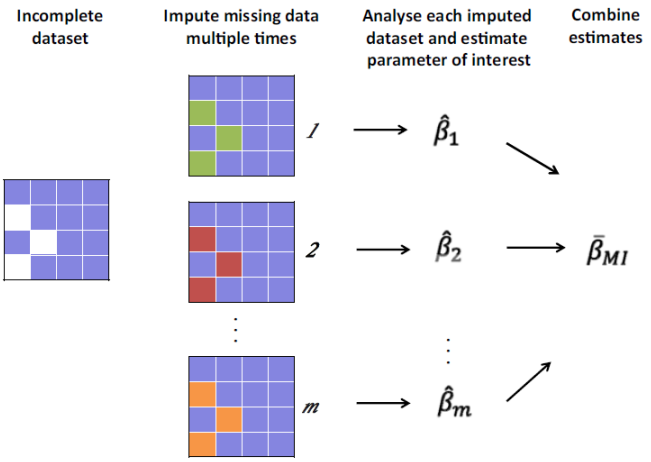


Figure 2.1: Illustration of the method of multiple imputation. Each box represents a data value where the columns are variables and the rows are individuals. Blank spaces represent the missing values. β_i is the estimate of interest from the completed dataset number i, β_{MI} is the estimate obtained from multiple imputation and m is the number of iterations. Taken from [27].

2.4 Receiver operating characteristic curve - ROC curve

The receiver operating characteristic (ROC) curve [28, 29], which is defined as a plot of the test sensitivity as the y coordinate versus its 1-specificity or false positive rate (FPR) as the x coordinate. It is an effective method of evaluating the quality or performance of diagnostic tests, and is widely used in medicine to evaluate the performance of many diagnostic tests. Basically, the ROC curve allows quantification of how accurate medical diagnostic tests (or systems) can discriminate between two patient states, typically referred to as ”diseased” and ”nondiseased”.

Sensitivity and specificity, which are defined as the number of true positive decisions (the number of actually positive cases) and the number of true negative decisions (the number of actually negative cases), respectively, constitute the basic measures of performance of diagnostic tests (see table 2.1).

Table 2.1: Decision Matrix.

Diagnostic test results	**Disease status**	
	Present	**Absent**
Positive	a (TP)	b (FP)
Negative	c (FN)	d (TN)
Total	n1 = a+c	n2=b+c

Note:
TPR = True positive rate (sensitivity)= TP/(TP+FN);
FNR = False negative rate (1-sensitivity)= FN/(TP+FN);
TNR = True negative rate (specificity) = TN/(TN+FP);
FPR = False positive rate (1-specificity) = FP/(TN+FP);
n1=patient with disease; n2=patients without disease.

Several summary indices are associated with the ROC curve. One of the most popular measures is the area under the ROC curve (AUC). AUC is a combined measure of sensitivity and specificity. It is a measure of the overall performance of a diagnostic test and is interpreted as the average value of sensitivity for all possible values of specificity.

The accuracy of the test is evaluated according to the following scale:

- **AUC = 0.9-1:** Excellent test accuracy;
- **AUC = 0.7-0.9:** Good test accuracy;
- **AUC = 0.6-0.7:** Moderate test accuracy but it is better than relying on pure chance;
- **AUC = 0.5-0.6:** If AUC is close to 0.5, it relies on pure chance to distinguish those subjects with versus those without a particular disease, the resulting ROC curve would fall along this diagonal line, which is referred to as the chance diagonal.

In summary, the ROC curves as close as possible to the superior left corner of the ROC plot are the most optimal. Figure 2.2 depicts three different ROC curves. Considering the area under the curve, test A is better than both B and C, and the curve is closer to the perfect discrimination. Test B has good validity and test C has moderate.

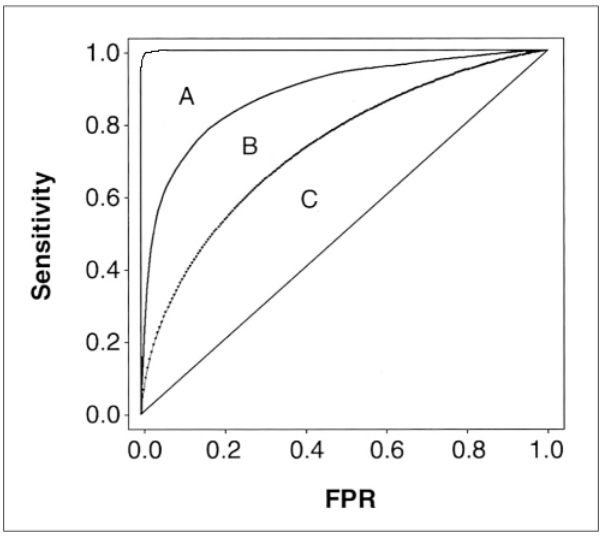


Figure 2.2: Comparision between ROC curves. Taken from [29].

2.4.1 Optimal cut-off values

ROC curve analysis has several advantages. First, AUC is not affected by decision criterion and it is also independent of prevalence of disease since it based on sensitivity and specificity. Second, we can easily obtain the sensitivity at a specific FPF by visualizing the curve. Another advantage and one of the most important tools of ROC is the possibility to determine the cut-off value for a certain parameter.

There are two methods to determine the optimal cut-off values. The first method gives equal weight to sensitivity and specificity with no ethical, cost and prevalence constraints. The second method gives different weight to sensitivity and specificity. For example, given a disease with low prevalence and high cost of false positive diagnosis, the cut-off point may be chosen at higher value to maximize the specificity.

Chapter 3

Bibliography Review

3.1 Introduction

Prostate cancer (PCa) is the second cause of death in men, the first one is the lung tumours. This is more frequent in older men ($\geq$ 50 years old). In Portugal, it is estimated that prostate cancer has an incidence of 108 cases per 100 000 inhabitants (in 2009) and the mortality rate is approximately 36 per 100 000 inhabitants (in 2012) [30]. It represents approximately 3.5% of all deaths and more than 10% of other types of cancer deaths [31]. In the Netherlands, 11 158 new cases of prostate cancer are diagnosed each year, and one out of 35 patients will die from prostate cancer [32] and expected to increase due to growth and ageing of the population. This kind of tumour can be treated by several techniques, for instance, radical prostatectomy, chemotherapy, External Beam Radiotherapy (EBRT), Intensity Modelated Radiotherapy (IMRT), Hormone Therapy and Brachytherapy (BT). In the last years, the most used technique is EBRT or IMRT. A new technique appeared in early twentieth century called Brachytherapy. This technique inserts the sources within the patients, so is only available for certain types of tumours as prostate tumours, gynaecology tumours and other superficial or interstitial tumours.

The first treatment that was used to treat prostate cancer was called Low-dose-rate Brachytherapy because they used low-dose rate sources (e.g. ^{125}I).They implanted seeds permanently in the prostate volume to achieve the desired dose distribution. Over a period of months, the level of radiation emitted by the seed sources will decline to almost zero. This technique continues to be used currently, but not with the same force.

There is an other type of BT called HDR, its uses high-dose rate sources. In the beginning, this technique had a big problem because of the radiation exposure to operators from the manual application of the radioactive sources. So, the huge growth only occurred in 1950s and 1960s with the development of remote afterloading machines. With this development the treatment can be delivered with no radiation exposure to the operators.

3.2 HDR BT for PCa

In the paper of **Kovács et al.** [11] several advantages of remote temporary afterloading brachytherapy are illustrated as:

- Accurate positioning of the source by first implanting non-active guide needles;
- Possibility to choose the source positions over the length of the needle;
- No target movement during radiation;

- Stepping source technology allowing for dose and volume adaptation due to adjustment of source dwell locations and times according to 3D imaging based individual dose prescription before irradiation.

Introducing a remote afterloading technique combined with the technological developments in 3D imaging, such as transrectal ultrasound (TRUS), as well as treatment planning software developments resulted in an appropriate target delineation and guidance of the needles.

After these technical developments, HDR brachytherapy started to be used in combination with conventional EBRT. In this way, the **GEC/ESTRO recommendation** had to be updated for HDR afterloading brachytherapy for localised prostate cancer in 2013 [12]. In this paper, they enumerated several advantages of this technique as:

- The use of image guided needle placement enables accurate implantation which can be extended to include extracapsular disease and seminal vesicles.
- It is possible to individualise the source positions over the full length of the prostate based on a defined planning target volume and organs at risk. Dose distribution optimization by inverse planning enables highly conformal dose delivery.
- The fixation of the prostate by the implant and rapid radiation delivery minimises the problems of target and OAR movement.
- The use of high dose per fraction has a biological dose advantages for tumours with a low α/β ratio of which prostate is a common example.
- The use of a singles source for all patients using a multipurpose facility makes HDR BT highly cost effective.

They also described some disadvantages as the use of fractionated schedule which results in more work load per patient. This paper is a important tool for HDR BT for prostate cancer because they show all requirements regarding to patient selection, organ delineation, implant procedure, planning aim and dose prescription and how the treatment should be delivered. So, this is the most recent guide for HDR BT for prostate cancer.

3.3 Results HDR BT for PCa

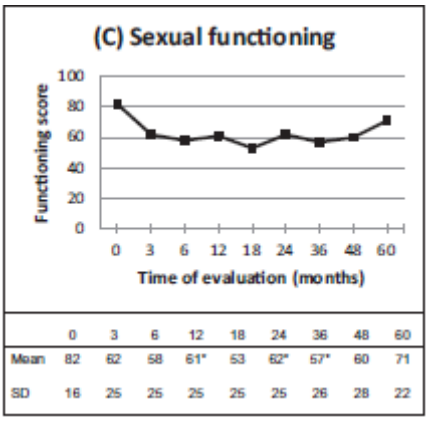
When we use these techniques to treat the patient, the main objective is to treat the prostate while protecting the organs at risk and the normal surrounding tissue. In this way, it is important to evaluate the toxicity in these organs due to this kind of treatment.

In 2008, the group of **Ishiyama et al.** [33] from Japan, sought to evaluate the severity of genitourinary (GU) toxicity HDR brachytherapy combined with hypofractionated external beam radiotherapy (EBRT) and they also looked for factors that might affect the severity of GU toxicity. They evaluated 100 Japanese patients and they observed that their patients have a high value of GU toxicity (a significant percentage 28%). After they applied the multiple logistic regression model, they found that the volume of the prostatic urethra is associated with the grade of acute GU toxicity and that urethral dose is associated with the grade of late GU toxicity.

Another group, **Aluwini et al.** [34] from The Netherlands in 2011, reported the clinical outcomes and early and late complications in patients with low- and intermediate- risk of prostate cancer who were treated with a combined technique (EBRT + HDR BT). They follow-up the patients treated between 2000 and 2007 and they show that the treatment with interstitial HDR BT + EBRT resulted in a low incidence of late complications and a favourable oncology outcome after 7 years follow-up. In this research group, the freedom

of biochemical failure was 97% and failure-free survival [1] was 96%. They found excellent results for low- and intermediate risk PCa patients using EBRT plus HDR BT and they suggested the use of less intensive treatment for this group, using monotherapy HDR BT. They also proposed that EBRT plus HDR BT should be used for high-risk prostate cancer.

Recently, **Aluwini et al.** [13] published another study where they reported their results on toxicity and quality of life after HDR BT monotherapy. Three months after treatment, acute GU and GI toxicities were reported in 10.8% and 7.2%. Late grade $\geq$ 2 GU and GI toxicity were reported in 19.7% and 3.3% of patients 12 months after HDR BT. They also observed a biochemical failure rate as 2.4% and the cancer-specific survival was 100%. An interesting result that they also found was that 8 patients needed an indwelling bladder catheter due to acute urinary retention. In this way, this group decided to change the criteria for patient selection regarding to IPSS score. Nowadays, in clinical practice in *Erasmus MC - Cancer Institute* (Rotterdam, Netherlands) only patients with IPSS $\leq$ 18/35 are selected for HDR BT as monotherapy . The last important result is regarding the erectile function after treatment. Their patients recovered almost to normal erectile function after 60 months of treatment as you can see in figure 3.1.



	0	3	6	12	18	24	36	48	60
Mean	82	62	58	61*	53	62*	57*	60	71
SD	16	25	25	25	25	25	26	28	22

Figure 3.1: Sexual Functioning score vs Time of Evaluation in months. Taken from [13].

So they concluded that HDR BT shows a good clinical outcome and acceptable acute and late toxicity. Even so, these types of toxicities still appear in a considerable amount of patients. Therefore,it is important to evaluate whether dosimetric values predict the occurrence of GI and/or GU toxicities.

In Berlin, **Ghadjar et al.** [35] published a study in 2009: their main goal was to evaluate the acute and late GU and GI toxicity after HDR BT as monotherapy for for low- and intermediate PCa patients. They found some association of late grade 3 GU toxicity with urethral V120 and V100 and also with $D90^2$ of PTV. So, they concluded that reduction of the irradiated urethral volume may reduce the GU toxicity and potentially improve the therapeutic ratio of this treatment. Six years later, the some group published a similar study where they used the same group of patients. **Ghadjar et al.** [36], in this current study, used other kind of statistical analysis (multivariate Cox regression) to find within a huge amount of DVH parameters which parameters are associated to grade 3 GU toxicity. Regarding the urethral V120 they found the same association but also found that GI toxicity was negligible and that erectile function preservation rate was excellent as **Aluwini at el.** [13] found recently.

Therefore, the main issue in this area is the urethral strictures, urinary retention and rectum toxicities due to HDR BT. A significant proportion of patients still have some acute and late toxicities associated to the GU system. Nowadays, an important research area is related to this problem, where the researcher try to find some correlation between DVH parameters and these kind of toxicities. In this way, in 2009, **Konishi et al.** [37], published a study where the main goal was to evaluate the correlation between dosimetric parameters and late rectal and urinary toxicities. They use 83 patients from Japan treated from 2001 through 2005. The

[1] **Failure-free Survival:** is defined as a percentage of patients still alive without evidence of biochemical or clinical failure
[2] D90: the dose that covers 90% of the target volume

total prescribed dose was 54Gy in 9 fraction over 5 days. They found some dosimetric parameters for rectum significantly high in 18 patients who presented with late rectal toxicities. Regarding to the urethral toxicities they did not find any correlation. The next tables in figure 3.2 show the main results of this paper.

parameters			
Parameters	Grade 0 (n = 65)*	Grade 1–3 (n = 18)*	p
V10 (cc)	29.9 ± 10.4	33.0 ± 8.4	0.210
V20 (cc)	21.1 ± 6.0	22.7 ± 3.2	0.153
V30 (cc)	12.7 ± 3.4	15.2 ± 2.5	0.001
V40 (cc)	6.9 ± 2.2	9.1 ± 2.1	< 0.001
V50 (cc)	3.5 ± 1.5	5.1 ± 1.6	0.001
V60 (cc)	1.6 ± 1.0	2.6 ± 1.1	0.002
V70 (cc)	0.67 ± 0.59	1.20 ± 0.78	0.014
V80 (cc)	0.24 ± 0.31	0.46 ± 0.50	0.088
V90 (cc)	0.07 ± 0.13	0.15 ± 0.26	0.227
V100 (cc)	0.07 ± 0.14	0.04 ± 0.10	0.322
D5 (Gy)	32.7 ± 5.3	35.2 ± 5.9	0.109
D10 (Gy)	28.1 ± 4.9	30.2 ± 5.6	0.161
D20 (Gy)	22.8 ± 4.3	24.3 ± 5.1	0.258
D30 (Gy)	19.4 ± 4.1	20.4 ± 5.0	0.411
D40 (Gy)	16.8 ± 3.9	17.5 ± 4.8	0.601
D50 (Gy)	14.6 ± 3.7	14.9 ± 4.6	0.773
D60 (Gy)	12.8 ± 3.4	12.6 ± 4.4	0.929
D70 (Gy)	11.0 ± 3.2	10.7 ± 4.1	0.783
D80 (Gy)	9.3 ± 3.0	8.9 ± 3.8	0.757
D90 (Gy)	7.5 ± 2.7	7.2 ± 3.2	0.737
D1cc (Gy)	34.9 ± 4.6	38.5 ± 4.4	0.005
D2cc (Gy)	30.8 ± 4.1	34.3 ± 3.7	0.002
D5cc (Gy)	24.0 ± 3.2	27.2 ± 2.8	< 0.001
D10cc (Gy)	18.3 ± 2.6	20.7 ± 2.3	0.001

D5–D90 and D1cc–D10cc are against the total prescribed dose (54 Gy).
* Mean ± SD.

(a) Comparison of mean values of rectal dosimetric parameters.

parameters			
Parameters	Grade 0 (n = 68)*	Grade 1–3 (n = 15)*	p
V100 (cc)	0.57 ± 0.17	0.55 ± 0.18	0.664
V110 (cc)	0.47 ± 0.19	0.44 ± 0.19	0.609
V120 (cc)	0.28 ± 0.19	0.22 ± 0.16	0.283
V130 (cc)	0.099 ± 0.12	0.069 ± 0.12	0.407
V140 (cc)	0.021 ± 0.055	0.026 ± 0.067	0.791
V150 (cc)	0.0044 ± 0.015	0.0073 ± 0.028	0.704
D5 (Gy)	70.7 ± 5.8	70.7 ± 5.9	0.963
D10 (Gy)	69.3 ± 5.4	69.5 ± 5.8	0.912
D20 (Gy)	67.7 ± 5.0	68.0 ± 5.7	0.840
D30 (Gy)	66.5 ± 5.0	67.0 ± 5.7	0.789
D40 (Gy)	65.4 ± 4.8	66.0 ± 5.7	0.764
D50 (Gy)	64.4 ± 4.8	64.8 ± 5.9	0.788
D60 (Gy)	63.0 ± 4.9	63.5 ± 6.0	0.785
D70 (Gy)	61.2 ± 5.0	61.7 ± 6.4	0.802
D80 (Gy)	58.4 ± 5.7	58.7 ± 6.8	0.903
D90 (Gy)	53.4 ± 7.0	54.4 ± 6.9	0.617

D5–D90 are against the total prescribed dose (54 Gy).
* Mean ± SD.

(b) Comparison of mean values of urinary dosimetric parameters.

Figure 3.2: Rectal and Urethral Dosimetric Parameters Evaluation. Taken from [37].

The statistical most significant difference was observed for V40 and D5cc[3] for rectum. In this way, they suggested that rectal V40 $\leq$ 8cc and D5cc $\leq$ 27Gy may be dose-volume constraints in HDR BT.

Recently, in 2014, a research group from UK, **Diez et al.** [38], tried also to evaluate and find some correlation between dosimetric parameters related to urethral strictures and dose schedule. They evaluated 4 different dose schedules and 213 patients. In these patients 10 urethral strictures were identified. For evaluation, they divided the urethra in prostatic urethra and membranous urethra . The first volume was further divided in 3 equal parts and the membranous urethra was defined from apex of prostate to the bulb of penis measuring approximately 1.2cm in length. They do this for checking whether some part of urethra is more sensitive to dose. As dosimetric parameters they use only six parameters as V10Gy (%)[4], V8.5Gy (%), D30 (Gy), D10 (Gy), Dmax (Gy) and Dmean (Gy). As results of their study, they did not find any difference between stricture cases and control cases (people without urethral strictures) in terms of dosimetric parameters.

The factors that predict which patients have a greater chance of developing acute urinary retention (AUR) are not very well known, mainly for HDR BT. In literature, there are several LDR BT studies reporting possible risk factors of AUR, mainly, related to clinical parameters. **Bucci et al.** [39], reported IPSS as important predictor of AUR. **Roeloffzen et al.** [40] and **Mabjeesh et al.** [41] , found IPSS and prostate volume before treatment as predictors of AUR. While, **Lee et al.** [42] reported number of needles and prostate volume after treatment as variables associated to AUR. More authors have reported prostate volume [43, 44] as an associated factor with AUR.

In 2010, **Roeloffzen et al.** [45] looked to assess the influence of dose in different prostate regions, and

[3]D5cc: The dose delivered to the 5 cubic centimeter volume.
[4]V10Gy: Percentage of volume that receive 10Gy

the influence of anatomic variation on the risk of acute urinary retention after ^{125}I prostate brachytherapy. They used 100 patients, 50 as considered as cases (with AUR) and other 50 as controls (without AUR). The dosimetric parameters analysed were D10, D50, D90, V100 [5] and V200 and they used the logistic regression analysis. The group found that AUR is associated with high dose in bladder neck mainly, they reported mean bladder neck D90 = 65Gy in cases versus 56Gy in controls (p=0.016), and mean bladder neck D10 = 128Gy vs. 107Gy in controls (p = 0.018).With this study, they also re-emphasized the need to avoid the insertion of needles and seeds into the bladder neck, in order to reduce the risk of AUR.

Most recently, in 2014, a research group from the Department of Radiation Oncology and Medical Physics in New York, **Hathout et al.** [46], reported that the dose to the bladder neck is the most important predictor for Acute and Late Toxicity after LDR BT. They evaluated 927 patients treated between 2002 and 2013. The clinical and dosimetric factors were evaluated with Cox regression, ROC curve and univariate and multivariate method. This group found that the bladder neck D2cc $\geq$ 50% is the strongest predictor for grade $\geq$ 2 acute and late urinary toxicities, so they suggested to include bladder neck constraints into the brachytherapy planning to decrease urinary toxicity (see figure 3.3).

Table 2 Univariate and multivariate analysis for acute urinary toxicity

Variable	Univariate		Multivariate	
	P value	HR (95% CI)	*P* value	HR (95% CI)
Baseline IPSS (continuous)	.30	-	-	-
Age (continuous)	.88	-	-	-
Prostate volume on pretreatment MRI (cm^3)	<.0001	1.01 (1.01-1.02)	.43	-
Prostate V100 (continuous)	.13	-	-	-
Prostate D90 (continuous)	.02	1.013 (1.002-1.023)	.09	
Prostate V150 (continuous)	.05	-	-	-
Urethra D20 (continuous)	.41	-	-	-
Urethra D5 (continuous)	.41	-	-	-
Urethra D1 (continuous)	.93	-	-	-
Bladder V100	<.0001	1.12 (1.05-1.19)	.29	-
Bladder D2cc (continuous)	<.0001	1.01 (1.00-1.01)	.54	-
Bladder D1 (continuous)	<.0001	1.01 (1.00-1.01)	.34	-
Bladder neck V100 (continuous)	.1	-	-	-
Bladder neck D2cc	<.0001	1.04 (1.03-1.04)	<.0001	1.03 (1.03-1.04)
HI ([Prostate V100−V150]/V100)	.07	0.56 (0.30-1.06)	.2	-
Use of neoadjuvant ADT (yes vs no)	.42	-	-	-
Choice of isotope (^{103}Pd vs^{125}I)	.94	-	-	-
Definitive treatment vs combined therapy with EBRT	<.0001	1.49 (1.25-1.78)	.008	1.32 (1.08-1.63)
Number of seeds (continuous)	<.0001	1.01 (1.01-1.02)	.24	-
Number of needles implanted (continuous)	<.0001	1.07 (1.04-1.10)	.12	-
Diabetes (yes vs no)	.35	-	-	-
Smoking habits (current vs former vs never vs unknown)	.64	-	-	-
Use of PDE-5I at diagnosis (yes vs no)	.66	-	-	-

Abbreviations: ^{103}Pd = Palladium 103; ^{125}I = Iodine 125; ADT = androgen-deprivation therapy; CI = confidence interval; HI = homogeneity index; HR = hazard ratio; EBRT = external beam modulated radiation therapy; IPSS = International Prostate Symptom Score; MRI = magnetic resonance imaging; PDE-5I = phosphodiesterase type 5 inhibitor.

Figure 3.3: Results of Univariate and Multivariate analysis. Taken from [46].

Another possible side effect of HDR BT is rectal bleeding. The factors that predict rectal bleeding are not very well known for HDR brachytherapy as monotherapy treatment but there are several studies reporting those after EBRT or LDRBT. In 2004, **Akimoto et al.** [47] investigated the incidence and severity of rectal bleeding after high-dose hypofractionated radiotherapy. They used a data set of 52 patients where 13 patients developed grade 2 or worse rectal bleeding. They evaluated clinical and dosimetric parameters by using univariate and multivariate analysis. On univariate method, they found diabetes mellitus ($p < 0.001$) and rectum V30 $\geq$ 60%, V50 $\geq$ 40% ($p < 0.05$), V80 $\geq$ 25% and V90 $\geq$ 15% ($p < 0.001$) as a significant risk factors for the occurrence of grade 2 or worse rectal bleeding. Only history of diabetes mellitus retained the significance value on multivariate method as the most important factor. **Herold et al.** [48] reported also diabetes as a significant risk of the development of late grade 2 GI and GU complications after EBRT.

In 1998, **Hu et al.** [49] did not find obvious difference in rectal wall radiation for patients who did or not experience resolution of their bleeding. In 2001, **Jackson et al.** [50], reported a significant correlation between grade 2-3 rectal bleeding and "intermediate doses" (around 40-50Gy) in a randomly chosen sample of patients treated with 70.2-75.6Gy conformal radiotherapy. They suggested large fractions of rectum receiving those doses may result in a loss of repair capacity of the mucosa cells, which may lead to bleeding. Later

[5] V100: Percentage of volume that receive at least 100% of prescribed dose.

on, one research group from Italy (**Fiorino et al.** [51]), found V50Gy $>$ 60-65% and V60Gy $>$ 50-55% as statistically significant variables associated to rectal bleeding after EBRT treatment. Another research group from Italy, **Cozzarini et al.** [52], reported that late rectal bleeding is associated with doses between 66.6-70.2Gy by using EBRT as treatment technique. Few investigations found a possible relationship between rectal volume and bleeding, [51, 53, 54], particularly, there are one inverse correlation between the rectum dimension and the fraction of that included in the high-dose region.

Regarding to LDRBT studies reporting some factors associated to this side effect, in 2004, **Sherertz et al.** [55] evaluated the contribution of various clinical and radiation treatment parameters to the likelihood of late rectal bleeding after LDR BT. They used univariate method and they found V100, V200 and V300 as statistically related to rectal bleeding. Later on, in 2007, **Ohashi et al.** [56] from Tokyo Medical Center, Japan, after multivariate analysis reported maximal rectal dose ($p < 0.001$) as the only significant factor associated to RB. Recently, in 2012, **Harada et al**. [57] from Japan, investigated the association between some clinical and dosimetric parameters and RB after LDR BT by using the data set of 24 patients with RB versus 65 without. They found as the most important factor the usage of anticoagulants ($p = 0.007$). Therefore, there are several LDR BT studies reporting some factor associated to this side effect and few studies reported this problem using HDR BT even combined with other types of brachytherapy. In 2006, one research group from department of radiation oncology from Japan explored the incidence of grade 2 or worse rectal bleeding after HDR brachytherapy combined with hypofractionated EBRT. Univariate analysis was performed to evaluated dosimetric parameters, such as rectal V10, V30, V50, V80, and some clinical variables as prostate volume, number of needles and patient age. This group, **Akimoto et al.** [58], found differences in the percentages of the entire rectal volume receiving 10%, 30% and 50% between those with and without bleeding.

Recently, in 2012, **Okamoto et al.** [59], evaluated the predictive risk factors for grade 2 or worse rectal bleeding after HDR BT combined with EBRT in 216 patients. They estimated the radiation doses delivered by HDR BT alone to 5% and 10% of rectum in patients with RB as 5.1Gy and 4.1Gy, respectively, and those results demonstrated that high dose areas, even if they include only small volume, should be carefully taken into consideration during HDR BT treatment planning suggesting V5 and V10 as risk factors for late rectal bleeding.

In conclusion, HDR and LDR BT are the most important technique for prostate cancer but still have some secondary problems related to dose delivery at OAR. This is the reason for many research groups to try to find some explanation or some new constraints in dose planning in order to improve the quality of life of the patients after the treatment.

Chapter 4

Predictive factors for acute urinary retention after HDR BT as monotherapy for low risk prostate cancer

4.1 Purpose

To evaluate clinical and dosimetric parameters related to acute urinary retention (AUR) needing a temporary bladder catheter (CAD) after high-dose rate brachytherapy as monotherapy treatment for prostate cancer.

4.2 Materials and Methods

In this study, patients with histological confirmed prostate carcinoma (PCa), clinical stage T1b-T2b, Nx-0, Mx-0, Gleason score $\leq$7, PSA $\leq$ 16 ng/ml and WHO performance [1]status 0-2 were treated with HDR BT monotherapy. HDR BT monotherapy was administered in four fractions of 9.5Gy with a minimum interval of six hours within 36 hours using one implant.

Predictive factors for AUR were investigated in 2 different groups, i.e., small group and large group. In the small group, the number of patients in evaluation was reduced because clinical and dosimetric data were selected and evaluated in more detail, e.g., we analysed dose in different regions of urethra. In the large group, we analysed data from HDR BT database for PCa of *ErasmusMC - Cancer Institute*. The following sections will explain the main differences between the small and the large group in detail.

4.2.1 Patients

Data set of 42 subjects - Small Group

The the small group is a selection from patients treated between 2007 and 2015 (210 patients). Fourteen of 210 (6.7%) patients received a CAD because of AUR after primary treatment for their PCa with HDR BT. These were analysed together with 28 other patients with grade $\leq$ 1 GU and GI toxicities [2]. Table 4.1 shows the patients and treatment characteristics.

[1]WHO performance status in *Appendix C*
[2]GU and GI classification in *Appendix B*

Data set of 210 subjects - Large Group

The large group consists of 210 patients treated between 2007 and March 2015. The treatment scheme and the number of cases with AUR after treatment are the same as it was mentioned before. These 14 patients who needed CAD were analysed together with all other 196 patients who did not receive a bladder catheter after treatment. Patients and treatment characteristics are shown in table 4.1.

Table 4.1: Patient, tumour and treatment characteristics. Small group = 14 CAD (2nd column)+ 28 no-CAD (3rd column); Large group = 14 CAD (2nd column) + 196 no-CAD (4th column).

Characteristic	CAD (n= 14 patients)	no-CAD (n= 28 patients)	no-CAD* (n= 196 patients)
Patient and tumour			
Age at implantation (y)	67.6 (57.8-74.7)	68.9 (53.2-79.3)	66.8 (44-79)
Clinical Tumour Stage			
T1 (n (%))	9 (64.3%)	16 (57.1%)	124 (63%)
T2 (n (%))	5 (35.7%)	12 (42.9)	68 (35%)
Gleason sum-score			
< 7 (n (%))	12 (85.7%)	25 (89.3%)	137 (70%)
=7 (n (%))	2 (14.3%)	3 (10.7%)	24 (12%)
iPSA (ng/ml) (mean (min-max))	8.4 (4.5-12)	7.8 (3.8-14)	7.9 (1.4-16)
Pretreatment IPSS (mean (min-max))	8 (2-19)	5 (0-16)	7 (0-24)
Baseline urinary flow (Qmax; ml/s) (mean (min-max))	12.4 (7-24.9)	16.2 (2-37)	16.1 (2-41.2)
Treatment			
TRUS volume (cm3) (mean (min-max))	38.5 (19.6-57.1)	32.5 (18.6-50)	33.5 (15-55)
Needles (mean (min-max))	18 (14-23)	17 (13-23)	17 (12-23)
PTV volume (mean (min-max))	67.1 (44.1-105.9)	49.1 (25.8-79.26)	55.7 (23.8-94.7)
Urethra length (mean (min-max))	57.9 (45-69)	60.7 (39-123)	59.7 (33-123)

Abbreviations:
CAD = patients needed a temporary bladder catheter after treatment;
iPSA = initial prostate-specific antigen level;
IPSS = international prostate symptom score; TRUS =transrectal ultrasound;
PTV = planning target volume at treatment;
* Data base is not complete: large amount of missing values.

4.2.2 Organs Delineation

Small Group

For all patients, organs at risk (bladder, urethra) were delineated. The urethra was divided into urethra membranous and urethra prostatic. Urethra prostatic was the urethra part within the boundaries of the prostate. The urethra prostatic was divided into superior, mid and inferior equals parts [38]. The membranous urethra was defined as 2 cm of the urethra caudal to the prostate apex. The bladder neck was defined as a bladder portion around the urethra opening adjacent to the prostate. Figure 4.1 illustrate the organs delineations. On the sagital view the bladder is represented as a dark blue contour, bladder neck as a green contour, prostate as a red contour, membranous urethra as a pink contour and prostatic urethra superior, mid and inferior as blue, violet and orange, respectively.

Large Group

In this group, bladder and urethra were not divided by different substructures and all delineations were made by the physicians and technicians at the treatment day according to the treatment protocol.

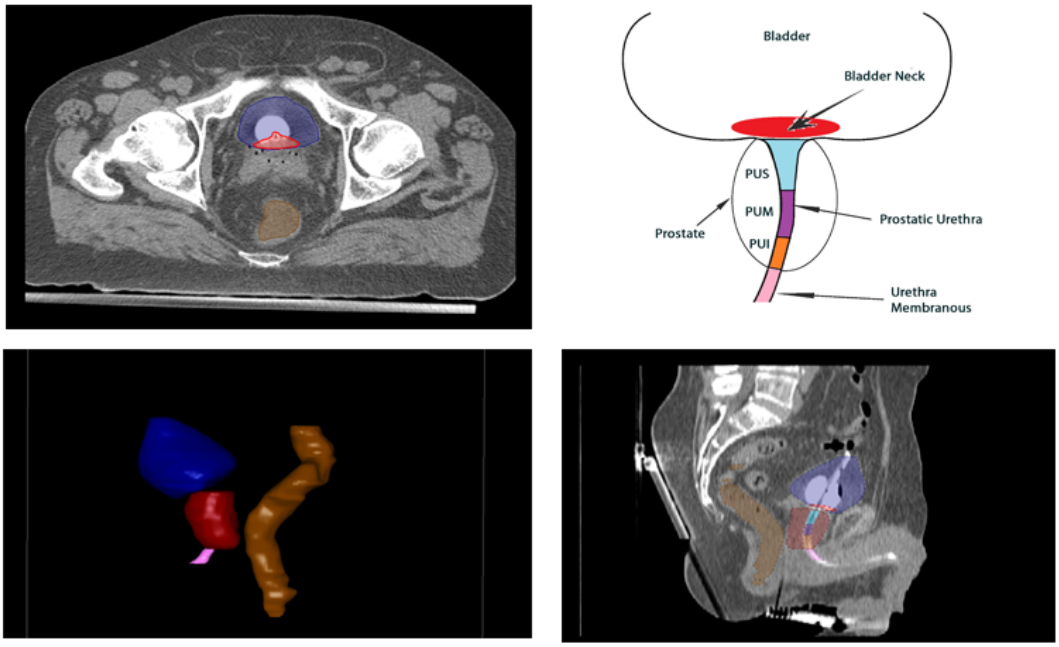


Figure 4.1: The delineated organs in the planning CT scan and a schematic representation of different parts of bladder and urethra. PUS = Prostatic Urethra Superior, PUM = Prostatic Urethra MID, PUI = Prostatic Urethra Inferior and UM = Membranous Urethra.

4.2.3 DVH and Clinical Parameters Selection

Small Group

For all delineated structures, the following dosimetric parameters were calculated as presented in table 4.2. The DVH parameters were defined as:

- **D*xcc***: dose received by x cc of volume;
- **D*x***: dose received by x % of volume;
- **V*x***: % or cm^3 (cc) of volume receiving x % of PD.

After the extraction from Treatment Planning System Oncentra by Elekta, only some of these parameters were used on univariate analysis and they are represented in table 4.2 in bold. Correlation table together with medical opinion were used to select the parameters. Table 4.3 presents the selected clinical variables and the summarized choices for cut-off points.

Table 4.2: List of DVH parameters

Organs	DVH Parameters
Bladder	**D1cc**, **D2cc**, D5cc, **D25**, D50, **V75**, **V80**, V90, V95, V100, V105, V110
Bladder Neck	**D0.1cc**, **D0.5cc**, D1cc, D2cc, **V80**, V90, V100, V105, V110
Prostatic Urethra Superior/Mid/Inferior	**D0.1cc**, D0.5cc, D1cc, D5, D10, D20, **D30**, V100, V105, **V110**, V120, V150
Membranous Urethra	**D0.1cc**, **D.05cc**, D1cc, D2cc, D5, D10, D15, D20, **D30**, **V100**, V120, V150

Table 4.3: List of cut-off values for clinical parameters.

Clinical Variables	Cut-off Values
TRUS volume*	40 cc
Qmax*	10 or 15 ml/s
IPSS*	10
Urinary residue*	30 ml
Nr. needles	17
PTV volume	50 cc
Urethra length	50 mm
Age	70 years

*Baseline variables

Large Group

The selection of DVH parameters for this group is different. For the delineated structures, the following dosimetric parameters were used: bladder D1cc, D2cc, bladder D10 and D25, urethra D1cc, urethra D1 and D5. The selection of clinical variables equal to small group. These parameters were extracted from *ErasmusMC - Cancer Institute* HDR BT for PCa database.

4.2.4 Statistical Analysis and Missing values

SPSS (version 21) was used for statistical analysis of the data. In this phase, DVH parameters and clinical variables have been selected to evaluate according to different methods.

On the first method (***Method A***), multivariate logistic regression (MVA) analysis was performed including all DVH parameters and clinical parameters with a threshold p-value of ≤ 0.2 on univariate logistic regression. MVA was built using stepwise backward elimination method. This technique consists in including all variables in the model. Then, it analyses each variable individually and if that parameter does not meet the criterion for inclusion, it is eliminated from the model. This procedure continues until all variables have been considered for elimination. The final model contains all of the independent variables that meet the inclusion criteria. P-values ≤ 0.05 were considered statistically significant on MVA.

On the second method (***Method B***), the association between AUR and independent dosimetric and clinical parameters was assessed using Mann-Whitney test for DVH variables and Chi-square/Fisher's exact test for clinical ones. The parameters showing p-values < 0.05 or parameters showing p-value close to assume statistical meaning and/or reported as important factor in previous studies were evaluated on multivariate logistic regression adjusted by the following confounders: IPSS, Age, needles, PTV volume and urinary residue. This approach is based on the method followed by **Roeloffzen et al.** [45].

Cross-validation using receiver operating characteristic (ROC) curve analysis was used to assess how well the found parameters were predicting for AUR. The area under ROC curve (AUC) shows the capability to distinguish no-AUR patients from AUR patients. Additionally, this method was used to confirm the cut-off points investigated in this study. This approach is explained in detail *in chapter 2 section 2.4.*

Qmax missing values in the small group were replaced using different techniques as Median Substitution, Single Imputation using EM and Multiple Imputation. The single imputation using EM and multiple imputation are tools of SPSS. In large group, missing values were replaced by using multiple imputation MCMC.

4.2.5 Statistical Analysis Methodology

The first step of this project was to apply ***Method A***. Therefore, several experiments were done called "**TESTA.**". Those were built according to the results from previous tests. With these tests, different techniques to handle missing values and the inclusion or not of certain parameters into the analysis were investigated. Following, we will summarize and explain the content of each test.

- **TEST.A1:** using original data;
- **TEST.A2:** replacing bladder neck D0.5cc missing values by zero;
- **TEST.A3:** replacing the Qmax missing values by the correspondent group median and using 15 ml/s as Qmax cut-off value;
- **TEST.A4:** replacing the Qmax missing values by the correspondent group median and using 10 ml/s as Qmax cut-off value;
- **TEST.A5:** not replacing the Qmax missing values and using 15 ml/s as Qmax cut-off value;
- **TEST.A6:** not replacing Qmax and using 10 ml/s as Qmax cut-off value;
- **TEST.A7:** replacing Qmax by the global median (overall median) and using 15 ml/s as Qmax cut-off value;
- **TEST.A8:** replacing Qmax by the global median and using 10 ml/s as Qmax cut-off value.

After all these tests and according to the results, bladder D25 was transformed in categorical variable. For this reason more tests were performed.

- **TEST.A9:** testing 30% of PD for bladder D25, replacing Qmax missing values by the global median and using 10 ml/s as Qmax cut-off value.
- **TEST.A10:** testing 30% of PD for bladder D25, replacing Qmax missing values by the correspondent group median and using 10 ml/s as Qmax cut-off value.
- **TEST.A11:** testing 30% of PD for bladder D25, replacing Qmax missing values by the global median and using 15 ml/s as Qmax cut-off value.
- **TEST.A12:** testing 30% of PD for bladder D25, replacing Qmax missing values by the correspondent group median and using 15 ml/s as Qmax cut-off value.
- **TEST.A13:** replacing Qmax missing values by using single imputation EM and using 10 ml/s as Qmax cut-off value;
- **TEST.A14:** using multiple imputation for Qmax missing values and using 10 ml/s as Qmax cut-off value; .
- **TEST.A15:** testing other cut-off values for bladder D25 as 28%, 32%, 35% of PD.

As last, other DVH parameters of bladder neck were analysed.

- **TEST.A16:** Investigating on univariate analysis bladder neck D5, D10, D15, D20 and D30.

The second step on this part was to apply ***Method B***. The main objective of this methodology was to investigate whether the final results will be the same or not using different statistical approach. Like in the previous step, we did some tests which will be described below.

- **TEST.B1:** Applying Chi-square and Mann-Whitney test. According to the results evaluate those parameters on MVA using confounders without replacing the missing values;
- **TEST.B2:** Investigating the cut-off values of parameters showing statistical significance without replacing the Qmax missing values.
- **TEST.B3:** Applying TEST.B1 and TEST.B2 approach but replacing the missing values. The missing values are replaced using MCMC MI.
- **TEST.B4:** Cross validation using ROC curve analysis.

Method A (TEST.A1-TEST.A16) was applied only in small group, while Method B (TEST.B1-TEST.B4) was performed in small and large group.

4.3 Results

In this section, the results related to the small and large group will be reported. Firstly, we will show the results using a small data set and then using a large sample. Within these subsections, the results will be show according to the methodology described in subsection 4.2.5.

4.3.1 Small Group

TEST.A1 + TEST.A2

The first experiment was to use the original data without replacing any missing values. Only variables that achieved p-value ≤ 0.2 are represented in the table 4.4. Other variables were included on univariate analysis but they were not found statistically significant. The complete results are in *Appendix D*.

The next step was to include all variables on multivariate analysis, but because of missing values in some parameters (bladder neck D0.5cc and Qmax), SPSS did not achieve the best model reporting convergence problems (it only use 64.3% of the data). In this way, it was not possible to perform the analysis

Afterwards, the missing values of bladder neck D0.5cc were replaced by 0 and it fixed the convergence problems (TEST.A2). On the other hand, replacing bladder neck D0.5cc by 0 does not make sense because these missing values are not a missing values caused by losing some patient file. In this case, the bladder neck is a small structure and some patients did not have that volume. Therefore, we decided to exclude bladder neck D0.5cc from the analysis because we cannot replace any value even using complex techniques.

The summarized outcome of univariate logistic regression is described in table 4.4.

Table 4.4: Summarized result of TEST.A1.

	Univariate	
Variables	**OR (95%CI)**	**p-value**
Bladder D1cc	1.08 (0.97-1.22)	0.144
Bladder D2cc	1.11 (0.98-1.27)	0.098
Bladder D25	1.25 (1.07-1.45)	0.005*
Bladder V75	3.36 (1.23-9.19)	0.018
Bladder V80	4.99 (1.13-22.03)	0.034
Bladder Neck D0.5cc	1.10 (0.96-1.27)	0.163
PUM V110 (cc)	71.37 (0.61-8354.1)	0.079
UM D0.5cc	1.09 (1.00-1.18)	0.043
UM V100	1.21 (0.99-1.47)	0.063
UM V100 (cc)	1.9E+8 (0.008-4.6E+18)	0.119
TRUS volume $\geq$ 40 cc	4.60 (1.11-19.14)	0.036
Qmax $<$ 15 ml/s	3.61 (0.79-16.35)	0.096
IPSS $\geq$ 10	3.33 (0.73-15.27)	0.121
Needles $\geq$ 17	3.67 (0.84-16.04)	0.084
PTV volume $\geq$ 50 cc	2.89 (0.73-11.43)	0.132

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

TEST.A3

In order to solve the convergence problems, Qmax missing values were replaced. The Qmax of the CAD group showed a skewed distribution and for that reason the replacements were made using median rather than mean. However, the Qmax of no-CAD showed a Gaussian distribution that means the median is roughly equal to the mean.Therefore, the median was used for both groups. Qmax missing values are distributed in this way: 1 missing value in CAD group that was replaced by 9.3 and 3 missing values in no-CAD that were replaced by 16.6. This technique allowed to run the univariate and multivariate analysis without convergence problems. Applying this method, bladder D25, Qmax $<$ 15 ml/s and IPSS $\geq$ 10 were statistically significant (see table 4.5). In other words, those variables might be related to the need of a bladder catheter after treatment due to AUR.

Table 4.5: Summarized result of TEST.A3.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.677	-
Bladder D2cc	0.098	0.691	-
Bladder D25	0.005	0.006*	1.32 (1.084-1.603)
Bladder V75	0.018	0.768	-
Bladder V80	0.034	0.807	-
PUM V110 (cc)	0.079	0.351	-
UM D0.5cc	0.043	0.333	-
UM V100	0.063	0.104	-
UM V100 (cc)	0.119	0.515	-
TRUS volume $\geq$ 40 cc	0.036	0.667	-
Qmax $<$ 15 ml/s	0.036	0.039*	39.82 (1.20 -1325.86)
IPSS $\geq$ 10	0.121	0.021*	74.11 (1.91-2879.46)
Needles $\geq$ 17	0.084	0.104	-
PTV volume $\geq$ 50 cc	0.132	0.323	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

TEST.A4

In this test, the usage of another cut-off value for Qmax was investigated, namely 10ml/s. The same technique in TEST.A3 to work with missing values was applied. As such in previous test, there were no convergence problems. Using Qmax cut-off as 10 ml/s, multivariate method provided bladder D25, Qmax $<$ 10ml/s and IPSS $\geq$ 10 as important predictors. Furthermore, the Qmax $<$ 10 ml/s p-value was smaller than Qmax $<$ 15 ml/s p-value. Therefore, Qmax $<$ 10 ml/s seems to be stronger related to AUR than with Qmax $<$ 15 ml/s. The results are presented in table 4.6.

Table 4.6: Summarized result of TEST.A4.

	Univariate	Multivariate	
Variables	**p-value**	**p-value**	**OR (95%CI)**
Bladder D1cc	0.144	0.427	-
Bladder D2cc	0.098	0.362	-
Bladder D25	0.005	0.006*	1.34 (1.09-1.66)
Bladder V75	0.018	0.702	-
Bladder V80	0.034	0.780	-
PUM V110 (cc)	0.079	0.105	-
UM D0.5cc	0.043	0.336	-
UM V100	0.063	0.217	-
UM V100 (cc)	0.119	0.264	-
TRUS volume $\geq$ 40 cc	0.036	0.221	-
Qmax $<$ 10 ml/s	0.025	0.020*	10.08 (1.44 – 70.54)
IPSS $\geq$ 10	0.121	0.021*	16.73 (1.53 – 183.29)
Needles $\geq$ 17	0.084	0.209	-
PTV volume $\geq$ 50 cc	0.132	0.624	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

TEST.A5 + TEST.A6

In TEST.A5, univariate and multivariate analysis were performed using the dataset without Qmax missing value replacements. SPSS did not report convergence problems and did not find any statistically significant variables either. SPSS used only 90.5% of the data and because of the small sample size losing patients has an important impact on results. In TEST.A6, only Qmax cut-off value was changed to 10 ml/s. The MVA output reported again statistically significant variables (bladder D25 and IPSS $\geq$ 10). The result are presented in table 4.7.

Table 4.7: Summarized result of TEST.A6.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.479	-
Bladder D2cc	0.098	0.412	-
Bladder D25	0.005	0.006*	1.33 (1.08-1.63)
Bladder V75	0.018	0.674	-
Bladder V80	0.034	0.759	-
PUM V110 (cc)	0.079	0.164	-
UM D0.5cc	0.043	0.360	-
UM V100	0.063	0.218	-
UM V100 (cc)	0.119	0.259	-
TRUS volume $\geq$ 40 cc	0.036	0.259	-
Qmax $<$ 10 ml/s	0.072	0.071	-
IPSS $\geq$ 10	0.121	0.044*	19.12 (1.08-338.01)
Needles $\geq$ 17	0.084	0.288	-
PTV volume $\geq$ 50 cc	0.132	0.820	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

TEST.A7 + TEST.A8

In these tests, Qmax missing values were replaced by the overall median (13.25) instead of by the correspondent group median. In TEST.A7 and TEST.A8 (see tables 4.8 and 4.9, respectively), Qmax cut-off value 15 ml/s and 10 ml/s was used, respectively. Once again, when the Qmax $<$ 10 ml/s was tested, the results recovered outcome obtained in TEST.A3.

Table 4.8: Summarized result of TEST.A7.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.611	-
Bladder D2cc	0.098	0.626	-
Bladder D25	0.005	0.008*	1.31 (1.07-1.60)
Bladder V75	0.018	0.697	-
Bladder V80	0.034	0.770	-
PUM V110 (cc)	0.079	0.210	-
UM D0.5cc	0.043	0.606	-
UM V100	0.063	0.165	-
UM V100 (cc)	0.119	0.833	-
TRUS volume $\geq$ 40 cc	0.036	0.624	-
Qmax $<$ 15 ml/s	0.125	0.113	-
IPSS $\geq$ 10	0.121	0.029*	15.76 (1.34-186.05)
Needles $\geq$ 17	0.084	0.081	-
PTV volume $\geq$ 50 cc	0.132	0.427	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

Table 4.9: Summarized result of TEST.A8.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.419	-
Bladder D2cc	0.098	0.333	-
Bladder D25	0.005	0.007*	1.31 (1.08-1.59)
Bladder V75	0.018	0.736	-
Bladder V80	0.034	0.807	-
PUM V110 (cc)	0.079	0.092	-
UM D0.5cc	0.043	0.265	-
UM V100	0.063	0.211	-
UM V100 (cc)	0.119	0.246	-
TRUS volume $\geq$ 40 cc	0.036	0.131	-
Qmax $<$ 10 ml/s	0.066	0.044*	6.83 (1.05-44.45)
IPSS $\geq$ 10	0.121	0.019*	18.34 (1.60-209-99)
Needles $\geq$ 17	0.084	0.134	-
PTV volume $\geq$ 50 cc	0.132	0.444	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

TEST.A9 + TEST.A10

In both these test, the first experiment was to transform the bladder D25 in a categorical variable. It is interesting to know what is the dose threshold that might be associated to the needing of bladder catheter after treatment. The cut-off value (30% of PD) for bladder D25 was chosen according to medical experience and data distribution through that variable. In both tests, 10 ml/s was used as cut-off value for Qmax. The difference between tests is the technique to work with missing values. In TEST.A9 and TEST.A10, the missing values were replaced using global median and subgroup median techniques, respectively. Bladder D25 $\geq$ 30% only showed p $\leq$ 0.05 in TEST.A10. The outcome of these tests are shown in table 4.10 and 4.11.

Table 4.10: Summarized result of TEST.A9.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.737	-
Bladder D2cc	0.098	0.855	-
Bladder D25 $\geq$ 30% of PD	0.023	0.148	-
Bladder V75	0.018	0.022*	5.61 (1.29-24.41)
Bladder V80	0.034	0.199	-
PUM V110 (cc)	0.079	0.056	-
UM D0.5cc	0.043	0.266	-
UM V100	0.063	0.163	-
UM V100 (cc)	0.119	0.225	-
TRUS volume $\geq$ 40 cc	0.036	0.103	-
Qmax $<$ 10 ml/s	0.066	0.027*	8.44 (1.27-56.22)
IPSS $\geq$ 10	0.121	0.026*	13.59 (1.37-134.76)
Needles $\geq$ 17	0.084	0.152	-
PTV volume $\geq$ 50 cc	0.132	0.604	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

Table 4.11: Summarized result of TEST.A10.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.264	-
Bladder D2cc	0.098	0.275	-
Bladder D25 $\geq$ 30% of PD	0.023	0.031*	26.73 (1.35-529.48)
Bladder V75	0.018	0.321	-
Bladder V80	0.034	0.438	-
PUM (cc)	0.079	0.050	-
UM D0.5cc	0.043	0.675	-
UM V100	0.063	0.141	-
UM V100 (cc)	0.119	0.374	-
TRUS volume $\geq$ 40 cc	0.036	0.561	-
Qmax $<$ 10 ml/s	0.025	0.023*	13.13 (1.43-120.85)
IPSS $\geq$ 10	0.121	0.013*	53.48 (2.35-1217.46)
Needles $\geq$ 17	0.084	0.191	-
PTV volume $\geq$ 50 cc	0.132	0.870	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

TEST.A11 + TEST.A12

The procedure for bladder D25, in TEST.A11 and TEST.A12, was equal to the previous test, but Qmax cut-off was changed to 15 ml/s. Based on these results and according to TEST.A3 + TEST.A4 and TEST.A7 + TEST.A8 results, Qmax $<$ 10 ml/s seems to be a better risk factor for CAD due to AUR than Qmax $<$ 15 ml/s.

Table 4.12 and 4.13 summarize the results in this stage.

Table 4.12: Summarized result of TEST.A11.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.279	-
Bladder D2cc	0.098	0.284	-
Bladder D25 $\geq$ 30% of PD	0.023	0.029*	20.26 (1.36-301.85)
Bladder V75	0.018	0.289	-
Bladder V80	0.034	0.419	-
PUM V110 (cc)	0.079	0.050	-
UM D0.5cc	0.043	0.978	-
UM V100	0.063	0.129	-
UM V100 (cc)	0.119	0.858	-
TRUS volume $\geq$ 40cc	0.036	0.882	-
Qmax $<$ 15 ml/s	0.125	0.169	-
IPSS $\geq$ 10	0.121	0.028*	32.98 (1.46-744.60)
Needles $\geq$ 17	0.084	0.090	-
PTV volume $\geq$ 50 cc	0.132	0.790	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

Table 4.13: Summarized result of TEST.A12.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.894	-
Bladder D2cc	0.098	0.953	-
Bladder D25 $\geq$ 30% of PD	0.023	0.364	-
Bladder V75	0.018	0.021*	5.03 (1.28-19.77)
Bladder V80	0.034	0.296	-
PUM V110 (cc)	0.079	0.112	-
UM D0.5cc	0.043	0.730	-
UM V100	0.063	0.210	-
UM V100 (cc)	0.119	0.467	-
TRUS volume $\geq$ 40 cc	0.036	0.284	-
Qmax $<$ 15 ml/s	0.036	0.044*	23.35 (1.09-501.17)
IPSS $\geq$ 10	0.121	0.063	-
Needles $\geq$ 17	0.084	0.137	-
PTV volume $\geq$ 50 cc	0.132	0.854	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

TEST.A13

In this test, the usage of a more complex technique to handle with missing values was investigated, namely, single imputation using EM. First of all, Qmax was tested if it would be classified as MCAR and SPSS software has one test to do that. The MCAR test outcome was: Chi-Square = 8.729 and p-value = 0.366; that means the assumption of MCAR was met because p-value > 0.05. To impute the missing values, this technique uses the relationships between variables and because of that assumption the complete list of clinical variables to impute missing values on Qmax was used. Afterwards, univariate and multivariate logistic regression were applied. In this test, bladder D25 $\geq$ 30% of PD, Qmax $<$ 10 ml/s and IPSS were statistically significant and this result is in agreement with the TEST.A8 and TEST.A9 (see table 4.14).

Table 4.14: Summarized result of TEST.A13.

	Univariate	Multivariate	
Variables	**p-value**	**p-value**	**OR (95%CI)**
Bladder D1cc	0.144	0.223	-
Bladder D2cc	0.098	0.231	-
Bladder D25 $\geq$ 30% of PD	0.023	0.029*	29.56 (1.41-621.51)
Bladder V75	0.018	0.277	-
Bladder V80	0.034	0.380	-
PUM V110 (cc)	0.079	0.056	-
UM D0.5cc	0.043	0.757	-
UM V100	0.063	0.155	-
UM V100 (cc)	0.119	0.456	-
TRUS volume $\geq$ 40 cc	0.036	0.426	-
Qmax $<$ 10 ml/s	0.046	0.048*	8.71 (1.02-74.46)
IPSS $\geq$ 10	0.121	0.016*	33.84 (1.91-600.55)
Needles $\geq$ 17	0.084	0.115	-
PTV volume $\geq$ 50 cc	0.132	0.682	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

TEST.A14

Multiple imputation like single imputation can only be used when the variables with missing values meet MCAR test and previous test confirmed that. Multiple Imputation has more than one technique to impute the missing values and Automatic and MCMC method were used in this test. Those methods are described in chapter 2 section 2.3. In both methods 5 iterations were used. Therefore, in TEST.A14 both method of multiple imputation were under investigation. The automatic method outcome showed bladder V75, Qmax $<$ 10 ml/s and IPSS as statistically significant and bladder D25 $\geq$ 30% of PD lost its significance. The summarized result is shown in table 4.15.

Table 4.15: Summarized result of Automatic MI.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.737	-
Bladder D2cc	0.098	0.855	-
Bladder D25 $\geq$ 30% of PD	0.023	0.148	-
Bladder V75	0.018	0.022*	5.61 (1.29-24.41)
Bladder V80	0.034	0.199	-
PUM V110 (cc)	0.079	0.056	-
UM D0.5cc	0.043	0.266	-
UM V100	0.063	0.163	-
UM V100 (cc)	0.119	0.225	-
TRUS volume $\geq$ 40 cc	0.036	0.103	-
Qmax $<$ 10 ml/s	0.066	0.027*	8.44 (1.27-56.22)
IPSS $\geq$ 10	0.121	0.026*	13.59 (1.37-134.76)
Needles $\geq$ 17	0.084	0.152	-
PTV volume $\geq$ 50 cc	0.132	0.604	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

The MCMC MI output showed again bladder D25 $\geq$ 30% of PD, Qmax $<$ 10 ml/s and IPSS $\geq$ 10 as factors associated with AUR, in accordance with TEST.A8 and TEST.A10. This result is shown in table 4.16.

Table 4.16: Summarized result of MCMC MI.

Variables	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144	0.223	-
Bladder D2cc	0.098	0.231	-
Bladder D25 $\geq$ 30% of PD	0.023	0.029*	29.56 (1.41-621.51)
Bladder V75	0.018	0.277	-
Bladder V80	0.034	0.380	-
PUM V110 (cc)	0.079	0.056	-
UM D0.5cc	0.043	0.757	-
UM V100	0.063	0.155	-
UM V100 (cc)	0.119	0.456	-
TRUS volume $\geq$ 40 cc	0.036	0.426	-
Qmax $<$ 10 ml/s	0.066	0.048*	8.71 (1.02-74.46)
IPSS $\geq$ 10	0.121	0.016*	33.84 (1.91-600.55)
Needles $\geq$ 17	0.084	0.115	-
PTV volume $\geq$ 50 cc	0.132	0.682	-

Abbreviations: * Statistically significant;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

Table 4.17 will summarize the result of different methods to impute missing values. MCMC MI is the most suitable method to impute missing values. However, median group substitution seems to be a simple method to replace few missing values in small datasets.

Table 4.17: Comparison between Imputation Methods.

Multivariate Logistic Regression					
Method / **Variables**	**Subgroup median p-value**	**Overall median p-value**	**Single Impuatation p-value**	**Automatic MI p-value**	**MCMC MI p-value**
Bladder D25 $\geq$ 30% PD	0.031	-	0.029	-	0.029
Qmax <10 ml/s	0.023	0.027	0.048	0.027	0.048
IPSS $\geq$ 10	0.013	0.026	0.016	0.026	0.016
Bladder V75	-	0.022	-	0.022	-

TEST.A15

According to the previous tests, bladder D25 $\geq$ 30% of PD is an important parameter associated with CAD due to AUR. Therefore, other cut-off values were evaluated, such as 28% of PD and 32% of PD. The result was not expected: bladder D25 $\geq$ 28% and 32% of PD lost the significance and other bladder parameter (bladder V75 (% of PD)) pops-up (see in table 4.18). One possible interpretation is the population size. When the cut-off value was changed, one or two patients switched over the group, that means small changes in our dataset resulting in big effects on final outcome.

Table 4.18: Summarized result of TEST.A15.

	Univariate (p-value)	**Multivariate (p-value)**
Bladder D25 $\geq$ 28% PD	0.075	0.277 –> bladder V75 (p=0.016)
Bladder D25 $\geq$ 30% PD	0.023	0.029
Bladder D25 $\geq$ 32% PD	0.034	0.750 –> bladder V75 (p=0.016)

TEST.A16

In TEST.A1, one parameter of bladder neck appeared statistically significant on univariate method but it was excluded from analysis because that variable contained many missing values. In this TEST.A16, other DVH parameters were analysed in order to assess whether there is some relationship between AUR and dose in bladder neck or not. On univariate method the following parameters were investigated: bladder neck D5, D10, D15, D20 and D30.

In this test, bladder neck was not associated with CAD due to AUR after treatment and the statistical significance observed in the first test (TEST.A1) was thus a reflective of missing values. The result is shown in table 4.19.

Table 4.19: Summarized result of TEST.A16.

	Univariate (p-value)
Bladder Neck D5	0.333
Bladder Neck D10	0.314
Bladder Neck D15	0.367
Bladder Neck D20	0.379
Bladder Neck D25	0.407

The following TESTS are related to results from application of a different statistical analysis methodology: ***Method B*** (see subsection 4.2.5).

TEST.B1

In this test, the association between CAD due to AUR and clinical parameters was assessed using Chi-square test or Fisher's exact test. When the assumption of expected counts for Chi square (see 2.1.1) is broken the result is a radical reduction in test power and the solution is the Fisher's exact test. In this test, the Qmax missing values were not replaced. The outcome is shown in table 4.20.

Table 4.20: Result of Chi-Square on TEST.B1.

Clinical Parameters	CAD (n=14)	no-CAD (n =28)	p-value
Qmax* **			0.084*
$<$ 10 ml/s	7 (50%)	6 (21%)	
$\geq$ 10 ml/s	6 (43%)	19 (73%)	
IPSS			0.113*
$<$ 10	9 (64%)	24 (86%)	
$\geq$ 10	5 (36%)	4 (14%)	
Age (years)			0.662**
$\geq$ 70	6 (43%)	14 (50%)	
$<$ 70	8 (57%)	14 (50%)	
PTV volume			0.125**
$\geq$50 cc	10 (71%)	13 (46%)	
$<$ 50 cc	4 (29%)	15 (54%)	
Needles			0.075**
$\geq$17	11 (79%)	14 (50%)	
$<$ 17	3 (21%)	14 (50%)	
Urinary residue			0.827**
$\geq$ 30 ml	7 (50%)	13 (46%)	
$<$30 ml	7 (50%)	15 (54%)	
Urethra length			0.650*
$\geq$ 50 mm	13 (93%)	24 (86%)	
$<$ 50 mm	1 (7%)	4 (4%)	
TRUS volume			0.067*
$\geq$ 40 cc	7 (50%)	5 (18%)	
$<$ 40 cc	7 (50%)	23 (82%)	

Abbreviations:
CAD = patients with bladder catheter;
* According to Fisher's exact test;
** According to Chi-square test;
*** 4 missing values: 1 case and 3 controls.

Then, Mann-Whitney test was applied in order to investigate the relationship between DVH parameters and CAD due to AUR. The outcome of this test is present in table 4.21.

Table 4.21: Result of Mann-Whitney on TEST.B1.

DVH Parameters	CAD (n=14) Median	no-CAD (n=28) Median	p-value
Bladder D1cc (% of PD)	73.87	71.36	0.023*
Bladder D2cc (% of PD)	68.78	65.26	0.020*
Bladder D25 (% of PD)	37.00	29.60	0.002*
Bladder V75 (%)	1.08	0.45	0.012*
Bladder V80 (%)	0.42	0.09	0.017*
Bladder V75 (cc)	0.84	0.53	0.040*
Bladder V80 (cc)	0.38	0.14	0.038*
Bladder Neck D0.1cc (% of PD)	75.42	74.48	0.308
Bladder Neck V80 (%)	1.29	0.69	0.362
Bladder Neck V80 (cc)	0.02	0.01	0.272
PUS D0.1cc (% of PD)	113.29	113.52	0.864
PUS D30 (% of PD)	111.67	112.54	0.518
PUS V110 (%)	42.70	47.20	0.452
PUS V110 (cc)	0.24	0.23	0.535
PUM D0.1cc (% of PD)	115.50	115.35	0.553
PUM D30 (% of PD)	114.05	114.37	0.968
PUM V110 (%)	91.42	93.33	0.989
PUM V110 (cc)	0.49	0.44	0.101
PUI D0.1cc (% of PD)	112.85	112.99	0.683
PUI D30 (% of PD)	111.52	111.47	0.927
PUI V110 (%)	46.82	47.85	0.762
PUI V110 (cc)	0.29	0.23	0.452
UM D0.1cc (% of PD)	90.11	89.19	0.782
UM D0.5cc (% of PD)	57.31	52.71	0.056
UM D30 (% of PD)	67.49	66.23	0.452
UM V100 (%)	0.98	0.86	0.947
UM V100 (cc)	0.01	0.01	0.702

Abbreviations:
* Statistically significant; CAD = patients with bladder catheter;
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra Mid;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra .

Clinical parameters, such as Qmax and DVH parameters with p-values ≤ 0.5 or close to that value, were selected to evaluate their relationship with CAD due to AUR using univariate and multivariate logistic regression. To investigate whether these parameters are independent factors or not, multivariate logistic regression was performed adjusted by the following confounder: IPSS, age, needles, PTV volume and urinary residue. Qmax < 10 ml/s, bladder D25 and UM D0.5cc retained their significance after performing MVA. However, only the ORs of bladder D25 and UM D0.5cc did not change substantially after adjustment for the confounders indicating that there was no confounding, while the ORs of Qmax < 10 ml/s changed. The result of this test is shown in table 4.22.

Table 4.22: UVA and MVA result of TEST.B1.

	UVA		MVA$^{\gamma}$	
Parameters	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Qmax $<$ 10 ml/s	3.69(0.88-15.37)	0.072	6.06 (1.18-31.1)	0.031*
Bladder D2cc	1.11 (0.98-1.27)	0.098	-	0.075
Bladder D25	1.25 (1.07-1.45)	0.005	1.34 (1.01-1.63)	0.004*
Bladder V75 (cc)	-	0.260	-	-
Bladder V80 (cc)	-	0.371	-	-
UM D0.5cc	1.09 (1.00-1.181)	0.043	1.17 (1.02-1.34)	0.029*

Abbreviations:
* Statistically significant; OR = Odd ratio; 95% CI = 95% Confidence interval;
γ Ajusted by IPSS, needles, age, urinary residue and PTV volume.

TEST.B2

In accordance with the results, it is interesting to know what the dose threshold is for bladder D25 and membranous urethra D0.5cc, like was performed in ***Method A***. Chi-square/ Fisher's exact test was applied to assess the relationship between CAD due to AUR and different cut-off values for both parameters. Bladder D25 $\geq$ 30% and 35% of PD and membranous urethra D0.5cc $\geq$ 55% of PD were statistically significant, but only the ORs of bladder D25 $\geq$ 35% of PD did not change substantially after adjustment for the confounders indicating that there was no confounding. The outcomes are presented in tables 4.23 and 4.24.

Table 4.23: Result of bladder D25 cut-off points.

cut-off values			UVA		MVA$^{\gamma}$	
Bladder D25	**p-value**	**Parameters**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
40% of PD	0.032*	Bladder D25 $\geq$ 40% of PD	-	0.999		-
35% of PD	0.036*	Bladder D25 $\geq$ 35% of PD	4.89 (1.22-19.65)	0.025	5.13 (1.18-22.28)	0.029
30% of PD	0.014**	Bladder D25 $\geq$ 30% of PD	6.92 (1.30-36.82)	0.023	18.09 (1.75-187.3)	0.015
25% of PD	0.283*	-	-	-	-	-

Abbreviations:* According to Fisher's exact test; ** According to Chi-square test; OR = Odd ratio;
95% CI = 95% Confidence interval; γ Adjusted for IPSS, needles, age, urinary residue and PTV volume.

Table 4.24: Result of membranous urethra D0.5cc cut-off points.

cut-off values			UVA		MVA$^{\gamma}$	
UM D0.5cc	**p-value**	**Parameters**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
40% of PD	0.545*	-	-	-		-
45% of PD	0.233*	-	-	-		-
50% of PD	0.495**	-	-	-		-
55% of PD	0.013**	UM D00.5cc $\geq$ 55% of PD	5.40 (1.35-21.64)	0.017	20.65 (2.21-192.74)	0.008
60% of PD	0.197*	UM D00.5cc $\geq$ 60% of PD	-	0.157		-
65% of PD	0.100*	UM D00.5cc $\geq$ 65% of PD	-	0.099		-

Abbreviations:* According to Fisher's exact test; ** According to Chi-square test; OR = Odd ratio;
95% CI = 95% Confidence interval; γ Adjusted for IPSS, needles, age, urinary residue and PTV volume.

TEST.B3

In this test, the same method of TEST.B1 was applied, but the Qmax missing values were replaced using MCMC MI. Therefore, Chi-square was performed for clinical parameters. The Mann-Whitney test is exactly the same that was reported in TEST.B1. In this stage, the cut-off value analysis (bladder and membranous

urethra) do not change because the confounders do not have missing values. No considerable differences were registered and the results and conclusions are in agreement with TEST.B1.

Table 4.25: Result of Chi-Square on TEST.B3.

Clinical Parameters	CAD (n=14)	no-CAD (n =28)	p-value
Qmax			0.040**
$<$ 10 ml/s	8 (57%)	7 (25%)	
$\geq$ 10 ml/s	6 (43%)	21 (75%)	
IPSS			0.113*
$<$ 10	9 (64%)	24 (86%)	
$\geq$ 10	5 (36%)	4 (14%)	
Age (years)			0.662**
$\geq$ 70	6 (43%)	14 (50%)	
$<$ 70	8 (57%)	14 (50%)	
PTV volume			0.125**
$\geq$50 cc	10 (71%)	13 (46%)	
$<$ 50 cc	4 (29%)	15 (54%)	
Needles			0.075**
$\geq$ 17	11 (79%)	14 (50%)	
$<$ 17	3 (21%)	14 (50%)	
Urinary residue			0.827**
$\geq$ 30 ml	7 (50%)	13 (46%)	
$<$ 30 ml	7 (50%)	15 (54%)	
Urethra length			0.650*
$\geq$ 50 mm	13 (93%)	24 (86%)	
$<$ 50 mm	1 (7%)	4 (4%)	
TRUS volume			0.067*
$\geq$ 40 cc	7 (50%)	5 (18%)	
$<$ 40 cc	7 (50%)	23 (82%)	

Abbreviations:
CAD = patients with bladder catheter;
* According to Fisher's exact test;
** According to Chi-square test.

Table 4.26: UVA and MVA result of TEST.B3.

	UVA		MVA$^{\gamma}$	
Parameters	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Qmax $<$ 10 ml/s	3.26 (0.76-14.01)	0.112	6.07 (1.28-28.81)	0.023*
Bladder D2cc	1.11 (0.98-1.27)	0.098	-	0.075
Bladder D25	1.25 (1.07-1.45)	0.005	1.34 (1.01-1.63)	0.004*
Bladder V75 (cc)	-	0.260	-	-
Bladder V80 (cc)	-	0.371	-	-
UM D0.5cc	1.09 (1.00-1.181)	0.043	1.17 (1.02-1.34)	0.029*

Abbreviations:
* Statistically significant; OR = Odd ratio; 95% CI = 95% Confidence interval;
γ Adjusted for IPSS, needles, age, urinary residue and PTV volume.

TEST.B4

In this test, we evaluated the accuracy of our results and tested the validity of the selected median cut-off values for the statistical significant parameters: bladder D25, membranous urethra D0.5cc and Qmax. Table 4.27 shows the AUC for each parameter and figure 4.2 presents the ROC curves. The cut-off points were obtained through the coordinates points of the plot where the sensitivity is equal to the specificity (see table 4.28). The result of this test confirm the validity of the cut-off points. Therefore, our analysis predict the increased risk of AUR better than by chance.

Table 4.27: AUC analyses for each statistical significant parameter.

	AUC	Std.Error	Asymptotic Sig.	95% CI
Qmax	0.65	0.10	0.120	0.464-0.844
Qmax*	0.68	0.09	0.090	0.504-0.856
Bladder D25	0.79	0.07	0.003	0.644-0.933
UM D0.5cc	0.68	0.09	0.050	0.501-0.866

Notes: * missing values replaced by MCMC MI.

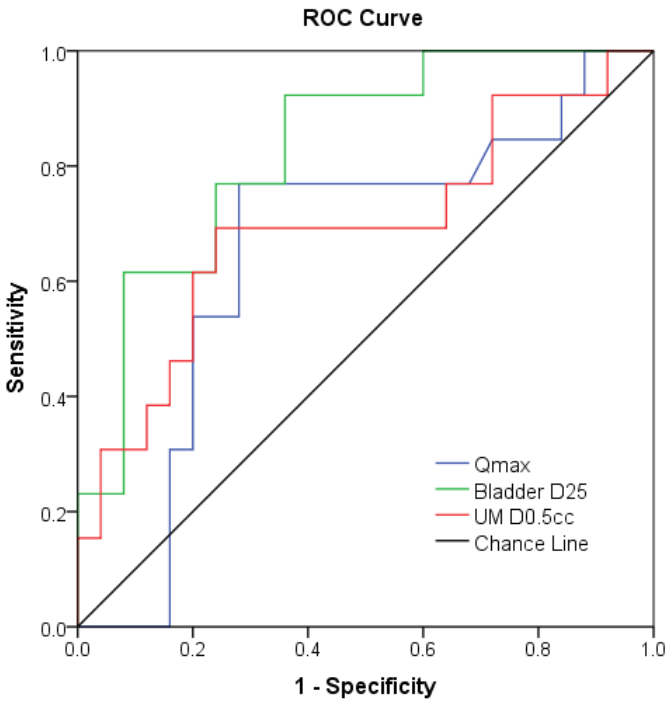


Figure 4.2: ROC curves for Qmax, bladder D25, membranous urethra D0.5cc.

Table 4.28: Comparison between ROC optimal cut-off points and previous cut-off points (TEST.B2).

	Sensitivity	Specificity	Cut-off point	Cut-off point (TEST.B2)
Qmax	0.77	0.72	12.6 ml/s	10 ml/s
Bladder D25	0.71	0.71	32.5% of PD	30/35% of PD
UM D0.5cc	0.64	0.64	54.5% of PD	55% of PD

4.3.2 Large Group

According to the results achieved in the previous section and according to statistical guidelines, only the ***Method B*** in this data set was applied .

First of all, in this dataset, there are more missing values and those are shown in table 4.29.

Table 4.29: Missing values distribution by variables.

Missing values	N	Percent
Qmax	54	25.70%
IPSS	42	20.00%
Age	1	0.50%
PTV volume	0	0.00%
Needles	0	0.00%
Urinary residue	43	20.50%
Urethra length	2	1.00%
TRUS volume	22	10.50%

TEST.B1

Firstly, the Mann-Whitney test was applied in order to evaluate the association between CAD due to AUR and dosimetric parameters (table 4.30). Then, clinical parameters were evaluated by using Chi-square test or Fisher's exact. The results are shown in table 4.31. Clinical and dosimetric variables with p-values $<$ 0.05 were considered statistically significant to include on univariate and multivariate analysis. In this case, Qmax, bladder D25, bladder D10 were investigated in UVA and MVA adjusted for the following confounders: IPSS, Age, needles, urinary residue and PTV volume. Qmax $<$ 10 ml/s, bladder D25 and bladder D10 are shown to be independent risk factor for CAD due to AUR after treatment. The outcomes are displayed in table 4.32.

Table 4.30: Result of Mann-Whitney test on TEST.B1.

DVH Parameters	CAD Median	no-CAD Median	p-value
Bladder D1cc (% of PD)	76.79	74.21	0.174
Bladder D2cc (% of PD)	70.56	68.39	0.091
Bladder D25 (% of PD)	36.12	32.79	0.026*
Bladder D10 (% of PD)	51.15	47.98	0.012*
Bladder V75 (cc)	1.11	0.92	0.262
Bladder V80 (cc)	0.65	0.35	0.286
Urethra D1cc (% of PD)	109.59	109.17	0.543
Urethra D1 (% of PD)	117.88	119.27	0.098
Urethra D5 (% of PD)	116.95	116.22	0.908
Urethra V100 (cc)	0.95	0.95	0.700
Urethra V120 (cc)	0.01	0.01	0.227

Abbreviations: * Statistically significant;
CAD = patients with bladder catheter.

Table 4.31: Result of Chi-Square on TEST.B1.

Clinical Parameters	CAD (n=14)	no-CAD (n =196)	p-value
Qmax			0.017*
$<$ 10 ml/s	7 (50%)	31 (16%)	
$\geq$ 10 ml/s	6 (43%)	112 (57%)	
IPSS			0.528*
$<$ 10	9 (64%)	114 (58%)	
$\geq$ 10	5 (36%)	40 (20%)	
Age (years)			0.715**
$\geq$ 70	6 (43%)	74 (38%)	
$<$ 70	8 (57%)	121 (62%)	
PTV volume			0.564**
$\geq$50 cc	10 (71%)	125 (64%)	
$<$ 50 cc	4 (29%)	71 (36%)	
Needles			0.133**
$\geq$17	11 (79%)	114 (58%)	
$<$ 17	3 (21%)	82 (42%)	
Urinary residue			0.870**
$\geq$ 30 ml	7 (50%)	73 (37%)	
$<$ 30 ml	7 (50%)	80 (41%)	
Urethra length			1.00*
$\geq$ 50 mm	13 (93%)	172 (88%)	
$<$ 50 mm	1 (7%)	23 (12%)	
TRUS volume			0.134*
$\geq$ 40 cc	7 (50%)	51 (26%)	
$<$ 40 cc	7 (50%)	123 (78%)	

Abbreviations:
CAD = patients with bladder catheter;
* According to Fisher's exact test;
** According to Chi-square test.

Table 4.32: UVA and MVA results of TEST.B1.

	UVA		MVA$^{\gamma}$	
Parameters	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
Qmax $<$ 10 ml/s	4.22 (1.32-13.46)	0.015	4.62 (1.42-15.01)	0.011*
Bladder D25	1.01 (1.00-1.02)	0.019	1.01 (1.00-1.02)	0.017*
Bladder D10	1.01 (1.00-1.02)	0.019	1.01 (1.00-1.02)	0.019*

Abbreviations:
* Statistically significant; OR = Odd ratio; 95% CI = 95% Confidence interval;
γ Ajusted by IPSS, needles, age, urinary residue and PTV volume.

TEST.B2

According to the results in the previous test, cut-off values for bladder D25 and bladder D10 were analysed. That relationship was assessed by using chi-square or Fisher's exact test and afterwards the UVA and MVA were performed to investigate possible confounders. Bladder D25 $\geq$ 30% of PD only showed a tendency to correlate with CAD due to AUR. Regarding to bladder D10, only the cut-off value $\geq$ 50% PD retained the significance after MVA adjusted for the confounders as independent factor because its OR did not

change substantially. The outcomes for bladder D25 and D10 are shown in tables 4.33 and 4.34, respectively.

Table 4.33: Results of bladder D25 cutt-off points.

cut-off values			UVA		MVA$^{\gamma}$	
Bladder D25	**p-value**	**Parameters**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
40% of PD	0.093*	Bladder D25 $\geq$ 40% of PD	3.53 (0.88-14.12)	0.075	-	0.126
35% of PD	0.122**	Bladder D25 $\geq$ 35% of PD	2.33 (0.77-6.98)	0.131	-	0.173
30% of PD	0.145**	Bladder D25 $\geq$ 30% of PD	3.51 (0.76-16.14)	0.106	4.00 (0.86-18.58)	0.077
25% of PD	0.224*	Bladder D25 $\geq$ 25% of PD	-	0.998	-	-

Abbreviations:* According to Fisher's exact test; ** According to Chi-square test; OR = Odd ratio; 95% CI = 95% Confidence interval; γ Adjusted for IPSS, needles, age, urinary residue and PTV volume.

Table 4.34: Results of bladder D10 cutt-off points.

cut-off values			UVA		MVA$^{\gamma}$	
Bladder D10	**p-value**	**Parameters**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
35% of PD	0.371*	-	-	-	-	-
40% of PD	0.077*	-	-	-	-	-
45% of PD	0.179**	Bladder D10 $\geq$ 45% of PD	2.39 (0.65-8.85)	0.191	-	-
50% of PD	0.038**	Bladder D10 $\geq$ 50% of PD	3.14 (1.01-9.75)	0.047	3.26 (1.03-10.30)	0.044
55% of PD	0.039**	Bladder D10 $\geq$ 55% of PD	4.18 (1.19-14.80)	0.026	3.44 (0.93-12.70)	0.064

Abbreviations:* According to Fisher's exact test; ** According to Chi-square test; OR = Odd ratio; 95% CI = 95% Confidence interval; γ Adjusted for IPSS, needles, age, urinary residue and PTV volume.

TEST.B3

In this test, missing values were replaced by using MCMC MI. Qmax, IPSS and urinary residue were the variables with a large amount of missing values and both of them were used in statistical analysis. Mann-Whitney test did not change because those variables do not have missing values. Only Qmax met the p-value < 0.05 (see table 4.35). After this test, Qmax, bladder D25 and bladder D10 were investigated on univariate and multivariate method adjusted for the confounders. Both bladder D25, D10 and Qmax showed a relationship as independent factors with CAD due to AUR after treatment. Qmax $<$ 10 ml/s was the most significant predictor of this side effect in this dataset.

Table 4.35: Result of Chi-Square on TEST.B3.

Clinical Parameters	CAD (n=14)	no-CAD (n =196)	p-value
Qmax			0.008*
$<$ 10 ml/s	8 (57%)	44 (22%)	
$\geq$ 10 ml/s	6 (43%)	152 (78%)	
IPSS			0.531*
$<$ 10	9 (64%)	149 (71%)	
$\geq$ 10	5 (36%)	61 (29%)	
Age (years)			0.704**
$\geq$ 70	6 (43%)	74 (38%)	
$<$ 70	8 (57%)	122 (62%)	
PTV volume			0.564**
$\geq$50 cc	10 (71%)	125 (64%)	
$<$ 50 cc	4 (29%)	71 (36%)	
Needles			0.133**
$\geq$ 17	11 (79%)	114 (58%)	
$<$ 17	3 (21%)	82 (42%)	
Urinary residue			0.767**
$\geq$ 30 ml	7 (50%)	106 (54%)	
$<$ 30 ml	7 (50%)	90 (46%)	
Urethra length			1.00*
$\geq$ 50 mm	13 (93%)	172 (88%)	
$<$ 50 mm	1 (7%)	23 (12%)	
TRUS volume			0.132*
$\geq$ 40 cc	7 (50%)	57 (29%)	
$<$ 40 cc	7 (50%)	139 (71%)	

Abbreviations:
CAD = patients with bladder catheter;
* According to Fisher's exact test;
** According to Chi-square test.

Table 4.36: UVA and MVA result of TEST.B3.

	UVA		MVA$^{\gamma}$	
Parameters	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
Qmax $<$ 10 ml/s	4.31 (1.36-13.70)	0.013	4.61 (1.52-13.98)	0.007*
Bladder D25	1.01 (1.00-1.02)	0.019	1.01 (1.00-1.02)	0.019*
Bladder D10	1.01 (1.00-1.02)	0.019	1.01 (1.00-1.02)	0.019*

Abbreviations:
* Statistically significant; OR = Odd ratio; 95% CI = 95% Confidence interval;
γ Ajusted by IPSS, needles, age, urinary residue and PTV volume.

Once again, it is interesting to know what the dose threshold is for these parameters. According to these results, bladder D25 $\geq$ 40% of PD showed only a tendency to associate with CAD due to AUR (see table 4.37). Bladder D10 $\geq$ 50% and 55% of PD have a relationship with this side effect, in this specific case, as independent factor (4.38).

Table 4.37: Results of bladder D25 cutt-off points.

cut-off values			UVA		MVA$^{\gamma}$	
Bladder D25	**p-value**	**Parameters**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
40% of PD	0.093*	Bladder D25 $\geq$ 40% of PD	3.53 (0.88-14.12)	0.075	-	0.06
35% of PD	0.122**	Bladder D25 $\geq$ 35% of PD	2.33 (0.77-6.98)	0.131	-	0.122
30% of PD	0.145**	Bladder D25 $\geq$ 30% of PD	3.51 (0.76-16.14)	0.106	-	0.106
25% of PD	0.224*	Bladder D25 $\geq$ 25% of PD	-	0.998	-	-

Abbreviations:* According to Fisher's exact test; ** According to Chi-square test; OR = Odd ratio; 95% CI = 95% Confidence interval; γ Adjusted for IPSS, needles, age, urinary residue and PTV volume.

Table 4.38: Results of bladder D10 cutt-off points.

cut-off values			UVA		MVA$^{\gamma}$	
Bladder D10	**p-value**	**Parameters**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
35% of PD	0.371*	-	-	-	-	-
40% of PD	0.077*	-	-	-	-	-
45% of PD	0.179**	Bladder D10 $\geq$ 45% of PD	2.39 (0.65-8.85)	0.191	-	-
50% of PD	0.038**	Bladder D10 $\geq$ 50% of PD	3.14 (1.01-9.75)	0.047	3.14 (1.01-9.75)	0.047
55% of PD	0.039**	Bladder D10 $\geq$ 55% of PD	4.18 (1.19-14.80)	0.026	4.18 (1.19-14.80)	0.026

Abbreviations:* According to Fisher's exact test; ** According to Chi-square test; OR = Odd ratio; 95% CI = 95% Confidence interval; γ Adjusted for IPSS, needles, age, urinary residue and PTV volume.

TEST.B4

In this test, cross validation was performed using ROC analysis such as for small group. Table 4.39 shows the AUC for each parameter and figure 4.3 presents the ROC curves. The cut-off points were obtained through the coordinates points of the plot where the sensitivity is equal to the specificity (see table 4.40).

The result of this test is less optimistic (lower sensitivity and specificity (see table 4.40)) than for small group because the large group has other GU and GI toxicities working as intrinsic confounders. In conclusion, our statistical analysis predicts the increased risk of AUR better than by chance (even for large group). The cut-off analysis still shows roughly the same values as found in TEST.B2.

Table 4.39: AUC analyses for each statistically significant parameter.

	AUC	**Std.Error**	**Asymptotic Sig.**	**95% CI**
Qmax	0.65	0.077	0.078	0.497-0.799
Qmax*	0.67	0.072	0.037	0.526-0.808
Bladder D25	0.68	0.067	0.026	0.547-0.808
Bladder D10	0.70	0.068	0.012	0.568-0.833

Notes: * missing values replaced using MCMC MI

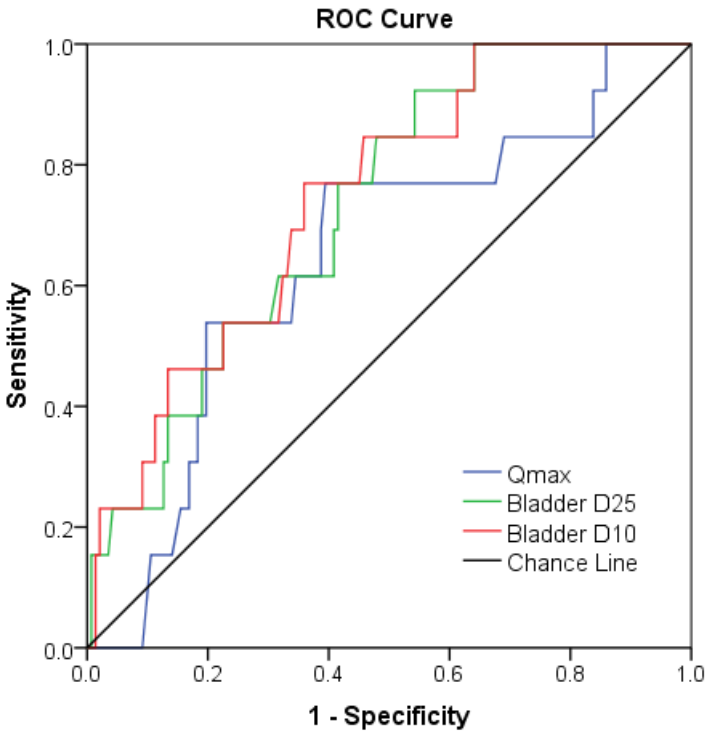


Figure 4.3: ROC curves for Qmax, bladder D25 and bladder D10.

Table 4.40: Comparison between ROC optimal cut-off points and previous cut-off points (TEST.B2).

	Sensitivity	Specificity	Cut-off point	Cut-off point (TEST.B2)
Qmax	0.69	0.61	12.5 ml/s	10 ml/s
Bladder D25	0.51	0.51	34.2% of PD	30% of PD
Bladder D10	0.64	0.64	50.0% of PD	50% of PD

4.4 Discussion

Patients treated with HDR BT usually have a long survival with 10 year biochemical recurrence free-survival (bRFS) [3] $> 90\%$. This underline the importance of reducing toxicity to improve quality of life (Qol) of these patients. As AUR is a serious grade 3 toxicity for patients treated with HDR BT, exploring the predictive clinical and dosimetric factors for AUR could improve Qol of those patients by reducing toxicity.

To the extent of our knowledge, this is the first study to investigate the association between dosimetric and clinical parameters and the occurrence of AUR (with the need for temporary CAD) in prostate cancer treated with HDR BT as monotherapy. For this study, we compared the dosimetric and clinical variables of the 14 PCa patients who needed a temporary CAD after HDR BT with other 28 selected patients (with grade $\leq$ 1 GU and GI toxicities) treated with the same HDR BT regimen who did not need a CAD. We found that having a pre-treatment baseline Qmax $<$ 10 ml/s and dose to 25% of bladder volume exceeding 30%-40% of PD were the two statistically significant factors associated with increased risk of AUR. The statistical significance of these two parameters was confirmed when the dataset of these 14 CAD patients was compared to all available patients (196) in our HDR BT monotherapy database who did not experience AUR. Furthermore, two different statistical methods (Method A and B) were tested and used to perform the statistical analysis and different methods were applied to deal with missing values. The usage of Method B and MCMC MI to deal with missing values was robust and finally used for our final results.

4.4.1 Imputation Methods & Methodology

Before embarking on the discussion of the results, some remarks have to be done regarding imputation methods and statistical methodology used in our study.

Comparing the imputation methods in ***Method A***, we conclude that the median substitution was a good and simple technique to handle missing values in this specific case. However, choosing more complex techniques, such as multiple imputation in case of a large amount of missing values could avoid biased outcomes [27, 60, 61]. Table 4.17 shows the general comparison between different techniques to deal with missing values. It can be concluded that the use of subgroup median substitution worked better to replace missing values than using overall median because we do not lose the population variance supporting previous findings [26].

When comparing more complex techniques (see table 4.17), such as Single Imputation EM, Automatic MI and MCMC MI, the results were slightly different. Only Single Imputation and MCMC MI recovered the same statistical parameters indicating that these are equivalent methods to impute missing values in small datasets. However, as described in *chapter 2*, SPSS automatically selected the method to impute the missing values, between monotone and MCMC MI methods, based on the pattern of missing values. In this case, the automatic method should have recovered the MCMC MI method because the missing values have an arbitrary pattern. Therefore, the automatic method is an interesting tool but it can result in different outcomes. In conclusion, evaluating clinical variables is challenging because the user is dependent on how complete that information is.

In our study, we also investigated two different methodologies. Method A is a powerful statistical method to analyse large dataset with large amount of events versus controls. In our case, the problem is the low number of AUR cases even when we use the entire cohort of patients. There is one role to apply the logistic regression method: 1 covariate per 10 events. For this reason, the ***Method A*** gives us unreliable conclusions because we included in the multivariate method more than 1 covariate. More clearly, that effect easily can be seen looking at large confidence intervals obtained by using ***Method A***. On the other hand, in the ***Method B***, we only looked at one variable in the multivariate method adjusted for the confounders. This seems to be more suitable for this study providing more reliable results. In conclusion, our study suggests the

[3] bRFS: means that after undergoing prostate cancer treatment, the patient PSA level does not rise significantly. If the patient relapse biochemically (PSA rises), it is a reasonable indicator of who will develop a recurrent prostate cancer

use of Method B and MCMC MI to impute missing values for datasets where a parameter with a low incidence rate is being investigated.

4.4.2 Acute Urinary Retention

Clinical Parameters

In this study, the importance of baseline function as selection criteria is illustrated by one of baseline clinical parameters selected. In our results, only the baseline Qmax was statistically associated with greater risk of AUR, this is in accordance with data from a previous study from *ErasmusMC - Cancer Institute* [13]. In that study the toxicity of the same group of patients was reported. Baseline urinary flow (Qmax) $>$ 15 ml/s (p = 0.047) was significantly correlated with lower incidence of grade 2 or higher acute GU toxicity. In our results, Qmax $<$ 10 ml/s was associated with AUR, which is a grade 3 acute GU toxicity. Qmax appeared in both methods (***Method A*** and ***B***) as factor associated with AUR, but it is important to highlight the fact that Qmax is not an independent factor according to the *Method B –Small group* because its OR changed after adjustment for the confounders(IPSS, needles, age, urinary residue and PTV volume). Lower baseline Qmax could be related to urinary symptoms and urinary dysfunction before treatment. Those patients with low Qmax reported mostly high IPSS and low urinary residue indicating limitations in daily functioning. Therefore, this strong correlation between Qmax and IPSS and urinary residue could explain the non-independence of Qmax observed in *Method B –Small group*. Qmax missing values were a limitation in our study. However, when missing data were replaced using MCMC MI, the results did not change substantially, and Qmax sustained as independent factor. Furthermore, ROC curve for Qmax showed that Qmax predicts well the correlation with AUR (better than by chance, AUC = 0.65/0.68). Additionally, the optimal cut-off value determined by ROC curve was 12.6ml/s which is not substantially different from 10ml/s. Therefore, ROC curve analysis validated the accuracy of this result. In conclusion, lower Qmax represents a pre-existing dysfunction and the cut-off point we defined, 10 ml/s, is helpful in selection patients for HDR BT monotherapy.

High scores of baseline International Prostate Symptom Score have been reported in several LDR BT studies [39, 40, 41] as predictor of AUR after treatment. In the previous study [13] IPSS $\geq$ 12 (p = 0.074) showed a tendency to correlate with grade 2 or higher late GU toxicity after HDR BT. In our current study, IPSS $\geq$ 10 showed statistical significance on multivariate analysis in ***Method A***. This is consistent with the previous LDR BT studies mentioned. However, in ***Method B***, IPSS did not achieve the p-value to consider as independent variable to investigate on multivariate analysis and it was used as confounder factor. It suggests that IPSS is less sensitive for AUR, which is an acute GU toxicity and not late GU toxicity. Additionally, baseline IPSS already is a patient selection criteria for this treatment. Patients with baseline IPSS $>$ 15/35 are rejected. This might indicate that the actual IPSS constraint is suitable and we do not expect an increased risk of AUR.

Several LDR BT studies addressed AUR and reported the following clinical and treatment related parameters; number of needles [42, 62], prostate volume [42, 43, 44, 40, 41] or hormone therapy [62] as statistically significant factors correlated with high risk of AUR. In our group of patients number of needles and prostate volume did not show a significant p-values ($\leq$ 0.05). In HDR BT the number of needles used is usually lower than that used for LDR BT, e.g. 17/18 [35, 13](in average) for HDR BT and 22/25 [45, 62] for LDR BT, which are related with less mechanical damage. It could explain the non-significance of number of needles in our study. Recently, [63, 64], a correlation between prostate volume ($>$ 50 cc) and late genitourinary is reported for EBRT. Prostate volume $>$ 50 cc is excluded for HDR BT regimen and we did not expect a volume effect on AUR.

DVH Parameters – Small Group

There are no studies evaluating DVHs parameters for HDR BT as monotherapy. In both methods, Method A and B, bladder D25 was a new predictor of AUR. This makes our findings clinically important such that one needs to restrict the dose to the bladder and that new sharper constraints should be applied.

Using method B, a dose to 25% of bladder volume exceeding 30%-35% of PD (11.4Gy-13.3Gy, in case of schedule 9.5Gy in 4 fractions) were statistically correlated with increased risk to develop AUR after treatment. However, only bladder D25 $\geq$ 35% of PD can be considered as independent factor because when we investigated the association on MVA adjusted for the confounders, the OR did not change considerably that allows the conclusion of independent factor. In the small dataset is hard to define a cut-off value, that explains the slight differences in independency for bladder D25 $\geq$ 35% of PD and bladder D25 $\geq$ 30% of PD. However, ROC curve analysis confirmed the strong relationship between bladder D25 and AUR (AUC = 0.79). Furthermore, the optimal cut-off point from ROC curve was 32.5% of PD and we previously found 30%/35% of PD as cut-off values, which might also explain the non-independence for bladder D25 $\geq$ 30% of PD. In literature, bladder dose constraints are related to high dose regions. It is already known that D1cc of bladder exceeding 80% of PD increase the risk of GU toxicity and it is commonly used as treatment constraint [12]. Peters at el. [65] reported bladder D2cc $\leq$ 70Gy constraint could reduce the risk on late GU toxicity after LDR BT. In conclusion, because the HDR treatment is given in very short period of time (36 hours) compared to 3 months in LDR, the role of lower dose in predicting toxicity is expected.

There is some hypothesis defending that the bladder trigone (bladder neck) plays an important role in voiding mechanism. Irritation or injury to the bladder trigone may lead to urinary retention [46, 66]. In our study, a possible relationship between dose to bladder neck and occurrence of AUR was not statistically significant. In contrast, **Roeloffzen et al.** [45] and **Hathout et al.** [46] reported dose to bladder neck as an important predictor of AUR after LDR BT. First, the correlation between AUR and bladder neck dosimetry observed in previous studies could be due to large variation in dose to OAR during the LDR BT compared with HDR BT, because the prostate size is being reduced over the time by retraction of edema or even due to organ movements. Second, the presence of relatively high dose in bladder neck implies that at least some seeds have been placed in the bladder muscle or due to seed migration's phenomenon. Therefore, the dosimetric accuracy of HDR compared to LDR could explain the lack of correlation between dose to bladder neck and AUR in our data. Other limitations are; the small sample size in our group and the great variation in the exact definition/delineation of bladder neck, which could bias the results.

Regarding all different regions of urethra in this analysis, only membranous urethra D0.5cc, particularly 0.5cc of volume exceeding 55% of PD, seems to be correlated with CAD due to AUR using the ***Method B***. However, its OR changed after adjustment for the confounders on MVA indicating non-independency. So, we can say that parameter might have some association with AUR but that small sample size makes it difficult to find a statistically significant correlation. **Díez et al.** [38] investigated the association between urethral strictures and different regions of urethra volume after HDR BT treatment (in four different treatment schedules, 34Gy in 4fr, 36Gy in 4fr, 31.5Gy in 4fr and 26Gy in 2fr). They identified 10 strictures in 213 patients and they found no correlations between volumetric and dosimetric urethra (including membranous urethra) parameters with that side effect. Our study suggests membranous urethra D0.5cc $\geq$ 55% of PD might have some association with AUR but its statistical power could not be confirmed in the large group because of lack of data on different areas of urethra in our database. However, ROC curve for UM D0.5cc showed that variable predicts well the relationship with AUR (AUC = 0.68). The optimal cut-off value (54.5% of PD) determined by ROC curve supported our findings.

DVH parameters - Large group

In the large group, we only applied Method B and MCMC MI to impute missing values. In this group of patients, bladder D25 $\geq$ 30% of PD (p = 0.077) showed only a tendency to associate with AUR. However, when Qmax, IPSS and urinary residue missing values were replaced, bladder D25 $\geq$ 30% of PD lost its statistical significance for bladder D25 $\geq$ 40% of PD. This results might translate the influence of missing values when we are doing statistical analysis. Another possible reason was the way how we selected the control's group in this large dataset. We did not restrict the controls to have grade $\leq$ 1 GU and/or GI toxicities and we took into account all patients without CAD but they might have other toxicities playing a role as intrinsic confounders explaining only the tendency to associate with. Even so, it suggests that the bladder D25 receiving $\geq$ 30%-

40% of PD is correlate with CAD due to AUR. ROC curve analysis suggested 34.2% of PD as cut-off point supporting our previous finding.

In this dataset, another DVH parameter for bladder was statistically significant: bladder D10 $\geq$ 50% of PD. However, this parameter was not evaluated in small dataset and is strongly correlated with bladder D25. Therefore, only one parameter might be used in clinical practice.

The usage of large dataset and ROC curve analysis confirmed our previous results and reinforced the association of bladder D25 and baseline Qmax with AUR.

4.4.3 Limitations

Although this study does support the definition of new dosimetric and clinical constraints, there are nonetheless several limitations. Foremost are the inherent biases associated, such as sample bias (systematic error due to a non-random sample of population), with any retrospective study. Another limitation is the small sample size and few events (patients with CAD due to AUR) to evaluate. This condition could affect the results reducing the real strength of the outcomes. Another limitation is the missing values in clinical variables because losing information with a small population reduces the possibility to find any association and replacing them could bias the results. Additionally, this study is based on "planned dose" and not "delivered dose" which means we did not take into account the treatment accuracy and anatomy variation during treatment course which is hard to do in HDR BT setting because of the short treatment time and difficulties of patients transfer with needles in the prostate during treatment.

4.5 Conclusion

Defining predictive factors for AUR as a serous grade 3 GU toxicity is important. Our results reporting a baseline Qmax $<$ 10 ml/s as predictive factor for AUR is helpful in selecting patients for HDR BT, and applying an extra dosimetric constraint as we found for bladder D25 in the daily clinic could reduce the risk of AUR. Furthermore these two parameters are important to be investigated in future studies with larger sample size.

Chapter 5

Predictive factors for late rectal bleeding after HDR BT as monotherapy for low risk prostate cancer

5.1 Purpose

To evaluate clinical and dosimetric parameters related to late rectal bleeding after high-dose rate brachytherapy as monotherapy treatment for prostate cancer.

5.2 Materials and Methods

In this study, patients with histological confirmed prostate carcinoma (PCa), clinical stage T1b-T2b, Nx-0, Mx-0, Gleason score ≤ 7, PSA ≤ 16 ng/ml and WHO performance [1]status 0-2 were treated with HDR BT monotherapy. HDR BT monotherapy was administered in four fractions of 9.5Gy with a minimum interval of six hours within 36 hours using one implant. In contrast to chapter 4 only one group was evaluated.

5.2.1 Patients

The small group is a selection from patients treated between 2007 and 2015 (210 patients). Fifteen of 210 (7.1%) developed rectal bleeding after primary treatment for their PCa with HDR BT. These were analysed together with 30 other patients with grade ≤ 1 GU and GI toxicities [2]. Table 5.1 shows the patient, tumour and treatment characteristics.

[1]WHO performance status in *Appendix C*

[2]GU and GI toxicities classification in *Appendix B*

Table 5.1: Patient, tumour and treatment characteristics.

Characteristic	RB (n= 15 patients)	no-RB (n= 30 patients)
Patient and tumour		
Age at implantation(y) [mean (min-max)]	69.1 (57.8-74.8)	68.9 (53.2-79.3)
Clinical Tumor Stage		
T1 [n (%)]	12 (80%)	18 (60%)
T2 [n (%)]	3 (20%)	12 (40%)
Gleason sum-score		
<7 [n (%)]	12 (80%)	25 (83%)
=7 [n (%)]	3 (20%)	5 (17%)
iPSA(ng/ml) [mean (min-max)]	8 (4-12)	8.5 (4.7-14.8)
Pretreatment IPSS [mean (min-max)]	8 (2-19)	5 (0-15)
Rectum Volume (cc) [mean (min-max)]	96.9 (50.6-227.8)	103.1 (62.9-197.6)
Treatment		
TRUS volume (cc) [mean (min-max)]	33.1 (18-45.5)	31.6 (18.6-50)
Needles [mean (min-max)]	17 (15-21)	16 (12-23)
PTV volume [mean (min-max)]	62.3 (41.1-90.9)	49.4 (26.6-79.2)

Abbreviations: RB = patients with rectal bleeding after treatment;
iPSA = initial prostate-specific antigen level;
IPSS = international prostate symptom score;
TRUS = transrectal ultrasound; PTV = planning target volume at treatment.

5.2.2 Organs Delineation

For all patients, organs at risk (bladder, urethra and rectum) were delineated. The rectum was divided into cranial rectum, caudal rectum and anus. The caudal part was defined up to 1 cm to the PTV volume and the cranial part is the cranial one from 1cm above the PTV volume until to the lower edge of sacroiliac joint. The anus volume was defined as the last 3 cm of the rectum volume. The cranial/caudal rectum and anus wall were defined as the internal margin from rectum of 5 mm thin. Figure 5.1 shows an example of patient delineation.

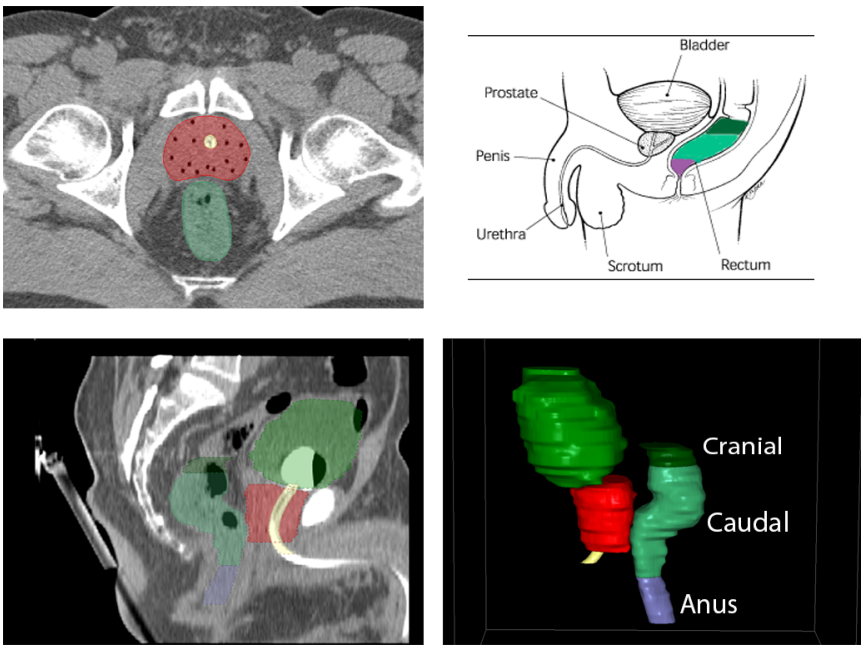


Figure 5.1: The delineated organs in planning CT scan and schematic representation of different parts of rectum. Green contour = bladder; red contour = PTV (prostate); yellow contour = urethra; dark green contour = cranial rectum; water green contour = caudal rectum and lilac contour = anus.

DVH and Clinical parameters selection

For all delineated structures, such as cranial, caudal, anus part and their walls, respectively, the following dosimetric parameters were calculated as presented in table 5.2.

Table 5.2: List of DVH parameters

Organs	DVH Parameters
Cranial/cranial wall rectum	Dmean, D1cc, D2cc, D10, D25, V10, V20, V30, V40
Caudal/caudal wall rectum	Dmean, D1cc, D2cc, D10, D25, V10, V20, V30, V40, V75, V80, V90
Anus/anus wall	Dmean, D1cc, D2cc, D10, D25, V10, V20, V30, V40

The majority of baseline clinical variables were selected as binary parameters with the median as cut-off values: TRUS volume (40 cc), number of needles (17), PTV volume (55 cc), age (70 years), rectum volume (85 cc), rectum diameter (35 mm), presence or absence of anticoagulants, diabetes and hypertension, mean prostate-rectum distance (4.5 mm) and number of needles in template row 1, 1.5 and 2 (see image 1.7c).

The rectum diameter was defined as the average diameter (3 measurements: top, mid, bottom) behind the PTV volume. The prostate-rectum distance was defined as the average distance between prostate and rectum. Both measurements were collected in the median prostate-sagital view of the CT-scan. These definitions needed small adjustments according to the anatomy by selecting the most representative measurements (see figure 5.2).

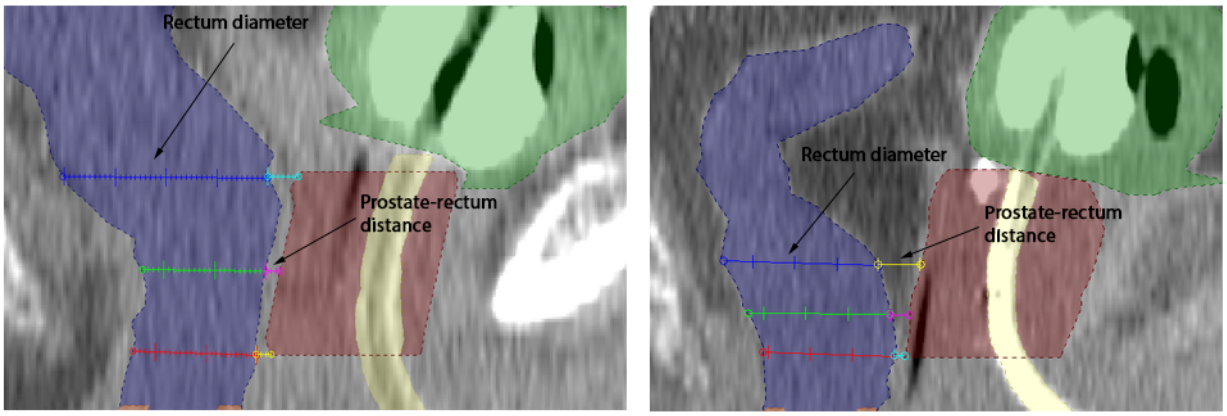


Figure 5.2: Rectum diameter and mean rectum-prostate distance: geometric definition. Left = nominal case, righ = adjusted case; blue contour = rectum; green contour = bladder; red contour = PTV (prostate); yellow contour = urethra.

5.2.3 Statistical Analysis and Missing Values

As described in *chapter 4 section 4.2.4*, SPSS statistical software was used to perform the statistical analysis. In this study, possible associations between DVH and/or clinical parameters and late rectal bleeding were explored using Method B (see section 4.2.4 and 4.2.5). Mann-Whitney and Chi-square test were applied and all variables showing p-value ≤ 0.05 were afterwards tested in univariate and multivariate logistic regression. Multivariate analysis was adjusted for the following confounders: age, TRUS volume, needles and rectum volume. Others confounders are dependent of Mann-Whitney and Chi-square test and for that reason the list of confounders might be extended. P-value ≤ 0.05 were considered statistically significant on MVA. Cross-validation using receiver operating characteristic (ROC) curve analysis was used to assess how well the found parameters were predicting for rectal bleeding. The area under ROC curve (AUC) shows the capability to distinguish no-RB patients from RB patients. This dataset does not have missing values.

5.3 Results

In this section, the results regarding all statistical analysis will be presented. First all, DVH parameters were analysed using Mann-Whitney test. Here, only Dmean and D25 for cranial part of rectum and Dmean, D10 and D25 for cranial wall part of rectum showed p-values ≤ 0.05. The complete outcome is in *Appendix E* and summarized results are shown in tables 5.3 and 5.4.

Table 5.3: Mann-Whitney test outcome for rectum divided in 3 regions.

DVH Parameters Rectum	RB (n=15) Median	no-RB (n=30) Median	p-value
Cranial Dmean (%PD)	11.90	8.90	0.028*
Cranial D1cc (% of PD)	15.39	15.35	0.665
Cranial D2cc (% of PD)	13.95	13.79	0.665
Cranial D10 (% of PD)	15.50	13.39	0.107
Cranial D25 (% of PD)	13.67	10.69	0.034*
Cranial V10-V40 (cc)	-	-	n.s.
Caudal Dmean (% of PD)	26.63	26.53	0.886
Caudal D1cc (% of PD)	71.96	73.96	0.312
Caudal D2cc (% of PD)	64.98	67.29	0.413
Caudal D10 (% of PD)	50.55	51.32	0.427
Caudal D25 (% of PD)	34.45	34.97	0.613
Caudal V10-V40 (cc)	-	-	n.s.
Caudal V75-V90 (cc)	-	-	n.s.
Anus Dmean (% of PD)	14.13	14.63	0.523
Anus D1cc (% of PD)	21.61	22.13	0.754
Anus D2cc (% of PD)	19.06	19.02	0.791
Anus D10 (% of PD)	20.32	21.71	0.773
Anus D25 (% of PD)	16.32	17.77	0.700
Anus V10-V40 (cc)	-	-	n.s.

Table 5.4: Mann-Whitney test outcome for rectum wall divided in 3 regions.

DVH Parameters Rectum Wall	RB (n=15) Median	no-RB (n=30) Median	p-value
Cranial Dmean (%PD)	11.79	8.48	0.010*
Cranial D1cc (% of PD)	16.13	14.45	0.132
Cranial D2cc (% of PD)	13.69	12.23	0.107
Cranial D10 (% of PD)	17.57	13.56	0.028*
Cranial D25 (% of PD)	13.61	10.03	0.011*
Cranial V10-V40 (cc)	-	-	n.s.
Caudal Dmean (% of PD)	26.53	27.63	0.962
Caudal D1cc (% of PD)	71.96	73.98	0.324
Caudal D2cc (% of PD)	64.95	66.44	0.386
Caudal D10 (% of PD)	58.48	60.63	0.258
Caudal D25 (% of PD)	34.52	37.20	0.248
Caudal V10-V40 (cc)	-	-	n.s.
Caudal V75-V90 (cc)	-	-	n.s.
Anus Dmean (% of PD)	14.34	14.42	0.596
Anus D1cc (% of PD)	20.21	20.75	0.754
Anus D2cc (% of PD)	16.97	17.62	0.754
Anus D10 (% of PD)	20.47	21.82	0.700
Anus D25 (% of PD)	16.27	17.62	0.665
Anus V10-V40 (cc)	-	-	n.s.

Abbreviations: RB = Rectal bleeding; no-RB= patients without RB;
* Statistically significant.

Rectum and rectum wall were also evaluated on Mann-Whitney test but taking into account the entire volume delineated. In other words, the rectum volume and rectum wall were not divided in different parts. Only rectum Dmean was statistically significant. The outcome of this test is shown in table 5.5 and 5.6 for rectum and rectum wall, respectively.

Table 5.5: Mann-Whitney test outcome for rectum as entire volume.

DVH Parameters Rectum	**RB (n=15) Median**	**no-RB (n=30) Median**	**p-value**
Dmean (% of PD)	22.74	20.70	0.029*
D1cc (% of PD)	72.29	74.28	0.360
D2cc (% of PD)	65.19	67.09	0.386
D5cc (% of PD)	52.15	53.20	0.754
D10 (% of PD)	43.42	42.76	0.923
D25 (% of PD)	28.27	27.24	0.283
V10 (cc)	69.01	65.60	0.773
V20 (cc)	37.30	33.52	0.656
V30 (cc)	19.19	17.74	0.885
V40 (cc)	11.03	10.16	0.942
V75 (cc)	0.71	0.92	0.289
V80 (cc)	0.23	0.43	0.373
V90 (cc)	0.00	0.02	0.489

Table 5.6: Mann-Whitney test outcome for rectum wall as entire volume.

DVH Parameters Rectum wall	**RB (n=15) Median**	**no-RB (n=30) Median**	**p-value**
Dmean (% of PD)	22.63	20.47	0.123
D1cc (% of PD)	72.04	74.10	0.312
D2cc (% of PD)	64.95	66.56	0.348
D5cc (% of PD)	41.28	45.64	0.399
D10 (% of PD)	46.34	47.36	0.700
D25 (% of PD)	26.54	25.29	0.727
V10 (cc)	36.09	34.03	0.312
V20 (cc)	17.07	16.62	0.926
V30 (cc)	9.08	9.46	0.596
V40 (cc)	5.20	6.22	0.555
V75 (cc)	0.68	0.90	0.233
V80 (cc)	0.18	0.42	0.335
V90 (cc)	0.00	0.01	0.340

Abbreviations: RB = Rectal bleeding;
no-RB= patients without RB;
* Statistically significant.

Regarding to the clinical variables in this analysis, only PTV volume $\geq$ 55 cc and hypertension were statistically correlated to rectal bleeding. The outcome of Chi-square test is shown in table 5.7. Afterwards, we also looked for any difference in the number of needles in different rows of the template between cases and controls. In that case, Mann-Whitney test was applied and no correlation was found. This result are shown in table 5.8.

Table 5.7: Result of Chi-square.

Clinical Parameters	RB (n=15)	no-RB (n =30)	p-value
Age (years)			0.673
≥ 70	8 (53%)	14 (47%)	
< 70	7 (47%)	16 (53%)	
TRUS volume			0.197
≥ 35 cc	8 (53%)	10 (33%)	
< 35 cc	7 (47%)	20 (67%)	
Needles			0.053
≥ 17	12 (80%)	15 (38%)	
< 17	3 (20%)	15 (62%)	
PTV volume			0.013*
≥ 55 cc	10 (67%)	8 (27%)	
< 55 cc	5 (33%)	22 (73%)	
Rectum diameter			0.526
≥ 35 mm	9 (60%)	15 (50%)	
< 35 mm	6 (40%)	15 (50%)	
Rectum Volume			0.399
≥ 85 cc	9 (60%)	16 (53%)	
< 85 cc	6 (40%)	14 (47%)	
Rectum wall volume			0.180
≥ 45 cc	8 (53%)	22 (73%)	
< 45 cc	7 (47%)	8 (27%)	
P-R distance			0.396
≥ 4.5 mm	8 (53%)	12 (40%)	
< 4.5 mm	7 (47%)	18 (60%)	
Hypertension			0.034*
yes	10 (67%)	10 (33%)	
no	5 (33%)	20 (67%)	
Diabetes			0.101
yes	3 (20%)	1 (4%)	
no	12 (80%)	29 (96%)	
Use of anticoagulants			0.454
yes	4 (27%)	5 (17%)	
no	11 (73%)	25 (83%)	

Abbreviations:
P-R distance = prostate-rectum distance;
* Statistically significant.

Table 5.8: Number of needles evaluation by row 1, 1.5 and 2 of the template.

Variables	RB (n=15) Median	no-RB (n=30) Median	p-value
Needles in row 1	2	1	0.312
Needles in row 1.5	4	4	0.454
Needles in row 2	5	4	0.167
Needles in row 1+1.5+2	10	10	0.348
Needles in row 1+1.5	6	6	0.712

Clinical and dosimetric variables with p-values ≤ 0.05 were considered statistically significant to include on univariate and multivariate analysis. All variables retained the statistical significance as independent factors. Only the OR of hypertension changed substantially after adjustment which means that parameter is not an independent factor associated with RB after treatment. The results are shown in table 5.9.

Table 5.9: UVA and MVA analysis.

	UVA		**MVA$^{\gamma}$**	
Parameters	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
Rectum: Cranial part				
Dmean	1.29 (1.05-1.60)	0.017	1.29 (1.05-1.60)	0.017*
D25	1.22 (1.01-1.48)	0.039	1.22 (1.01-1.48)	0.039*
Rectum Wall: Cranial part				
Dmean	1.33 (1.07-1.65)	0.010	1.33 (1.07-1.65)	0.010*
D10	1.18 (1.01-1.38)	0.035	1.18 (1.01-1.38)	0.035*
D25	1.25 (1.04-1.51)	0.018	1.25 (1.04-1.51)	0.018*
Rectum (entire vol)				
Dmean	1.27 (0.99-1.63)	0.059	1.27 (0.99-1.63)	0.059
PTV volume $\geq$ 55 cc	5.50 (1.43-21.10)	0.013	5.50 (1.43-21.10)	0.013*
Hypertension (yes)	4.00 (1.07-14.90)	0.039	7.46 (1.53-36.44)	0.013*

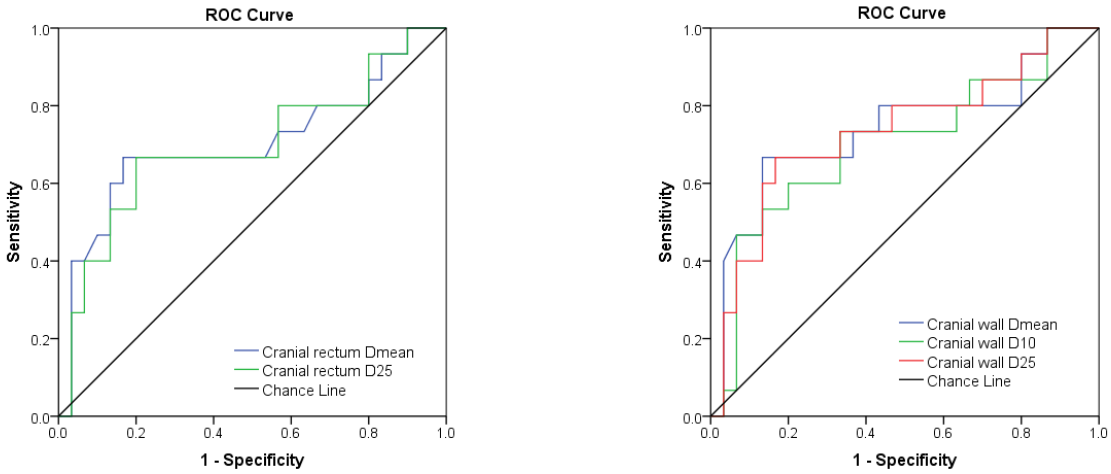
Abbreviations: * Statistically significant;
UVA = univariate analysis; MVA = multivariate analysis;
OR = Odd ratio; 95% CI = 95% Confidence Interval;
γ Adjusted for age, TRUS volume, needles, anticoagulants and rectum volume.

Within all parameters under investigation, only PTV volume can be tested in large dataset (210 patients treated between 2007-2015). In this phase, we excluded from control group all patients with grade 2-3 GI toxicity because that patients might play a role as intrinsic confounders. Differences between cases (15 RB) and controls (180 no-RB) in terms of PTV volume were tested using Chi-square test. In this test, PTV volume $\geq$ 55 cc lost the statistically significant with p-value of 0.300 which means that there were no differences in terms of PTV volume between bleeders and no-bleeders in large dataset.

After this analysis, cross-validation was performed using ROC curve analysis. For this project work, the optimal cut-off point for statistical significant parameters (see table 5.9)was not explored. The reason for that will be explained in following sections. Table 5.10 and table 5.11 show the AUC for each DVH parameter and clinical variables, respectively. Figure 5.3 and figure 5.4 show the ROC curves. However, this method proves the accuracy of our results. The statistically significant variables still have a modest correlation with rectal bleeding.

Table 5.10: AUC analyses for each statistical significant parameter.

	AUC	**Std.Error**	**Asymptotic Sig.**	**95% CI**
Cranial Dmean	0.70	0.093	0.028	0.521-0.886
Cranial D25	0.69	0.091	0.034	0.517-0.874
Cranial wall Dmean	0.74	0.088	0.010	0.567-0.911
Cranial wall D10	0.70	0.089	0.028	0.528-0.876
Cranial wall D25	0.73	0.085	0.011	0.566-0.900



(a) ROC curves for cranial rectum.

(b) ROC curves for cranial wall rectum.

Figure 5.3: ROC curves for DVH parameters of cranial/cranial wall of rectum.

Table 5.11: AUC analyses for each statistical significant clinical parameter.

	AUC	Std.Error	Asymptotic Sig.	95% CI	Cut-off point
PTV	0.75	0.077	0.006	0.603-0.903	54 cc
PTV*	0.61	0.071	0.146	0.474-0.751	-
Hypertension	0.67	0.087	0.071	0.496-0.837	-

Notes: * large group

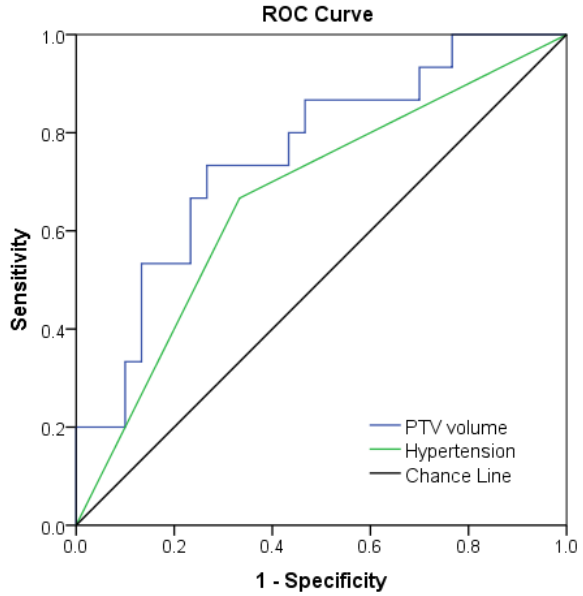


Figure 5.4: ROC curves for PTV volume and Hypertension.

5.4 Discussion

To the best of our knowledge, this is the first study to investigate predictive risk factors of rectal bleeding after HDR BT as monotherapy. For this study, we compared the DVH and clinical variables of 15 PCa patients with RB with those of 30 PCa patients without RB. We found that prostate PTV volume $\geq$ 55 cc and hypertension were significantly associated with increased risk of RB. Additionally, some DVH parameters of cranial/cranial wall part of rectum were statistically significant. However, the clinical relevance of this result is still unclear.

DVH Parameters

In our study, we evaluated dose to different parts of rectum (cranial, caudal and anus) to investigate the possible correlation with rectal bleeding. In the MVA, we found that Dmean and D25 for cranial part of rectum and Dmean, D10 and D25 for cranial rectum wall were statistically significant. However, analysing the median values for those parameters (see table 5.3-5.6), RB patients received more dose than no-RB patients but only low dose level of 11%-18% of prescribed dose (1-1.7Gy per fraction). Cranial rectum showed huge anatomy variations (see figure 5.2) which might explain the differences in cranial rectum dose between RB patients and no-RB patients. In summary, although ROC curve analysis confirmed the results with high AUC values for cranial and cranial wall parameters, those low dose levels could not well explain the expected dose effect relation to induce rectal bleeding.

In literature, there are several studies reporting "intermediate" doses as cause-effect of rectal bleeding after EBRT. **Jackson et al.** [50] suggested a correlation between late rectal bleeding and the volume irradiated at an "intermediate" dose approximately of 40-50Gy. **Fiorino et al.** [67] found an association with grade 1-3 bleeding and larger rectum volume receiving doses of 50-60Gy (EQD_2[3]). Over the years, other studies have confirmed that hypothesis [52, 68, 69, 70] for EBRT. However, we cannot directly compare these findings with our study because the treatment modality and fractionation schema is different. Using EQD_2, the 60Gy in EBRT can be converted roughly in 30Gy for HDR BT using $\alpha/\beta_{(rectum)}$ = 3Gy [9]. This value (30Gy) represents roughly 80% of prescribed dose for HDR BT (9.5Gy in 4fr). Due to excellent dose fall-off, the 30Gy dose is limited to $<$ 1 cc of rectum volume in HDR BT whereas the volume receiving 60Gy in EBRT is 50/55% ($\simeq$ 50 cc).

LDR BT studies [56, 55, 71, 72] have been reported associations between rectal volumes receiving higher doses ($>$ 100% of PD) and RB. Recently, **Okamoto et al.** [59] investigated separately the effect of EBRT and HDR (in combination therapy). They suggested the estimated radiation doses delivered during HDR BT to 5% and 10% (D5 and D10) of rectum volume in patents with late rectal bleeding were 48% (5.1Gy) and 44% (4.6Gy) of PD (10.5Gy as boost), respectively. In our study, we did not observed differences between RB patients and no-RB patients in rectum dose $>$ 18% of PD.

The DVH parameters of cranial part of rectum were statistically significant but clinically hardly relevant to be related to rectal bleeding. The small sample size and the sharp constraints followed in our HDR protocol may gave difficulties to extract dosimetric correlations.

Clinical Parameters

Clinical variables , such as rectum volume, rectum diameter, rectum wall volume, TRUS volume, total of needles used and number of needles in row 1, 1.5 and 2 of the template, were investigated and did not reveal any correlation with RB.

EBRT studies [50, 53, 54, 73] reported possible correlation between small rectum volume and risk of rectum bleeding, because small volumes could receive higher concentrated dose on smaller volume disturbing

[3]Equivalent dose in 2Gy fractions: $EQD_2 = nd(\frac{d+\alpha/\beta}{2+\alpha/\beta})$; n = number of fractions; d = dose per fraction.

repair capacity. In our study, the mean rectum volume in patients who developed RB was smaller than in those without bleeding (see table 5.1). Although, these results showed a slightly tendency on that inverse relationship, the differences was not statistically significant. In our study, prostate-rectum distance was not correlated with RB. In contrast, **Kang et al.** [74], suggested small distances between prostate and rectum (measured in median sagittal view on MRI) as predictive factor for late rectal complications after LDR BT. One possible reason of our results is the accuracy of the measurement by using CT-scan images comparing with previous study where they used MRI. Other possible cause is the anatomy variance among the patients which makes this measurement difficult to have a clear definition.

Our study also focused on the relation between RB and some specific comorbidities such as Diabetes Mellitus (DM) and Hypertension. These comorbidities cause damage to the microvasculature by inducing endothelial and vascular smooth muscle dysfunction and may prevent optimal repair after radiation acute damage. This suggest that radiation-induced pathologic changes that will be aggravated in patients with DM [75, 76]. However, our results did not found a correlation between DM and RB as other studies [56, 57, 70, 72, 77]. The lower reported incidence of DM in our data (4%) compared to normal population (12-20% of man population between 60 and 80 years [78]) and the small sample size could be two limitations that prevent representative results.

Our dataset has a representative sample (33% of no-RB patients) compared to the incidence rate of Hypertension in Dutch man (37.4%) [79] and it was significantly correlated with the increase risk of RB after HDR BT as monotherapy. However, its OR changed after adjustment for the confounders which might be caused by the correlation of that parameter with age. Despite this, the ROC curve analysis showed AUC = 0.67 which indicates that Hypertension predicts better rectal bleeding than by chance. In literature, to the extent of our knowledge, studies [59, 72] reported no correlation between Hypertension and RB. Reasons for this is the fact that Hypertension is mostly not well reported and the lack of information on well treated Hypertension and not treated Hypertension. In conclusion, for both Diabetes and Hypertension, it is worthy to investigate these 2 factor in a larger data set.

In this investigation, we also assessed the link between the usage of anticoagulants and the occurrence of RB after HDR BT. Anticoagulation therapy is required for many patients with cardiovascular disorders and that prolongs the time that it takes for blood to clot. Treatment with anticoagulant medications can result in episodes of bleeding itself, and for men undergoing radiotherapy that chance is expected to be greater. Our findings are in accordance with those from other published studies [59, 70, 77], where they also did not find any correlation between taking anticoagulants and occurrence of RB. However, **Harada et al.** [57] found the usage of anticoagulants as the most significant predictive factor of RB after LDR BT. One possible reason for our results was that the anticoagulants were checked only at the time HDR BT started.

In our study, patients with PTV volume exceeding 55 cc have more change to develop RB after HDR BT as monotherapy. In addition, PTV volume is the only factor that we can test by using the entire dataset of patients (treated between 2007 until March 2015) and PTV volume $\geq$ 55 cc lost its statistical significance. Although, ROC analysis confirmed the cut-off point for PTV volume, our results are inconclusive and suggest that PTV volume might have a relationship with RB but because of the few patients with RB, the statistical power is reduced.

In EBRT studies [70, 77], the investigators often look at prostate volume before treatment and no correlation with that variable and occurrence of RB was observed. **Skwarchuk et al.** [53] also investigated PTV volume and it was not statistically significant. LDR BT studies [58, 57, 56, 80] investigated the relationship with RB and prostate volume based on ultrasound images but no correlation between these two parameters was found.

In conclusion, we did not observe any reliable correlation with DVH parameters or PTV volume and RB. Hypertension was the most significant factor associated to RB but it cannot be considered as predictive factor.

Limitations

This study does not support the definition of new clinical or dosimetric constraints but there are nonetheless limitations. First of all, in this study, like in other retrospective studies, there are inherent biases. As discussed in the previous chapter, the few events (patients who developed RB) compromise the statistical power of these results. Additionally, this study is based on "planned dose" and not "delivered dose" which means we did not take into account the treatment accuracy and anatomy variation during treatment course. However, this study is an important tool for new investigations because it gives indication in what we should look at in future researching.

5.5 Conclusion

In our study, either in terms of DVH parameters or clinical variables, the results are inconclusive. In the cranial rectum, some DVH parameters were statistically correlated with RB but without clinical relevance. The PTV volume did not show clear relationship when tested in the large dataset. Hypertension was statistically associated with RB. However, the number of events is small and thus the power of these observations is limited and requires confirmation in a larger cohort of patients with RB. In conclusion, this study is an interesting guideline for future investigations in this area.

Chapter 6

Discussion and conclusions

6.1 Summary of Thesis

Fractionated HDR BT is being increasingly used for low/intermediate PCa. This treatment modality offers direct delivering of high doses to the prostate while sparing the normal surrounding tissue, in particular, bladder and rectum. This makes HDR BT one of the most efficient and effective technique to treat organ confound PCa in few large fractions. However, there are some side effects, such as: AUR and RB. Therefore, the main objective of this thesis was to investigate clinical and dosimetric parameters related to those secondary effects after HDR BT as monotherapy. This thesis is separated in the following chapters:

- Introduction of HDR BT modality: Physical aspects up to clinical procedure and side effects (*chapter 1*);
- General statistical procedures in this research area (*chapter 2*);
- Bibliography review about the technique itself and their outcomes in terms of toxicities (*chapter 3*);
- Risk factors for AUR and RB (*chapter 4* and *5*, respectively).

6.2 General Discussion

EBRT and LDR treatments are the well-known treatment modalities for PCa and there are several studies that investigated the causes of side effects, such as: AUR and RB. Nowadays, the sources of that side effects are well-known for EBRT/LDR BT. However, HDR BT as monotherapy is a relatively recent technique and further investigation is required to understand and establish the secondary effects. Therefore, to the best of our knowledge, our study is the first study investigating the predictive factors of AUR and RB for HDR monotherapy.

Although GU and GI toxicities rates after HDR BT are generally low, AUR and RB are well-established potential toxicities. These side effects, although often transient, cause a great deal of anxiety and discomfort effecting the patient's quality of life. These complications are also an issue for physicians and medical physicists because they want to minimize as much as possible those side effects. Therefore, this study added new information about what we can do to improve the daily routine of those patients after treatment.

Clinical and Dosimetric Data

In these investigations, clinical and dosimetric information are often used to assess to the factor that rises the chance of development a certain side effect. Dosimetric data is collected from treatment planning software's which means we use "planned doses" and not "delivered doses".

Therefore, one interesting field to discuss is the treatment accuracy. The uncertainties during brachytherapy treatments and their occurrence rates are not well known and it becomes crucial for patients with target and/or OAR doses closes to constraint values. In addition, the high dose gradient in HDR BT makes the treatment delivery challenging and since even small geometric/dosimetric uncertainties may result in large dose discrepancies from the original plan.

First of all, our results are based on the planning CT assuming neither needles displacements nor organ movements. This HDR BT treatment is administrated in four fractions of 9.5Gy with a minimum interval of 6h within 36h. Because of short treatment time per fraction ($\approx$ 10-15 minutes) we do not expect large differences in anatomy/organ motion. However, there are natural displacements of needles in caudal direction and this effect was already studied [81]. Therefore, in *ErasmusMC - Cancer Institute*, a lateral x-ray is made before each fraction to check the position of the tip of the catheters relative to the markers. Displacements exceeding 3mm are corrected by pushing the catheters to the planned depth as indicated by their position relative to the markers. In our study was not possible to include this factor in analysis and there is no way to know exactly how accurate the treatment was. There are always some accuracies, such as organ motion, source positioning, contouring or dose delivery, which are not quantified. Furthermore, BT treatments are not monitored with independent control systems from the delivery unit that make the possibility of that uncertainties remain undetected during the entire treatment course.

Regarding to clinical data, we can split that up into two domains: data reported by patient (questionnaires, e.g. IPSS score) or medical information reported by physicians (medical examination, e.g. Qmax or urinary residue). Periodically, in *ErasmusMC - Cancer Institute*, questionnaires are sent to the patients. It is an easy tool to assess the patient's qualify of life in several domains (e.g. urinary function or sexual function) before and after treatment. The usage of questionnaires allows to assess the toxicity not only reported by the physician but also by the patients improving the report of toxicities [13]. It also allows to analyse the toxicity behaviour in function of time.

On the other hand, clinical data (patient and medical information) has some problems associated. It is often not very well recorded resulting in incomplete data. Therefore, another big issue is the missing values. When we try to analyse clinical variables, sometimes those parameters are not filled in causing troubles in performing statistical analysis. First, the majority of statistical software excludes the patient if it finds one missing value in one parameter. This technique has some limitations, mainly, reducing the sample size and the variability. Second, there are some techniques to replace those missing values by estimated measures using simple or complex techniques: Mean/Median replacements or Multiple Imputation. However, those techniques calculate the values based on data available which might boost the presence of some relationship providing an overestimation of results. Consequently, when we analyse clinical variables it is important to have a large sample to cover the effect of missing values. In *chapter 2*, some techniques to replace missing values were shown. Those methods were evaluated and their results are shown in *chapter 4*. Because of this problem, Qmax, one of the most important risk factor for AUR according our results, has a modest statistical power.

Sample Size and Methodology

This study provides new clinical and dosimetric parameters but it is important to discuss the validity of these results. First of all, this study is limited by the number of events (patients who developed AUR or RB) which makes the statistical analysis less powerful. The incidence of each side effect is small in total of patients treated, in other words, we always have far more controls than cases to include in the analysis. Therefore, including more patients is in one side (controls) only. The question is: *Will it boost the statistical analysis?*

Rik Bijman, master student in *ErasmusMC - Cancer Institute*, developed NTCP models for each symptom of late GU and GI toxicities after EBRT for prostate cancer [82]. He modelled NTCP [1] models based on

[1]NTCP: Normal tissue complication probability.

clinical and DVH parameters of 800 patients (HYPRO study [2]) and he concluded that even with a large amount of patients it is hard to model certain symptoms. Again, the main reason is that the number of cases for each side effect is not well distributed among the number of patients treated. Therefore, one of the questions is: *What is the ideal number of patients to analyse?*. If we have a small sample size, the results will be based on one part of the entire population. However, if we have a large sample simple, we will have large variety which will introduce noise that might mask some relationship. In conclusion, the main issue in this kind of study is not the sample size but it is the number of cases versus controls.

In our study, we used two different methods to analyse the data: Method A and Method B. As discussed in *chapter 4*, to apply multivariate logistic regression, 1 covariate per 10 events is required. Our data study does not allow to perform multivariate logistic regression and in that case we tested Method B. We believe that Method B is suitable in case of datasets with small percentage of cases versus controls resulting in more powerful outcomes. Even though, the results are always limited by small events in this dataset. Despite this, it is always important prevent side effects that cause a great discomfort and change the daily routine of the patients even when their incidence rate is low.

Clinical Relevance and Applicability of Results

In chapter 4 and 5, it was shown that there are some important parameters which might be correlated with AUR and RB. Patients with Qmax before treatment lower than 10 ml/s have higher risk of AUR and it will be useful in selecting patients for HDR BT.

When patients already have bad urinary condition before treatment, one common procedure is the prescription of medicaments which prevent and improve urinary retention, such as α-blockers or corticosteroids. This effect is very well illustrated by prospective randomized trial [83] where they evaluated in a total of 234 patients, 142 patients who received an α-blocker 1 week prior to treatment versus the remaining patients who did not take it. Only 1.5% of patients who took these medication and 4% of patients who did not had urinary retention. Therefore, Qmax provides additional information and might help in prescription of that medication before treatment.

One of the big issues in radiotherapy is always the balance between acceptable PTV coverage and sparing OAR. We never optimize both sides, if we want to increase, for instance, PTV coverage from 95% to 99% of PD, we always will deliver more dose in OAR. First of all, this is the first study reporting DVH parameters as risk factors of AUR which means more studies will be necessary to prove this relationship. Even more, the patient will benefit if the side effects can be minimized, but it might reduce the tumour control rate. Therefore, our study suggests that we should pay attention to those parameters and try to minimize them without degrading PTV coverage. One of the next steps is to simulate the effect of adding those parameters in treatment constraints and evaluating how much the PTV coverage is affected – planning study. This is the simplest way to evaluate how much the dose distribution will be changed.

In conclusion, in this kind of studies it is always important when we find new predictors but even more meaningful is to understand how we can use that in clinical point-of-view.

6.3 General Conclusion

The risk factors of acute urinary retention and rectal bleeding are likely multifactorial in nature and we only evaluated those side effects by using the available candidates to be a risk factors. For that reason, further research in different cancer institutes might lead to an extension and improvement of the predictive variables for those secondary effects. In conclusion, although this study has some limitations, it provides the first predictive factors for AUR.

[2]Randomized controlled trial for intermediate and high risk Prostate cancer patient treated in two arms: 39 x 2Gy and hypofractionated scheme 19 x 3,4Gy; 7 participating Dutch radiotherapy departments.

6.4 Future Perspectives

In this section I will provide the most recent developments and areas of interest in HDR brachytherapy.

6.4.1 Single fraction HDR BT

In *ErasmusMC - Cancer Institute* (Rotterdam, The Netherlands) a study to treat patients with HDR BT in one fraction is being developed. Hypofractionation regimens with curative intend of prostate cancer have been shown good results, specially, HDR BT delivered in 4 fractions over short period of time [13]. Applying HDR BT in one fraction will improve patient comfort during treatment, improve treatment accuracy excluding needles displacements correction and save time, costs and human resources.

One of the main research groups in brachytherapy and more recently in HDR BT in one fraction, **Hoskin et al.** [84], published in 2014 one study about early urinary and gastroinstestinal adverse events after two or one fraction of HDR BT. Their study suggested that single dose HDR BT delivering 19Gy or 20Gy is associated with higher rates of acute toxicity than seen with two-fraction schedule. They also observed a significant increase in catheter use in the first 12 weeks after implant of 19Gy or 20Gy compared with 2 x 13Gy. Single fraction HDR BT always has an argument of that single dose is less traumatic with the implant in place for only a few hours compared to 24h for two fraction or 36h for 4 fractions schedule. Therefore, single dose HDR BT remains an attractive treatment possibility with an acceptable level of acute complications. However, it is not clear yet what is the best dose level and the search for the optimal HDR BT schedule for prostate cancer remains a challenge.

Recently, in 2016, one research group from Spain published their study [85] where they evaluated acute and late genitourinary, gastrointestinal toxicity and the long-term biochemical control after HDR BT monotherapy in one fraction (19Gy). No severe toxicities were reported and overall survival was 90% ($\pm$ 5%) and biochemical control was 66% ($\pm$ 6%) at 6 years . Therefore, they concluded that single fraction schedule (19Gy) is feasible and well tolerated but not with the same level of LDR biochemical control at 6 years.

In conclusion, this modality of HDR BT seems to be a promising schedule and our study about risk factor for CAD due to AUR might be helpful for those studies.

6.4.2 Brachytherapy uncertainties and *in vivo* dosimetry (based on [1, 86, 87])

One of the most challenging areas in brachytherapy is related to how to measure and take into account the uncertainties during BT treatment. This is the field that still have lot of research work to do. HDR BT is known to have high dose gradients that makes accurate dose measurements and treatment delivery challenging because even small uncertainties may result in large dose discrepancies. Therefore, the main problem is that uncertainties can remain undetectable because those systems do not use independent tools to monitor that.

BT uncertainties come from treatment planning, imaging, anatomical variations and dose delivery. It is essential to identify these uncertainties, their magnitude and their impact on the overall uncertainty of dose delivery to the patients. One example of treatment planning uncertainties is that treatment planning system (TPS) incorporate AAPM Task Group No.43 dose calculation protocol [6]. This assumes that patient is made of water and neglects tissue heterogeneities introducing uncertainties in dose calculation.

Imaging uncertainties are related to organs contouring (user's dependency) and also addressed to computational limitations and assumptions due to the finite slice thickness. Anatomical variation in HDR BT is not a big issue because the treatment delivery time is short. However, for LDR BT, the target volume changes substantially during the time of relevant dose delivery and that is not taken into account in dose calculations. Another source of uncertainty is the post-implant oedema that might overestimate the total dose administered. Dose delivery accuracy depends on the consistency of the patients and delivery geometry

between treatment planning and treatment delivery. Systematic effects of afterloader accuracy is verified and checked by the system itself and it is possible to calibrate and eliminate those discrepancies. However, the precision of the source positioning during treatment remains to be quantified. A new field in brachytherapy will add a significant improvement in this domain named *in vivo* dosimetry (IVD).

Initially, *in vivo* dosimetry was used only to verify dose delivered to the OARs and tumour, namely in techniques administering large dose per fraction as HDR BT, placing the dosimeter inside patients. Nowadays, IVD may be also a tool to detect needles/catheter displacements and organ motions between fractions. Over the years, detectors and sensors have been developed in order to improve the *in vivo* dosimetry but physical and mechanical problems, such as energy or temperature dependence, have been precluded that usage in clinical practice. The implementation of this systems is used mostly in research and development because it might introduce potential risk and discomfort to the patient as well as extra human effort and potential interference with the existing clinical workflow. Therefore, *in vivo* dosimetry is a promising research area in brachytherapy and it still have lot of research to do because there is a lack of good infrastructure in terms of commercially available dosimetry systems with straightforward procedures.

For interested reader in *in vivo* dosimetry and BT uncertainties, we recommend **Kertzscher et al.** [86], **Tanderup et al.**[88] and **Kirisits et al.** [87]. In conclusion, it is important to understand the sources of BT uncertainties and to quantify them in order to improve the outcome in terms of local control and better OAR sparing.

Appendix A

UICC TNM Classification of Prostate Tumors (2009)

Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically inapparent tumor not palpable or visible by imaging
T1a	Tumor incidental histological finding in 5% or less of tissue resected
T1b	Tumor incidental histological finding in more than 5% of tissue resected
T1c	Tumor identified by needle biopsy (e.g., because of elevated PSA)
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
T2c	Tumor involves both lobes
T3	Tumor extends through the prostatic capsule
T3a	Extracapsular extension
T3b	Tumor invades seminal vesicle(s)
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles: bladder neck, external sphincter, rectum, levator muscles, or pelvic wall
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph nodes
N1	Regional lymph node metastasis
M0	No distant metastasis
M1	Distant metastasis

Appendix B

Adapted RTOG/EORTC Late Radiation Morbidity Scoring

Lower Gastro-Intestinal (GI)

Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
None.	Mild diarrhea. Mild cramping. Bowel movement up to 5 times daily. Slight rectal discharge or bleeding	Moderate diarrhea and colic. Bowel movement more than 5 times daily. Excessive rectal mucus or intermittent bleeding. Single laser treatment and/or transfusion.	Watery diarrhea. Obstruction requiring surgery. Bleeding requiring surgery or two or more laser treatments and/or transfusions.	Necrosis. Perforation. Fistula. Abdominal pain or tenesmus requiring tube decompression or bowel diversion.	Death directly related to radiation late effects

Genito-Urinary (GU)

Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
None	Frequency once/≥ 2 hrs. Nocturia 2-3. Slight dysuria or microscopic hematuria requiring no medication. Slight epithelial atrophy. Minor teleangiectasia. Bladder capacity > 300 ml.	Frequency once/1-2 hrs. Nocturia 4-6. Moderate dysuria or intermittent hematuria requiring medication. Moderate teleangiectasia. Bladder capacity 150-300 ml.	Frequency once/<1 hr. Nocturia > 6. Severe dysuria. Severe teleangiectasia. Frequent hematuria. Bladder capacity 100-150 ml. Benign urethral strictures, requiring a TURP, dilation, suprapubic or permanent catheter.	Necrosis. Contracted bladder, capacity <100 ml. Severe hemorrhagic cystitis	Death directly related to radiation late effects

Appendix C

WHO performance status classification

Grade	Explanation of activity
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

Appendix D

Complete results of Chapter 4: *Method A - Small Group*

Table D.1: Completed Result of TEST.A1.

Original Data : TEST.A1		
	Univariate	
Variable	**p-value**	**OR (95%CI)**
Bladder D1cc	0.144*	1.09 (0.97-1.22)
Bladder D2cc	0.098*	1.11 (0.98-1.27)
Bladder D25	0.005*	1.25 (1.07-1.45)
Bladder V75	0.018*	3.36 (1.23-9.19)
Bladder V80	0.034*	4.99 (1.13-22.03)
Bladder V75 (cc)	0.260	-
Bladder V80 (cc)	0.371	-
Bladder Neck D0.1cc	0.747	-
Bladder Neck D0.5cc	0.163*	1.10 (0.96-1.27)
Bladder Neck V80	0.450	-
Bladder Neck V80 (cc)	0.718	-
PUS D0.1cc	0.284	-
PUS D30	0.647	-
PUS V110	0.434	-
PUS V110 (cc)	0.441	-
PUM D0.1cc	0.250	-
PUM D30	0.299	-
PUM V110	0.962	-
PUM (cc)	0.079	71.37 (0.61-8354.10)
PUI D0.1cc	0.363	-
PUI D30	0.645	-
PUI V110	0.745	-
PUI V110 (cc)	0.371	-
UM D0.1cc	0.622	-
UM D0.5cc	0.043*	1.09 (1.00-1.18)
UM D30	0.309	-
UM V100	0.063	1.21 (0.99-1.47)
UM V100 (cc)	0.119*	1.9E+8 (0.008-4.6E+18)
TRUS volume $\geq$ 40 cc	0.036*	4.60 (1.11-19.14)
Qmax $<$ 15 ml/s	0.096*	3.61 (0.79-16.35)
Urinary residue $\geq$ 30 ml	0.910	-
IPSS $\geq$ 10	0.121*	3.33 (0.72-15.26)
Needles $\geq$ 17	0.084*	3.67 (0.83-16.04)
PTV Volume $\geq$ 50 cc	0.132*	2.89 (0.73-11.43)
Urethra length $\geq$ 50 mm	0.509	-
Age $\geq$ 70 year	0.663	-

Abbreviations: * Statistically significant (p-value $\leq$ 0.2);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95 % Confidence Interval.

Table D.2: Completed Result of TEST.A3.

Variable	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144*	0.677	-
Bladder D2cc	0.098*	0.691	-
Bladder D25	0.005*	0.006**	1.32 (1.08-1.60)
Bladder V75	0.018*	0.768	-
Bladder V80	0.034*	0.807	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110(cc)	0.079*	0.351	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043	0.333	-
UM D30	0.309	-	-
UM V100	0.063*	0.104	-
UM V100 (cc)	0.119*	0.515	-
TRUS volume $\geq$ 40 cc	0.036*	0.667	-
Qmax $<$ 15 ml/s	0.036*	0.039**	39.82 (1.19 -1325.86)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.021**	74.11 (1.91-2879.46)
Needles $\geq$ 17	0.084*	0.104	-
PTV Volume $\geq$ 50 cc	0.132*	0.323	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.3: Completed Result of TEST.A4.

Variable	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144*	0.427	-
Bladder D2cc	0.098*	0.362	-
Bladder D25	0.005*	0.006	1.34 (1.09-1.66)
Bladder V75	0.018*	0.702	-
Bladder V80	0.034*	0.780	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110 (cc)	0.079*	0.105	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUi V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043*	0.336	-
UM D30	0.309	-	-
UM V100	0.063*	0.217	-
UM V100(cc)	0.119*	0.264	-
TRUS volume $\geq$ 40 cc	0.036*	0.221	-
Qmax $<$ 10 ml/s	0.025*	0.020**	10.08 (1.44-70.54)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.021**	16.73 (1.53-183.29)
Needles $\geq$ 17	0.084*	0.209	-
PTV Volume $\geq$ 50 cc	0.132*	0.624	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.4: Completed Result of TEST.A6.

Variable	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144*	0.479	-
Bladder D2cc	0.098*	0.412	-
Bladder D25	0.005*	0.006**	1.33 (1.08-1.63)
Bladder V75	0.018*	0.674	-
Bladder V80	0.034*	0.759	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110(cc)	0.079*	0.164	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043*	0.360	-
UM D30	0.309	-	-
UM V100	0.063*	0.218	-
UM (cc)	0.119*	0.259	-
TRUS volume $\geq$ 40 cc	0.036*	0.259	-
Qmax $<$ 10 ml/s	0.025*	0.071	-
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.044**	19.12 (1.08-338.00)
Needles $\geq$ 17	0.084*	0.288	-
PTV Volume $\geq$ 50 cc	0.132*	0.820	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.5: Completed Result of TEST.A7.

Variable	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144*	0.611	-
Bladder D2cc	0.098*	0.626	-
Bladder D25	0.005*	0.008**	1.31 (1.07-1.60)
Bladder V75	0.018*	0.697	-
Bladder V80	0.034*	0.770	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110 (cc)	0.079*	0.210	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043	0.606	-
UM D30	0.309	-	-
UM V100	0.063*	0.165	-
UM V100 (cc)	0.119*	0.833	-
TRUS volume $\geq$ 40 cc	0.036*	0.624	-
Qmax $<$ 15 ml/s	0.125*	0.113	-
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.029**	15.76 (1.36-186.05)
Needles $\geq$ 17	0.084*	0.081	-
PTV Volume $\geq$ 50 cc	0.132*	0.427	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.6: Completed Result of TEST.A8.

Variable	Univariate p-value	Multivariate p-value	OR (95%CI)
Bladder D1cc	0.144*	0.419	-
Bladder D2cc	0.098*	0.333	-
Bladder D25	0.005*	0.007**	1.31 (1.08-1.59)
Bladder V75	0.018*	0.736	-
Bladder V80	0.034*	0.807	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110(cc)	0.079*	0.092	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043*	0.265	-
UM D30	0.309	-	-
UM V100	0.063*	0.211	-
UM V100 (cc)	0.119*	0.246	-
TRUS volume $\geq$ 40 cc	0.036*	0.131	-
Qmax $<$ 10 ml/s	0.066*	0.044**	6.84 (1.05-44.45)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.019**	18.34(1.60-209.99)
Needles $\geq$ 17	0.084*	0.134	-
PTV Volume $\geq$ 50 cc	0.132*	0.444	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.7: Completed Result of TEST.A9.

Variable	Univariable p-value	Multivariable p-value	OR (95% CI)
Bladder D1cc	0.144*	0.737	-
Bladder D2cc	0.098*	0.855	-
Bladder D25 $\geq$ 30% PD	0.023*	0.148	-
Bladder V75	0.018*	0.022**	5.61 (1.29-24.41)
Bladder V80	0.034*	0.199	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110 (cc)	0.079*	0.056	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043*	0.266	-
UM D30	0.309	-	-
UM V100	0.063*	0.163	-
UM V100 (cc)	0.119*	0.225	-
TRUS volume $\geq$ 40 cc	0.036*	0.103	-
Qmax $<$ 10 ml/s	0.066*	0.027**	8.44 (1.27-56.23)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.026**	13.59 (1.37-134.76)
Needles $\geq$ 17	0.084*	0.152	-
PTV Volume $\geq$ 50 cc	0.132*	0.604	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.8: Completed Result of TEST.A10.

Variable	Univariable p-value	Multivariable p-value	OR (95% CI)
Bladder D1cc	0.144*	0.264	-
Bladder D2cc	0.098*	0.275	-
Bladder D25 $\geq$ 30% PD	0.023*	0.031**	26.73 (1.35-529.48)
Bladder V75	0.018*	0.321	-
Bladder V80	0.034*	0.438	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110 (cc)	0.079*	0.050	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043	0.675	-
UM D30	0.309	-	-
UM V100	0.063*	0.141	-
UM V100 (cc)	0.119*	0.374	-
TRUS volume $\geq$ 40 cc	0.036*	0.561	-
Qmax $<$ 10 ml/s	0.066*	0.023**	13.14 (1.43-120.85)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.013**	53.48 (2.35-1217.46)
Needles $\geq$ 17	0.084*	0.191	-
PTV Volume $\geq$ 50 cc	0.132*	0.870	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.9: Completed Result of TEST.A11.

Variable	Univariable p-value	Multivariable p-value	OR (95% CI)
Bladder D1cc	0.144*	0.279	-
Bladder D2cc	0.098*	0.284	-
Bladder D25 $\geq$ 30% PD	0.023*	0.029**	20.26 (1.36-301.85)
Bladder V75	0.018*	0.289	-
Bladder V80	0.034*	0.419	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110 (cc)	0.079*	0.050	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043*	0.978	-
UM D30	0.309	-	-
UM V100	0.063*	0.129	-
UM V100 (cc)	0.119*	0.858	-
TRUS volume $\geq$ 40 cc	0.036	0.882	-
Qmax $<$ 15 ml/s	0.125*	0.169	-
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.028**	32.98 (1.46-744.56)
Needles $\geq$ 17	0.084*	0.090	-
PTV Volume $\geq$ 50 cc	0.132*	0.790	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.10: Completed Result of TEST.A12.

Variable	Univariable p-value	Multivariable p-value	OR (95% CI)
Bladder D1cc	0.144*	0.894	-
Bladder D2cc	0.098*	0.953	-
Bladder D25 $\geq$ 30% PD	0.023*	0.364	-
Bladder V75	0.018*	0.021**	5.03 (1.28-19.77)
Bladder V80	0.034*	0.296	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110 (cc)	0.079*	0.112	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043*	0.730	-
UM D30	0.309	-	-
UM V100	0.063*	0.210	-
UM V100 (cc)	0.119*	0.467	-
TRUS volume $\geq$ 40 cc	0.036*	0.284	-
Qmax $<$ 15 ml/s	0.036*	0.044**	23.35 (1.09-501.17)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.063	-
Needles $\geq$ 17	0.084*	0.137	-
PTV Volume $\geq$ 50 cc	0.132*	0.854	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.11: Completed Result of TEST.A13.

Variable	Univariable p-value	Multivariable p-value	OR (95% CI)
Bladder D1cc	0.144*	0.233	-
Bladder D2cc	0.098*	0.231	-
Bladder D25 $\geq$ 30% PD	0.023*	0.029**	29.56 (1.41-621.51)
Bladder V75	0.018*	0.277	
Bladder V80	0.034*	0.380	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.25	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110(cc)	0.079*	0.056	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
Urethra Membranous D0.1cc	0.622	-	-
Urethra Membranous D0.5cc	0.043*	0.757	-
Urethra Membranous D30	0.309	-	-
Urethra Membranous V100	0.063*	0.155	-
Urethra Membranous V100 (cc)	0.119*	0.456	-
TRUS volume $\geq$ 40 cc	0.036*	0.426	-
Qmax $<$ 10 ml/s	0.046*	0.048**	8.71 (1.02-74.46)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.016**	33.84 (1.91-600.55)
Needles $\geq$ 17	0.084*	0.115	-
PTV Volume $\geq$ 50 cc	0.132*	0.682	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.12: Completed Result of TEST.A14. - Automatic MI

Variable	Univariable p-value	Multivariable p-value	OR (95% CI)
Bladder D1cc	0.144*	0.737	-
Bladder D2cc	0.098*	0.855	-
Bladder D25 $\geq$ 30% PD	0.023*	0.148	-
Bladder V75	0.018*	0.022**	5.61 (1.29-24.41)
Bladder V80	0.034*	0.199	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.25	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110 (cc)	0.079*	0.056	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043*	0.266	-
UM D30	0.309	-	-
UM V100	0.063*	0.163	-
UM V100 (cc)	0.119*	0.225	-
TRUS volume $\geq$ 40 cc	0.036	0.103	-
Qmax $<$ 10 ml/s	0.046*	0.027**	8.44 (1.27-56.22)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.026**	13.59 (1.37-134.76)
Needles $\geq$ 17	0.084*	0.152	-
PTV Volume $\geq$ 50 cc	0.132*	0.604	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Table D.13: Completed Result of TEST.A14. - MCMC MI

Variable	Univariable p-value	Multivariable p-value	OR (95% CI)
Bladder D1cc	0.144*	0.223	-
Bladder D2cc	0.098*	0.231	-
Bladder D25 $\geq$ 30% PD	0.023*	0.029**	29.56 (1.41-621.51)
Bladder V75	0.018*	0.277	-
Bladder V80	0.034*	0.380	-
Bladder V75 (cc)	0.260	-	-
Bladder V80 (cc)	0.371	-	-
Bladder Neck D0.1cc	0.747	-	-
Bladder Neck V80	0.450	-	-
Bladder Neck V80 (cc)	0.718	-	-
PUS D0.1cc	0.284	-	-
PUS D30	0.647	-	-
PUS V110	0.434	-	-
PUS V110 (cc)	0.441	-	-
PUM D0.1cc	0.250	-	-
PUM D30	0.299	-	-
PUM V110	0.962	-	-
PUM V110 (cc)	0.079*	0.056	-
PUI D0.1cc	0.363	-	-
PUI D30	0.645	-	-
PUI V110	0.745	-	-
PUI V110 (cc)	0.371	-	-
UM D0.1cc	0.622	-	-
UM D0.5cc	0.043*	0.757	-
UM D30	0.309	-	-
UM V100	0.063*	0.155	-
UM V100 (cc)	0.119*	0.456	-
TRUS volume $\geq$ 40 cc	0.036*	0.426	-
Qmax $<$ 10 ml/s	0.046*	0.048**	8.71 (1.02-74.46)
Urinary residue $\geq$ 30 ml	0.827	-	-
IPSS $\geq$ 10	0.121*	0.016**	33.84 (1.91 -600.55)
Needles $\geq$ 17	0.084*	0.115	-
PTV Volume $\geq$ 50 cc	0.132*	0.682	-
Urethra length $\geq$ 50 mm	0.509	-	-
Age $\geq$ 70 year	0.663	-	-

* Statistically significant (p-value $<$0.2): Pre-selection;
**Statistically significant (p-value $\leq$0.05);
PUS = Prostatic Urethra Superior; PUM = Prostatic Urethra MID;
PUI = Prostatic Urethra Inferior; UM = Membranous Urethra;
OR = Odd ratio; 95% CI = 95% Confidence interval.

Appendix E

Complete results of Chapter 5: *Rectal Bleeding Project*

Table E.1: Complete result of Mann-Whitney test.

Rectum Variables Cranial Part	RB (n=15) Median	no-RB (n=30) Median	p-value	Rectum Wall Variables Cranial Wall Part	RB (n=15) Median	no-RB (n=30) Median	p-value
Dmean (% of PD)	11.90	8.90	0.028*	Dmean (% of PD)	11.79	8.48	0.010*
D1cc (% of PD)	15.39	15.35	0.665	D1cc (% of PD)	16.13	14.45	0.132
D2cc (% of PD)	13.95	13.79	0.665	D2cc (% of PD)	13.69	12.23	0.107
D10 (% of PD)	15.50	13.38	0.107	D10 (% of PD)	17.57	13.56	0.028*
D25 (% of PD)	13.67	10.69	0.034*	D25 (% of PD)	13.61	10.03	0.011*
V10 (cc)	5.33	7.77	0.682	V10 (cc)	4.93	4.585	0.360
V20 (cc)	0.09	0.05	0.672	V20 (cc)	0.24	0.05	0.183
V30 (cc)	-	-	-	V30 (cc)	-	-	-
V40 (cc)	-	-	-	V40 (cc)	-	-	-
Caudal Part				**Caudal Wall Part**			
Dmean (% of PD)	26.63	26.53	0.886	Dmean (% of PD)	26.53	27.63	0.962
D1cc (% of PD)	71.96	73.96	0.312	D1cc (% of PD)	71.96	73.98	0.324
D2cc (% of PD)	64.98	67.29	0.413	D2cc (% of PD)	64.95	66.44	0.386
D10 (% of PD)	50.55	51.32	0.427	D10 (% of PD)	58.48	60.63	0.258
D25 (% of PD)	34.45	34.97	0.613	D25 (% of PD)	34.52	37.20	0.248
V10 (cc)	51.27	48.87	0.981	V10 (cc)	24.54	23.17	0.373
V20 (cc)	32.78	31.40	0.613	V20 (cc)	14.72	14.63	0.942
V30 (cc)	19.05	17.27	0.895	V30 (cc)	8.13	9.32	0.516
V40 (cc)	10.46	10.10	0.923	V40 (cc)	5.21	6.21	0.605
V75 (cc)	0.70	0.90	0.253	V75 (cc)	0.70	0.89	0.238
V80 (cc)	0.21	0.43	0.323	V80 (cc)	0.23	0.41	0.360
V90 (cc)	0.00	0.02	0.481	V90 (cc)	0.00	0.02	0.481
Anus				**Anus Wall Part**			
Dmean(% of PD)	14.13	14.63	0.523	Dmean (% of PD)	14.34	14.42	0.596
D1cc(% of PD)	21.61	22.13	0.754	D1cc (% of PD)	20.21	20.76	0.754
D2cc (% of PD)	19.06	19.02	0.791	D2cc (% of PD)	16.97	17.26	0.754
D10 (% of PD)	20.32	21.71	0.773	D10 (% of PD)	20.47	21.82	0.700
D25 (% of PD)	16.32	17.77	0.700	D25 (% of PD)	16.27	17.62	0.665
V10 (cc)	9.70	8.39	0.500	V10 (cc)	5.77	5.82	0.571
V20 (cc)	1.53	1.59	0.691	V20 (cc)	1.04	1.15	0.718
V30 (cc)	0.03	0.04	0.844	V30 (cc)	0.03	0.03	0.844
V40 (cc)	-	-	-	V40 (cc)	-	-	-

Abbreviations: * Statistically significant.

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