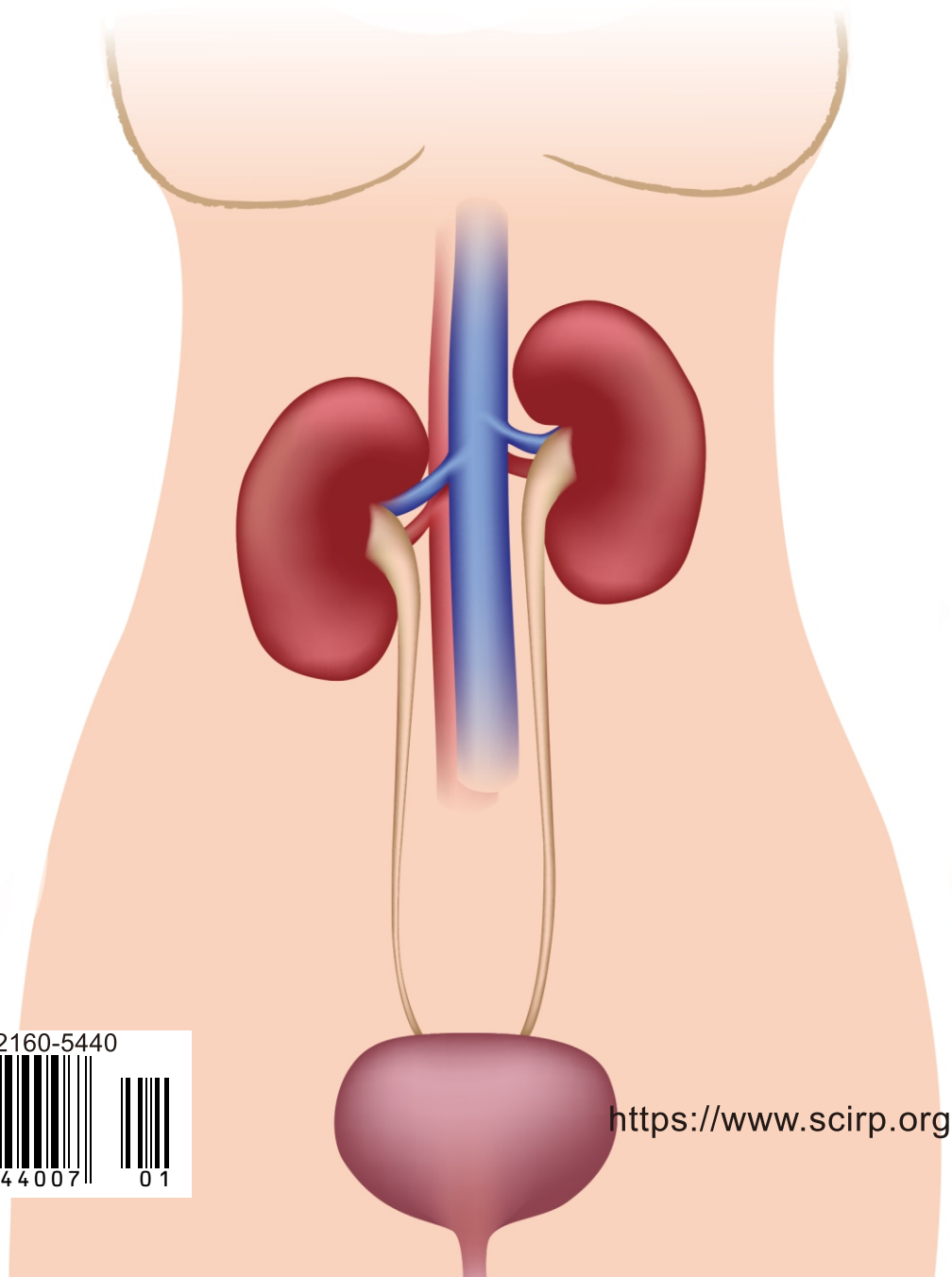
ISSN: 2160-5440 Volume 11, Number 1, January 2021

Scientific Research Publishing

Open Journal of Urology



ISSN: 2160-5440

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ISSN 2160-5440 (Print) ISSN 2160-5629 (Online)
https://www.scirp.org/journal/oju

Scientific Research Publishing

Open Journal of Urology, 2021, 11, 1-29
https://www.scirp.org/journal/oju
ISSN Online: 2160-5629
ISSN Print: 2160-5440

Table of Contents

Volume 11 Number 1 January 2021

Open Journal of Urology (OJU)
Journal Information

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Open Journal of Urology, 2021, 11, 1-5
https://www.scirp.org/journal/oju
ISSN Online: 2160-5629
ISSN Print: 2160-5440

Penile Metastasis of Colorectal Cancer Mimicking Priapism with Acute Urinary Retention: A Rare Case

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How to cite this paper: Xian, G.L.S., Tan, L.K. and Lee, G.E.G. (2021) Penile Metastasis of Colorectal Cancer Mimicking Priapism with Acute Urinary Retention: A Rare Case. *Open Journal of Urology*, **11**, 1-5.
https://doi.org/10.4236/oju.2021.111001

Received: November 24, 2020
Accepted: January 11, 2021
Published: January 14, 2021

Abstract

Background: Penile metastasis of colorectal carcinoma is a rare phenomenon in clinical setting. They normally manifest as penile lesion and acute urinary retention. However, presentation of priapism is exceedingly rare. **Aims:** Discussion of this rare presentation as well as the diagnostic processes and subsequent management. **Case Presentation:** A 54-year-old male with a history of colorectal cancer presents with acute urinary retention. Examination of the patient demonstrates a semi-erect penis, with multiple palpable nodules on the shaft and penile meatus. Histological and imaging findings indicate penile metastasis of colorectal cancer. **Conclusion:** Biopsy via cystoscopy is used to obtain definitive diagnosis of penile metastasis. Urinary drainage followed by further cancer intervention or palliative care is crucial for effective management.

Keywords

Metastatic Colorectal Cancer, Urinary Retention, Priapism, Secondary Penile Malignancy

1. Introduction

Colorectal carcinoma is a common malignancy affecting large demographic of populations across the world. Secondary colorectal cancers are also well documented in literature, with common sites of metastasis to the lungs, liver, bones and brain. Although prostate and penile tissues are in close proximity to sigmoid colon and have an extensive circulatory connection, metastases of colorectal cancer to them are rare. The first reported case was by Eberth in 1870 when he

reported penile metastasis from the rectum. In total, there have been about 400 cases of penile malignancy due to metastasis reported with only less than 60 cases related to colorectal cancer to have ever been reported in literatures [1] [2] [3]. Penile metastasis of colorectal cancer has clinical manifestation, such as penile nodules, priapism, urinary retention, and skin nodules. However, presentation of skin nodules and priapism are exceptionally rare [4] [5]. We present a rare case of such metastasis mimicking priapism, resulting in acute urinary retention. The diagnostic process and subsequent management are also discussed.

2. Case Presentation

A 54-year-old male with a history of colorectal cancer, who had undergone hemicolectomy 2 years ago, presents with acute urinary retention. The histological and post-operative imaging taken during his initial diagnosis 2 years ago revealed a T2 adenocarcinoma with no evidence of lymph node involvement (T2N0M0). Present examination of the patient demonstrates a semi-erect penis, with multiple palpable nodules on the shaft and penile meatus. Urethral catheterization was not possible due to the persistent erection and occluding metastatic nodules. Intra-cavernosal aspiration of penis arterial blood sample was analysed, demonstrating no evidence of high or low flow priapism. Pelvic CT scans also confirmed multiple lesions in corpus cavernosum, likely derived from the local recurrence of the colorectal cancer **Figure 1**. Doppler Ultrasound study of the patient shows no vascular abnormality of the caversonal arteries of corpus cavernosum and corpus spongiosum which excludes priapism. The ultrasound imaging also revealed multiple ill-defined lesions invading corpus cavernosum as well as on the subcutaneous tissues **Figure 2**. The patient subsequently underwent emergency cystoscopy to facilitate urethral drainage, relieving the patient of the retention of urine as well as obtaining subcutaneous and urethral penile samples for biopsy to aid the diagnosis. The histological analysis with haematoxylin and eosin stained of the urethral biopsy revealed tissue lined by urothelial epithelium with underlying malignant tumour and lympho-vascular permeation. Specimen from exterior penile biopsy shows ulcerated mucosa (stratified squamous epithelium)

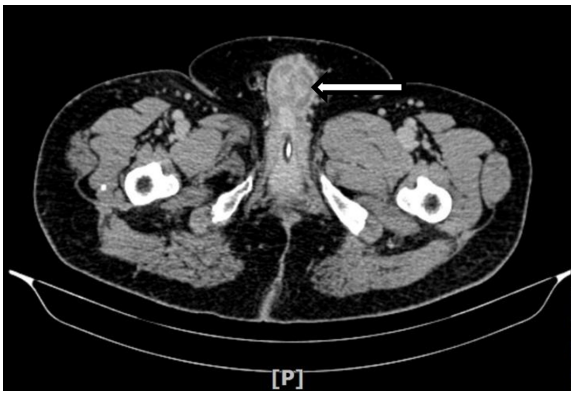

Figure 1. Pelvic CT scan showing marked thickening and heterogenous enhancement within corpus cavernosa.

with moderate neutrophils infiltration. Further immunohistochemistry differentiation also confirmed specimens to be CDX 2 and CK 20 positive, while CK 7 and PSA were negative Figure 3. The histological analysis definitively indicates that both lesions originated from the metastasis of colorectal carcinoma. Following confirmative diagnosis, the patient underwent abdomino-perineal resection followed by 6 months of adjuvant chemotherapy, Xeloda. Patient's routine CT scan and colonoscopy was done 6 months after the end of chemotherapy which shows no recurrences.

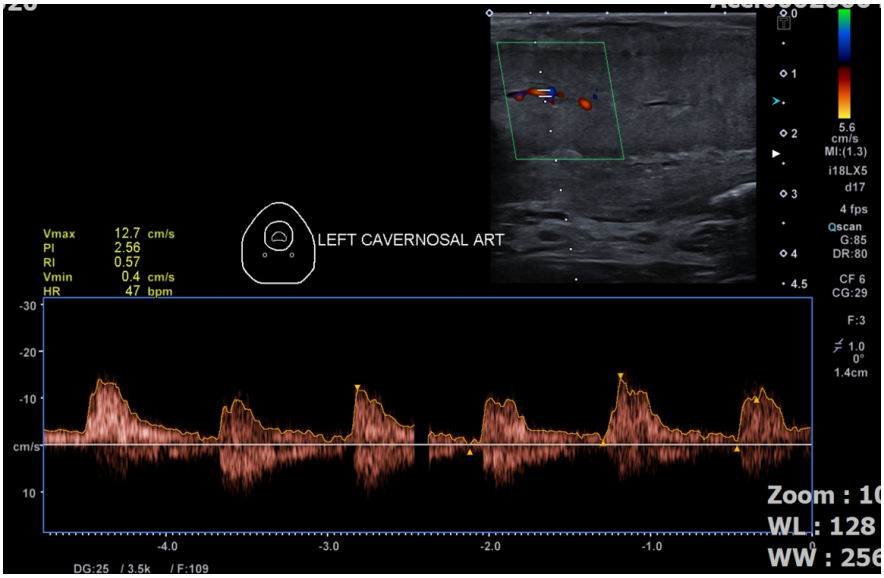

Figure 2. Doppler Ultrasound multiple ill-defined masses in corpus cavernosum bilaterally and subcutaneous tissues.

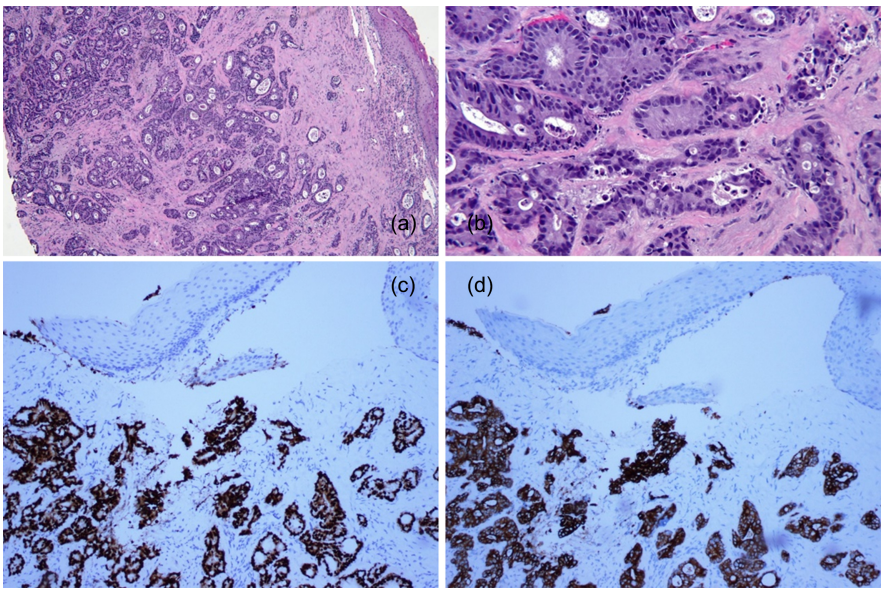

Figure 3. (a) H&E staining on urethral biopsy specimen; Lympho-vascular permeation is noted (original magnification ×100); (b) H&E staining on penile biopsy specimen; No Pagetoid spread seen (original magnification ×400); (c) CDX2 positive; (d) CK20 positive.

3. Discussion

Penile metastasis was first described by Eberth in 1870 [3]. Since the initial publications, only 400 such cases were recorded in literature. The penis is a rare site for metastasis. The stipulated mechanisms of metastasis can either be by venous route, lymphatic system, arterial spread, direct extension or by iatrogenic implantation as described by Paquin and Roland in 1956 [2] [5]. As a result of rich communication between the dorsal penile venous system and the pelvic organ, venous spread is the most likely mechanism of metastasis [6].

The clinical manifestation associated with penile metastasis are as acute urinary retention, priapism, penile nodules, skin nodules, generalize swelling, and oedema [3]. The penile nodules presented in secondary penile malignancy are usually deep within the corpus cavernosa rather than superficially like primary penile cancers. Imaging modalities such as Ultrasound, CT and MRI scans are ideal non-invasive methods to evaluate characteristics of lesion.

The presentation mimicking priapism in this case is believed to be due to neoplastic invasion of the corpus cavernosum which has cause impairment of venous return of the penis, causing accumulation of blood at the penis. Intra-cavernosal aspiration of penis arterial blood sample was analysed, demonstrating no evidence of high or low flow priapism. The patient had also presented with urinary retention, attempts of catherization to relieve the retention was not possible due to neoplastic lesion causing a blockage, restricting the access for the catheter.

As part of emergency management, cystoscopy is highly recommended as the procedure relieves the patient of retention of urine as well as providing an opportunity to obtain biopsy samples as the only definitive method of diagnosing penile metastasis requires fine-needle aspiration biopsy of the lesion for further histopathology and immunohistochemistry confirmation [3]. It is important to note that the process of diagnosing a patient with penile metastasis are by the process of elimination as it is crucial to first exclude other differential diagnosis such as primary penile cancer, chancre, chancroid, non-tumorous priapism, Peyronie's disease, tuberculosis and other inflammatory and suppurative diseases [1] [2].

The prognosis of the patient is generally described as poor as overall survival for patients with secondary penile malignancy is approximately nine month with one study describing 100% mortality rate [4]. Treatment plan may vary depending on the general health of the patient, as well as the site of primary, extent of metastatic spread and the severity of symptoms [2]. Options includes local excision, penectomy, chemotherapy and radiotherapy. However due to the poor prognosis of secondary penile malignancy, Palliative treatment and improvement of quality of life are main treatments for these patients. Invasive treatments such as partial or complete penectomy are not suggested because the survival rate enhancement is unremarkable, except in patients with small lesions which may yield a positive outcome [5]. The primary aim in the management of patient with metastasis penile malignancy is early detection, precise diagnosis and

non-invasive treatment for improvement of quality of life [1].

4. Conclusion

Penile metastasis of colorectal cancer is a rare phenomenon as there have only been around 400 cases reported since 1870. However, penile metastasis that mimics priapism resulting in acute urinary retention has not been previously reported. Non-invasive investigation such as imaging is ideal to evaluate the penile lesion and exclusion of the possibility of priapism. Fine-needle aspiration biopsy is required for definitive histopathology and immunohistochemistry confirmation. Following definitive diagnosis, urinary drainage is necessary to relieve urinary retention followed by further cancer intervention or palliative management to improve the quality of life of the patient. The prognosis for metastatic penile malignancy is poor.

Statement of Informed Consent

A verbal informed consent was obtained from the patient for the information and images used in this publication.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Scientific Research Publishing

Open Journal of Urology, 2021, 11, 6-16
https://www.scirp.org/journal/oju
ISSN Online: 2160-5629
ISSN Print: 2160-5440

Prediction of Intraoperative Trifecta Achievement during Laparoscopic Partial Nephrectomy

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How to cite this paper: Barnoiu, O.-S., Baron, F.J., Sæter, T., Tysland, A.O. and Andersen, A. (2021) Prediction of Intraoperative Trifecta Achievement during Laparoscopic Partial Nephrectomy. *Open Journal of Urology*, **11**, 6-16.
https://doi.org/10.4236/oju.2021.111002

Received: December 27, 2020
Accepted: January 24, 2021
Published: January 27, 2021

Abstract

Purpose: We introduce the concept of intraoperative Trifecta during laparoscopic partial nephrectomy (LPN) as the simultaneous achievement of estimated blood loss (EBL) < 500 ml, warm ischemia time (WIT) < 20 minutes and minimal changes of the intraoperative course. The study's aim was to find preoperative factors that could predict the likelihood of achieving intraoperative Trifecta and build a surgical nomogram. **Methods:** We retrospectively evaluated 122 patients who underwent LPN. Preoperative factors like age, sex, body-mass index (BMI), kidney function, tumor characteristics (R.E.N.A.L. score) and Charlson-Comorbidity-Index (CCI) were recorded. Intraoperative complication (IOC) was graded according to the Rosenthal classification. R software was used to find a predicting model for achievement of Trifecta using preoperative variables and a nomogram was built. **Results:** The surgical features include median EBL of 100 ml having 6.5% bleed > 500 ml, median WIT of 12 minutes having 7.3% more than 20 minutes. There was recorded a 12.3% IOC with a mean Rosenthal's grade of 0.2. Intraoperative Trifecta was achieved in 105 patients (86%) and three preoperative factors were chosen for the predictive model: BMI ($p = 0.041$), CCI ($p = 0.037$) and RENAL score ($p = 0.002$). A nomogram was generated and the ROC-AUC of the model was 75.8%. **Conclusion:** We have defined an intraoperative Trifecta concept as the achievement of EBL < 500 ml, WIT < 20 minutes and minimal changes of the intraoperative course. A nomogram was developed from preoperative factors like BMI, CCI and R.E.N.A.L. score. It can be used to estimate the probability of Trifecta achievement in patients treated with LPN.

Keywords

Intraoperative Complications, Laparoscopic Partial Nephrectomy, Prediction, Trifecta

1. Introduction

Partial nephrectomy (PN) represents the standard of care for patients diagnosed with T1a kidney cancer [1]. Minimally invasive nephron-sparing surgery should be performed if this approach does not compromise oncological, functional and perioperative outcomes. However, these approaches are technically challenging and are associated with a high rate of complications that has been reported in up to 30% of cases [2]. Hemorrhage and transient renal insufficiency are the most common concerns during PN. An increased hospital mortality [3] and risk for intraoperative transfusion [4] were found in those patients whose estimated blood loss (EBL) exceeded 500 ml. Vascular clamping during PN is associated with kidney function impairment and attempts should be done to limit warm ischemia time (WIT) to 20 minutes [5].

Many trials fail to report intraoperative complications (IOC). Rosenthal *et al.* defined in 2015 and classified IOC depending on the need for treatment and the severity of complication [6]. We tried to evaluate these complications by using a Trifecta concept and we defined the intraoperative Trifecta as the achievement of EBL < 500 ml, WIT < 20 minutes and no other change of normal intraoperative course/or changes without any consequences.

The study's aim was to find preoperative factor that could predict the likelihood of achieving intraoperative Trifecta and build a surgical nomogram.

2. Materials and Methods

We retrospectively evaluated the patients who underwent laparoscopic PN (LPN) at our institution between January 2015-December 2018 and 122 patients had registered the IOC directly after surgery and qualified for the statistical analysis.

In order to achieve our study's aim, we first did a univariate analysis of preoperative variables and a multivariate one, having achievement of Trifecta as the main variable, and then built a nomogram for those factors that showed to be predictable for our newly introduced intraoperative Trifecta.

We analyzed the preoperative factors like age, sex, body-mass index (BMI), kidney function evaluated by estimated glomerular filtration rate (eGFR) and tumor characteristics, like size, side, type (solid or cystic) and number. Morphometric score like R.E.N.A.L.-score (Radius, Exophytic/endophytic, Nearness, Anterior/posterior, Location), as described by Kutikov [7], was assigned in an unblinded manner by the same urologist (OSB). Comorbidity status was evaluated using the Charlson Comorbidity Index (CCI) and physical status by the American Society of Anesthesiologists (ASA) classification system. All the data

were collected from patients' electronic medical record, in a retrospective manner, and stored in our kidney cancer database reviewed and accepted by our Institution's Research and Ethics Committee. Variables like BMI were obtained using an online calculator (https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/english_bmi_calculator/bmi_calculator.html) the same way as CCI (https://www.mdcalc.com/charlson-comorbidity-index-cci). GFR was automatically calculated by our Laboratory Unit.

There were to surgeons that performed the procedures and both of them have overcome a learning curve of more than 300 LPN before the observational period. Both surgeons started doing LPN in our Unit in 2004 and performed more than 30 procedures per year, each. The procedures were performed using both pure laparoscopic or hand-assisted technique.

The intraoperative covariates consisted of total operation time, WIT and EBL, both recorded and agreed between the surgeon and the anesthesiologist, the usage of drainage tube, and performance of standard or hand-assisted technique.

Any deviation from the ideal intraoperative course occurring between skin incision and skin closure, regardless whether it was related to surgery or anesthesia, was considered as an IOC and graded according to the Rosenthal classification [6]. The classification includes four grades depending on the need for treatment (no need, grade 1; need for treatment, grade 2) and the severity of the complication (life-threatening/permanent disability, grade 3; death, grade 4). Because of expected variability in reporting complications of grade 1, both grade 0 (no deviation from the ideal intraoperative course) and 1 (any deviation without need of treatment) war taken together for subsequent analysis. No cystic lesion were cut or ruptured during the resection.

Statistical Analysis

Demographic and clinical outcomes were analyzed using descriptive statistics. R Core Team (2019) software was used to find the best predicting model for the achievement of intraoperative Trifecta using preoperative variables. A nomogram was built and receiver operating curve (ROC) and areas under the curve (AUC) were calculated and used to quantify predictive discrimination. Statistical significance was set at $p < 0.05$. Internal validation and variance of AUC-ROC processes were performed using bootstrapping with 10,000 repetitions.

3. Results

The clinical characteristics of the population are summarized in **Table 1**. The cohort comprised 65.6% males, mean BMI was 27.5 kg/m^2 and median CCI was 5. Most of the patients had ASA II with a median preoperative kidney function of 89.5 ml/min/1.73 m^2. Most of the tumors were solid (73.8%) with a median tumor diameter of 2.45 cm and a median RENAL score of 6.5 points. The surgical features include a median operation time of 157 minutes, median EBL 100

ml having 6.5% (eight patients) bleed more than 500 ml, median WIT of 12 minutes having 7.3% (nine patients) more than 20 minutes. There was used a drainage tube in 82% of the patients and a hand-assisted technique was performed in 23%. General and surgical postoperative complications (POC) occurred in 27% and 4% respectively (three patients with bleeding, one bowel injury and one chylous ascites).

There was recorded a 12.3% (15 patients) IOC with a mean Rosenthal's grade of 0.213. Of these 15 patients, six had a grade 1, six had grade 2 and three had grade 3 (two conversions to nephrectomy and one splenectomy). Some patients (three) had more than one complication but only the highest grade was assigned.

Table 1. Patient demographics.

	Overall (n = 122)
Age	
Mean (SD)	61.5 (12.2)
Median [Min, Max]	65.0 [18.0, 85.0]
Sex	
Man	80 (65.6%)
Woman	42 (34.4%)
BMI (kg/m^2)	
Mean (SD)	27.5 (4.78)
Median [Min, Max]	27.1 [17.3, 47.9]
CCI	
Mean (SD)	4.58 (1.55)
ASA	
ASA I	6 (4.9%)
ASA II	71 (58.2%)
ASA III	44 (36.1%)
ASA IV	1 (0.8%)
Tumor type	
Solid	90 (73.8%)
Bosniak 2F	0 (0%)
Bosniak 3	13 (10.7%)
Bosniak 4	19 (15.6%)
Tumor number	
Mean (SD)	1.04 (0.237)
Side	
Right	66 (54.1%)
Left	56 (45.9%)
CT diameter	
Mean (SD)	2.69 (1.20)
R.E.N.A.L. score	
Mean (SD)	6.42 (1.79)
Median [Min, Max]	6.50 [4.00, 10.0]

BMI: body mass index, CCI: Charlson Comorbidities Index, ASA: American Society of Anesthesiologists classification system.

Intraoperative Trifecta was achieved in 105 patients (86%) and four preoperative factors correlated with Trifecta: BMI (p = 0.041), CCI (p = 0.037), CT diameter (p = 0.036) and R.E.N.A.L. score (p = 0.002) as shown in Table 2. The best nomogram generated of R software included just BMI, CCI and R.E.N.A.L.-score (Figure 1). The ROC AUC of the model was 75.8%. After a bootstrapping with 10,000 repetitions, the model reported a bias of −0.022 and a standard error of 0.216.

Table 2. Multivariate analysis assessing the association between intraoperative Trifecta achievement and preoperative factors.

	Trifecta NOT achieved (n = 17)	Trifecta achieved (n = 105)	p-value
Age			
Mean (SD)	63.9 (11.2)	61.1 (12.4)	0.348
Sex			
Man	14 (82.4%)	66 (62.9%)	0.196
Woman	3 (17.6%)	39 (37.1%)	
BMI (kg/m^2)			
Mean (SD)	29.0 (3.70)	27.3 (4.90)	**0.041**
CCI			
Mean (SD)	5.29 (1.86)	4.47 (1.47)	**0.037**
Number			
Mean (SD)	1.06 (0.243)	1.04 (0.237)	0.746
Side			
Right	9 (52.9%)	57 (54.3%)	0.912
Left	8 (47.1%)	48 (45.7%)	
CT diameter			
Mean (SD)	3.55 (1.77)	2.56 (1.02)	**0.036**
R.E.N.A.L. score			
Mean (SD)	7.59 (1.46)	6.23 (1.77)	**0.002**
Surgeon			
1	14 (82.4%)	75 (71.4%)	0.518
2	3 (17.6%)	30 (28.6%)	
Drainage			
No	0 (0%)	22 (21.0%)	0.081
Yes	17 (100%)	83 (79.0%)	
Hand-assisted			
Yes	3 (17.6%)	25 (23.8%)	0.803
No	14 (82.4%)	80 (76.2%)	
Tumor (Solid)			
FALSE	4 (23.5%)	28 (26.7%)	1
TRUE	13 (76.5%)	77 (73.3%)	

BMI: body mass index, CCI: Charlson Comorbidities Index, ASA: American Society of Anesthesiologists classification system.

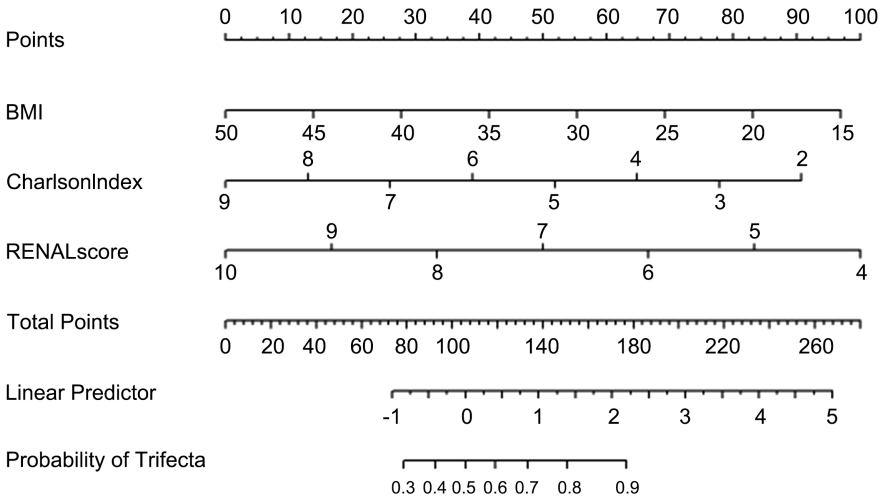

Figure 1. Nomogram predicting intraoperative Trifecta achievement. BMI: body mass index, CCI: Charlson Comorbidities Index.

4. Discussion

Various trifecta combinations exist, all of which are mostly used to measure postoperative outcomes after PN; for example Buffi [8] and Porpiglia [9] using the term MIC (negative Margin, Ischemia time < 20 minutes and no major Complications), Khalifeh [10] encompassed no positive surgical margin, zero complications and WIT < 25 minutes. Hung [11] defined Trifecta as a composite outcome of negative margin, no urological complications and no renal function loss of > 90%. We are the first to define a Trifecta only using intraoperative variables and tried to correlate it with preoperative factors in order to predict its accomplishment. Our intraoperative Trifecta was achieved in 86% of the patients attesting to the efficacy of LPN in the minimally invasive surgical treatment of kidney cancer.

EBL is the first component in our definition and intraoperative hemorrhage from the PN bed is an important concern. In no case in our group did significant hemorrhage occur during parenchymal and tumor resection, and in just two cases critical bleeding occurred after hilar unclamping. An increased risk for intraoperative transfusion [4] was found in those patients whose EBL exceeded 500 ml, the value chosen for our Trifecta. Most of the patients bleed < 100 ml and only eight bleed > 500 ml with need of transfusion in five patients (three of them with grade 2 according with Rosenthal classification and the 2 patients that needed nephrectomy and intraoperative transfusion).

Our second component was WIT with the cut-off value of 20 minutes as first used by Thompson *et al.* [5]. Zargar [12] and Khalifeh [10] used the 25 minutes cut-off to define their Trifecta and Propiglia [13] have shown no renal function impairment in WIT < 30 minutes. Ficarra [14] reported a group of patients with 36% having a WIT over 20 minutes and found tumor size, PADUA score and surgeon experience to be predictors of a WIT more than 20 minutes and IOC. In our study median WIT was 12 minutes and only in 7.3% was more than 20 mi-

nutes and in two patients more than 30 minutes, which, again, suggests that LPN is a feasible technique.

Third, and last component, included in our Trifecta was the common variableused in all previous definitions being that the presence of complications, in our case intraoperative complications. The classification most widely used and with most evidence in literature we found to be the Rosenthal classification [6]. Grade 0 and 1 were taken together as both no complication (grade 0) and complication with no need for treatment (grade 1), have no consequence for the surgical outcome. The six patients assigned as grade 1 included four patients with bleeding from trocar site, kidney dissection or kidney vein injury without any need for transfusion and two patients with respiratory distress or atrial flutter recorded intraoperatively without any hemodynamic consequence or need for treatment. Only nine patients had complications grade 2 and 3 and no death (grade 4) was recorded in our series.

BMI, CCI and R.E.N.A.L.-score were the preoperative factors that were predictive for achievement of intraoperative Trifecta. It is worldwide believed that high BMI can increase the operation time and blood loss but data regarding association between elevated BMI and POC after LPN is controversial. Wiens [15] showed that obese patients undergoing LPN are not at significantly increased risk of complication relative to non-obese patients and that comorbidity status and R.E.N.A.L.-score should be the main criteria to take into account to evaluate feasibility for LPN. On the contrary, Kott [16] found that a BMI over 30 kg/m^2 was a significant factor for POC associated with robot assisted LPN. In our analysis there was a relative significant correlation between achievement of intraoperative Trifecta and BMI. Comorbidity status, assessed with CCI, was a predictive factor for our Trifecta, and there are authors like Larcher [17] showing a correlation between CCI and complications after LPN. As for patients performance status we included also ASA score that failed to reach significance at multivariable analysis. ASA score was found to be significant predictive factor in a nomogram used by Mari [18] in the RECORD2 project in order to predict the likelihood of POC. Tumor anatomy is also a well-known factor that correlates with POC and anatomical characteristics of the renal tumor could be evaluated by many morphometric scores like, for example, the R.E.N.A.L.-score by Kutikov [7], PADUA by Ficarra [19] and C-index by Simmons [20]. Other anatomical characteristics like renal tumor invasion [21] were not assessed by the RENAL score that we used in our study and chosen as the standard morphometric score in our unit because of its worldwide demonstrated reproducibility and validity [22] [23]. This was the preoperative factor that most significantly correlated with the intraoperative Trifecta. We did not find a correlation between the suffix of the R.E.N.A.L.-score and the Trifecta as seen by Reddy [24]. There are few reports evaluating the true value of a single component included in the anatomical scores [25] and, in our analysis, the tumor density, side and number were not significant variables while tumor diameter was.

Other preoperative factors like age, sex, and surgeon, use of drainage tube or use of hand-assisted technique did not correlate with intraoperative Trifecta achievement. Age is a factor found to predict POC by two main nomograms of Larcher [17] and Mari [18]. Median age for these studies was 73 and 64 years respectively, compared to a younger population in our series with median age of 61 years. Age is a factor included in the CCI that correlated better in our study than age alone. Bindayi and RESURGE group [26] analyzed the Trifecta outcomes in elderly patients over 75 years and found a 40% Trifecta achievement and less transfusion and lower intraoperative complications in the Trifecta patients.

In our group, no difference was seen between the two surgeons as both have overcome alearning curve of more than 300 procedures, being 70 procedures needed according to Buffi to achieve Trifecta in 87.9% of the patients [8]. No difference between hand-assisted and pure laparoscopic technique was seen in order to achieve all three components of our Trifecta. Azawi [27] studied the impact of using a hand-assisted technique on the learning curve and found that the surgeon must perform 40 procedures to obtain a WIT of five minutes. Our study did not individually analyze the correlation between hand-assisted laparoscopic PN (HALPN) and R.E.N.A.L.-score alone but the three factors of Trifecta together. Elsamra [28] compared HALPN with the robot-assisted and found no significant advantage of robot-assisted over HALPN in short-term outcomes.

Our study is the first, in our knowledge, evaluating the factors that could predict IOC alone, and building a nomogram out of preoperative risk factors. A predictive tool, such as this, can enable clinicians to evaluate the risk of IOC according to specific patient and tumor related factors. It can also estimate more accurately the risk stratification on each individual case before treatment and could guide the learning curve of future kidney cancer surgeons allowing to choose the right patient for the right step in the learning curve.

The limitations of the study are the relative small sample size. The tumors operated on were relatively small and low to intermediate complexity, which we believe represents patients we typically treat in our daily practice in this era of CT-diagnosed abdominal symptoms and incidental finding of early kidney cancer. The study offers several opportunities for future research by using the intraoperative Trifecta concept in the robot-assisted field and in bigger national kidney cancer registers. It can be also used to analyze the correlation with postoperative outcomes and thereby measure the effect of its achievement.

The nomogram from our study should to be tested on multicenter cohorts in order to externally validate and generalize our findings. We believe there was a high-quality report of IOC that was guaranteed by the rigorous recording of data of both surgeon and anesthesiologist in our complications register.

5. Conclusion

We have defined an intraoperative Trifecta concept to evaluate the IOC during

LPN as the achievement of EBL < 500 ml, WIT < 20 minutes and no other changes or changes without any consequences over the normal intraoperative course. A nomogram was developed from preoperative predictive factors including BMI, CCI and R.E.N.A.L.-score and it can be used to estimate the probability of intraoperative Trifecta achievement in patients treated with LPN.

Acknowledgements

The study was approved by the local ethics committee. All patients signed informed consent.

Conflicts of Interest

The authors report no conflicts of interest and no funding for the study.

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Scientific Research Publishing

Open Journal of Urology, 2021, 11, 17-29
https://www.scirp.org/journal/oju
ISSN Online: 2160-5629
ISSN Print: 2160-5440

Automatic Computer Analysis of Digital Images of Triple-Antibody-Stained Prostate Biopsies

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How to cite this paper: Wilander, E., de la Torre, M., Wilhelmsson, U. and Nygren, S. (2021) Automatic Computer Analysis of Digital Images of Triple-Antibody-Stained Prostate Biopsies. *Open Journal of Urology*, **11**, 17-29.
https://doi.org/10.4236/oju.2021.111003

Received: December 12, 2020
Accepted: January 26, 2021
Published: January 29, 2021

Abstract

Background: Worldwide, prostatic adenocarcinoma is the most common tumour type among men. **Aim:** The aim of the present investigation was to develop a computer program to identify normal prostate biopsies and distinguish them from biopsies showing premalignant alterations (LGPIN, HGPIN) and adenocarcinoma. **Method:** Prostate biopsies (n = 2094) taken from 191 consecutive men during 2016 were stained with triple immunehistochemisty (antibodies to AMACRA, p63 and CK 5). Digital images of the biopsies were obtained with a scanning microscope and used to develop an automatic computer program (Cellda^{TM}), intended to identify the morphological alterations. Visual microscopic finding was used as a reference. **Result:** Of the 191 men, 121 (63.4%) were diagnosed as having prostate adenocarcinoma and 70 (36.6%) as having no malignancy on the basis of the visual microscopy. In comparison, computer analysis identified 134 (70.2%) men with malignant disease and 57 (29.8%) with non-malignant disease after exclusion of artifacts, which constituted 10.4% of areas (indicated as malignant disease). Discrepant results were recorded in 15 (7.9%) men, and in 14 of these cases, HGPIN and areas suggestive of early invasion were common. Thus, it was uncertain whether these cases should be regarded as malignant or not. The agreement between the visual examination and the computer analysis was 92.1% (kappa value 0.823, sensitivity 99.2 and specificity was 0.80). **Conclusion:** It seems that computer analysis could serve as an adjunct to simplify and shorten the diagnostic procedure, first of all by ensuring that normal prostate biopsies are sorted out from those sent for visual microscopic evaluation.

Keywords

Prostate, Adenocarcinoma, LGPIN, HGPIN, Antibody, Computer, Digital, Images, Automatic, Analysis, AMACR, P504S, Microscopy, Scanning

1. Introduction

Prostatic adenocarcinoma is one of the most common tumour types throughout the world, and consequently accurate histological diagnosis is an important issue worldwide [1]. However, the visual diagnosis of prostatic adenocarcinoma by light microscopy is associated with several challenges. Ordinary microscopy is to some extent subjective, and this is reflected in high intra-pathologist and inter-pathologist variability, resulting in both over- and under-diagnosis of prostate cancer [2] [3].

Prostatic adenocarcinoma is the most prevalent type of cancer in men in Sweden, with over 10,000 new cases diagnosed every year [4]. It makes up around 30% of all male cancer cases and occurs mainly in older men; accordingly, 70% of the tumours are diagnosed in men aged 70 or older. Over 2000 men die from prostatic cancer every year, and prostatic adenocarcinoma is the most common cause of death due to a malignant tumour among men in Sweden.

There is no organised screening for prostatic adenocarcinoma in Sweden, but middle-aged and older men are recommended to screen themselves for prostatic adenocarcinoma by having a blood test for analysis of PSA (prostatic-specific antigen). High levels of PSA indicate an increased risk of prostatic adenocarcinoma. Men with elevated levels of PSA are recommended to obtain a referral to a urological surgeon so that biopsy samples can be taken from the prostate gland. Such biopsies form the ultimate basis of a diagnosis of prostatic adenocarcinoma, although other visual methods such as ultrasonography and data tomography have a role as adjuncts [5] [6].

In Sweden, around 20,000 men are examined by means of biopsies from the prostate gland every year; since in most cases 12 biopsies are collected from every man, this means that around 250,000 biopsies from prostate glands are examined every year by light microscopy, by doctors trained in surgical pathology.

Currently, rapid progress is being made in the use of digital techniques such as scanning microscopy and automatic analysis of digital images in the field of laboratory medicine. It is likely that these techniques will play a much more dominating role as an adjunct to ordinary visual microscopy in the near future [7] [8] [9].

The time interval from presentation at a hospital out-patient department to treatment for men with prostate cancer in Sweden is around 6 months, due to lack of available resources, and among the latter, the lack of surgical pathologists is quite an important component. The situation is similar internationally [10].

The aim of the present article is to describe a method for rapid screening of prostate biopsies by automatic computer analysis of digital images obtained by scanning microscopy. The analysis is performed after triple antibody immuno-staining of the biopsies. The study focused on developing a method for identifying and separating out all normal biopsies, and indicating different pathological changes, such as low-grade prostatic intraepithelial neoplasia (LGPIN), high-grade prostatic intraepithelial neoplasia (HGPIN) and adenocarcinoma,

using different colour frames on the images, thus making it possible to markedly reduce the number of biopsies that have to be sent for careful visual microscopic examination. This will allow more rapid diagnosis of pathological changes by a surgical pathologist. Notably, as a rule, the majority of the prostate biopsies show normal tissue, not malignant or pre-malignant morphological changes [5].

2. Materials and Methods

The study was carried out at the Department of Pathology and Cytology, County Hospital, Gävle, Sweden, a department equipped with facilities for digital pathology and a scanning microscope (Hammamatsu, Nano Zoomer S360) allowing a magnification of ×800. Accordingly, histological sections are examined on a data screen and not by ordinary visual light microscopy.

Prostate biopsies are carried out on men being investigated on the basis of a blood test showing elevated PSA levels (as a rule, 12 ultrasound-assisted biopsies are obtained in each patient). The needle biopsies (0.9 mm in diameter, 18 ga) are fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned in about 4-µm-thin sections. The sections are routinely stained with haematoxylin-eosin and examined on a data screen. In selected cases the examination is completed with triple immuno-staining of the biopsies (see below).

During 2016, all prostate biopsies were immuno-stained in addition to ordinary staining with haematoxylin-eosin. For the immune-stain (Ventana instrument), three different antibodies were used: AMACR (alphamethylacyl-CoA racemase) antibody (clone name P504S), p63 and CK5 (cytokeratin 5) according to a certified protocol (Roche). Each glass slide was labelled with a serial number and personal identification code. The glass slides also contained antibody control sections from normal kidney (AMACR) and normal skin (p63 and CK5). Digital images of the triple-immune-stained biopsies were obtained using a scanning microscope (Hammamatsu, Nano Zoomer S 360) allowing a magnification of ×800.

The prostate gland is formed of glandular epithelium surrounded by connective tissue. Myoepithelial cells are located at the periphery of the gland. For pathological analysis, myoepithelial cells are immuno-stained with p63 antibodies in the nucleus (diaminobenzidine) and CK5 antibodies in the cytoplasm (red alkaline phosphatase). The gland's epithelial cells do not stain with AMACR. However, the AMACR antibody does stain pre-malignant epithelial cells, such as LGPIN and HGPIN, and those that have undergone transformation to adenocarcinoma. LGPIN is, relatively, the mostly weakly stained, whereas HGPIN and cancer stain more strongly. The myoepithelial cells do not constitute a component of malignant prostate tissues and thus cannot be identified. Consequently, in prostatic adenocarcinoma the glandular cells are as a rule strongly immuno-stained with AMACR (brown staining) whereas the peripheral myoepithelial cells have disappeared (**Figure 1(C)**). In normal prostate gland the glandular cells are unstained with AMACR and the peripheral myoepithelial cells are im-

mune-stained with antibodies to p63 and CK5 (brown nucleus and red-stained cytoplasm) (**Figure 2(B)**). Thus, in the staining pattern with triple immuno-staining (antibodies to AMACR, p63 and CK5), the two colours used, brown and red, show fundamentally different pictures in normal prostate gland and in prostatic adenocarcinoma. This discrepant staining pattern can be put to use by constructing an automatic computer program that can be used to analyse digital images. Haematoxylin was used for background staining (light blue) of the sections.

In total, biopsies from 564 men were collected during 2016 and digital images were obtained by a scanning microscope. From the digital archive of prostate images, consecutive, non-selected biopsies from 191 men (corresponding to 2094 biopsies) were collected and the digital images were used for automatic computer analysis (Cellda™, MM18 medical AB, Uppsala, Sweden). The computer program is based on a classic analysis system for measuring colour saturation, colour type and colour distribution. Images from biopsies recorded as normal by the computer program were indicated by a green frame around the edge of the image, while LGPIN changes were indicated by a blue frame and HGPIN changes by a yellow frame. Areas in biopsies identified as prostate adenocarcinoma were indicated by one or more red frames. Tissue artifacts observed by the computer were also indicated by a red frame.

3. Image Analysis

In order to assign the patient to the "normal" or "abnormal" category, the program must determine whether any image belonging to that patient contains signs of cancer. Thus, the program runs image analysis on the input images.

The analysis is based on defining cancer colours (by means of a list of possible value combinations for hue, saturation and brightness) and then searching the images for sufficient quantities of pixels within the cancer colour range. The detection result is further refined by looking for red colour in the image (indicating healthy cells) and reducing the weighting of cancer detection near it. In addition, reduction of false positives is needed, and is achieved by:

1) Detecting and removing intestine (artifacts) by shape analysis (a high concentration of tiny white vacuoles in one location indicates intestine).

2) Removing thin outer edges of prostate biopsies from analysis, because they contain disproportionate amounts of cancer colour false positives (usually connective tissue) and are therefore ignored even if there is no cancer anywhere else.

In the Cellda program, the cancer colour definition is input from a cancerColor.png input file. Cellda does not itself change or determine the cancer colour definition. We use a separate program for creating and refining the cancer colour definition, and then saving that as the cancerColor.png file.

The results of the automatic computer analysis performed by Cellda were compared with the original visual-microscopy anatomic pathology diagnosis given at the time the biopsies were collected, which were used as a reference. The

original diagnosis was mainly based on haematoxylin-eosin-stained sections, but triple-antibody-stained sections had occasionally been used as an adjunct in cases where changes of uncertain significance occurred, such as atypical changes or those suggestive of malignancy.

All prostate adenocarcinomas were originally classified according to the Gleason grading system. Gleason 3 + 3 occurred in 18 cases (24%), Gleason 3 + 4 in 24 cases (31%), Gleason 4 + 3 in 10 cases (13%), Gleason 4 + 4 + in 0 cases (0%), Gleason 3 + 5 in 8 cases (10%), Gleason 5 + 3 in 1 case (1%), Gleason 4 + 5 in 8 cases (10%), Gleason 5 + 4 in 8 cases (10%) and Gleason 5 + 5 in 1 case (1%).

4. Results

One aim of the study was to investigate to what extent automatic computer analysis of digital images of immuno-stained histological sections could be used to identify normal (benign) and non-malignant prostate tissue and distinguish it from prostate tissue with pre-malignant and malignant changes. Another aim was to examine to what extent the different pre-malignant alterations such as LGPIN and HGPIN could be identified and distinguished from each other and from invasive adenocarcinoma. Various kinds of benign changes such as inflammation, fibro-myo-glandular hyperplasia and metaplasia in the prostate gland were not the focus of the analysis.

Of the 191 men included in the study, 121/191 (63.4%) were diagnosed as having prostate adenocarcinoma and 70/191 (36.6%) as having no malignancy on the basis of visual microscopy (Table 1). A total of 2174 biopsy samples were visually examined, of which 660/2174 (30.4%) were malignant and 1514/2174 (69.6%) non-malignant (Table 2). In comparison, the Cellda computer program identified 134/191 (70.2%) men as having cancer (Figure 1(A), Figure 1(B) and Figure 1(C)) and 57/191(29.8%) as having no malignancy (Figure 2(A) and Figure 2(B)). On the biopsy level, 761/2094 biopsies (36.3%) were regarded as malignant and 1333/2094 (63.7%) as non-malignant after exclusion of red frames showing tissue artifacts; the artifacts were mainly caused by folding of the tissue section, which occurred in 262/2524 (10.4%) areas with red frames.

Table 1. Correlation* in 191 men between a Cellda computer analysis of digital images of prostate biopsies, after antibody staining (AMACRA, p63 and CK5), and ordinary visual microscopy.

Cellda analysis	Visual microscopy		
	Cancer	Benign**	Total
Cancer	120	14	134
Benign**	1	56	57
Total	**121**	**70**	**191**

*Agreement 92.1% and *kappa value* 0.823 (almost perfect agreement) [26]; **Includes LGPIN (low-grade prostatic intraepithelial neoplasia) and HGPIN (high-grade prostatic intraepithelial neoplasia) alterations.

Table 2. Correlation between Cellda computer analysis of digital images of prostate biopsies after antibody staining (AMACR/p63/CK5) and ordinary visual microscopy of biopsies from 191 men.

	Cellda analysis	Visual microscopy*
Benign	1092 (52.1%).	1514 (69.6%)**
LGPIN	87 (4.2%)	
HGPIN	154 (7.4%)	
Cancer	761 (36.3%)	660 (30.4%)
Total	**2094 (100%)**	**2174 (100%)**

*The Cellda program and the visual microscopy did not always examine identical number of biopsies; **Benign cases by visual microscopy included cases with LGPIN (low-grade prostatic intraepithelial neoplasia) and HGPIN (high-grade prostatic intraepithelial neoplasia).

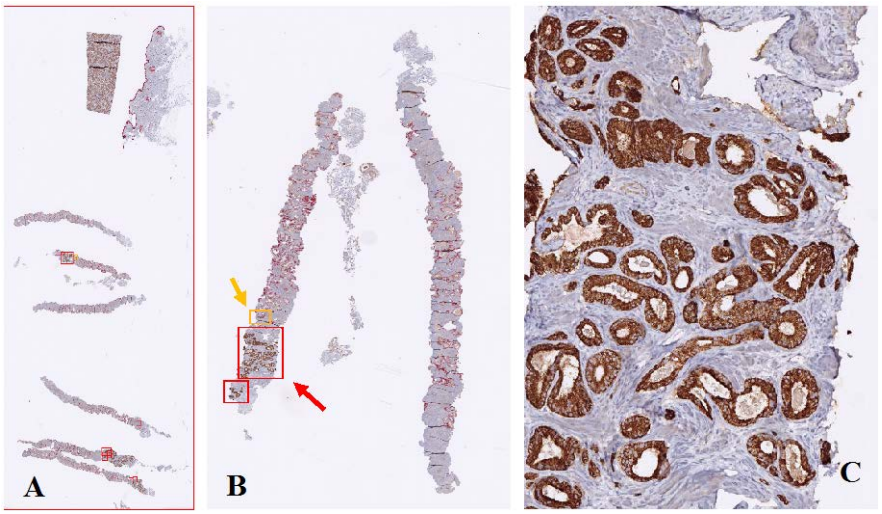

Figure 1. A glass slide analysed by Cellda surrounded by a red frame indicating the presence of abnormal biopsies (A). A number of smaller red frames (B) indicate prostate cancer (red arrow) and one yellow frame indicates HGPIN (yellow arrow). An area within one red frame, (C) is shown at higher magnification and demonstrates the presence of prostate cancer (Gleason 3 + 3).

The computer program identified 1092/2094 (52.1%) biopsies as benign, *i.e.* it surrounded the glass slide image of these biopsies with a green frame (**Figure 2(A)** and **Figure 2(B)**). All these biopsies were also benign according to the result of the visual microscopic analysis (reference), indicating 100% agreement with visual microscopy among these cases.

Computer analysis of biopsies classified by visual microscopy as benign also identified LGPIN in 87/2094 (4.2%) (**Figure 3(A)** and **Figure 3(B)**) and HGPIN in 154/2094 (7.4%) (**Figure 4(A)** and **Figure 4(B)**). When these biopsies were included among the benign biopsies, the proportion of benign biopsies increased to 63.7%. The discrepancy between the Cellda analysis and the visual microscopic analysis was only 5.9% and could mainly be explained by the finding that

the computer analysis identified HGPIN lesions with borderline changes, throwing a suspicion on invasive cancer, leading to a computer diagnosis of adenocarcinoma. The discrepancy was partly due to the observation that the computer program grouped HPGIN lesions with areas showing partly undefined borders and a lack of red stained myoepithelial cells as malignant (Figure 5(A) and Figure 5(B)). Accordingly, the Cellda analysis identified more biopsies with malignant changes or changes suggestive of malignancy.

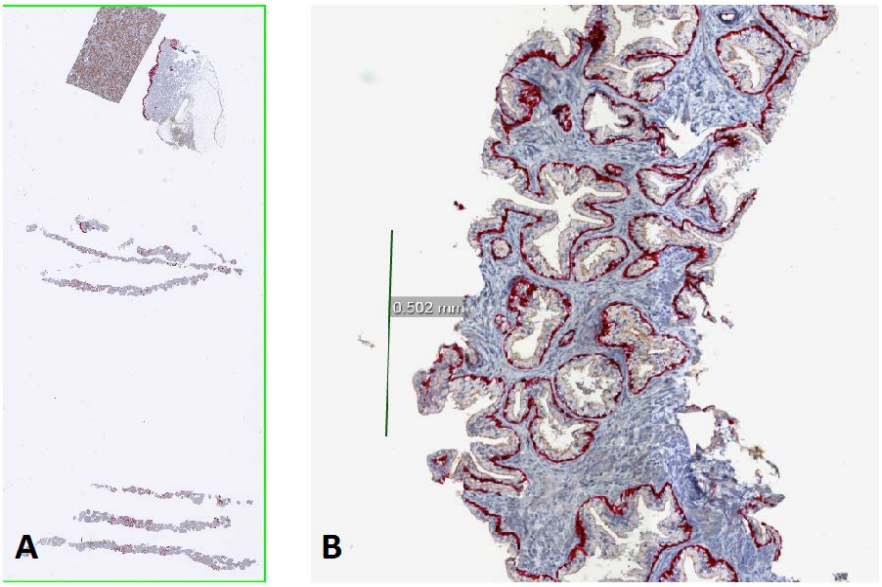

Figure 2. A glass slide analysed by Cellda with prostate biopsies surrounded by a green frame (A) indicating that all biopsies on the slide are normal, as is shown at a higher magnification (B). The two tissue sections at the top of A represent control sections of the antibody-staining.

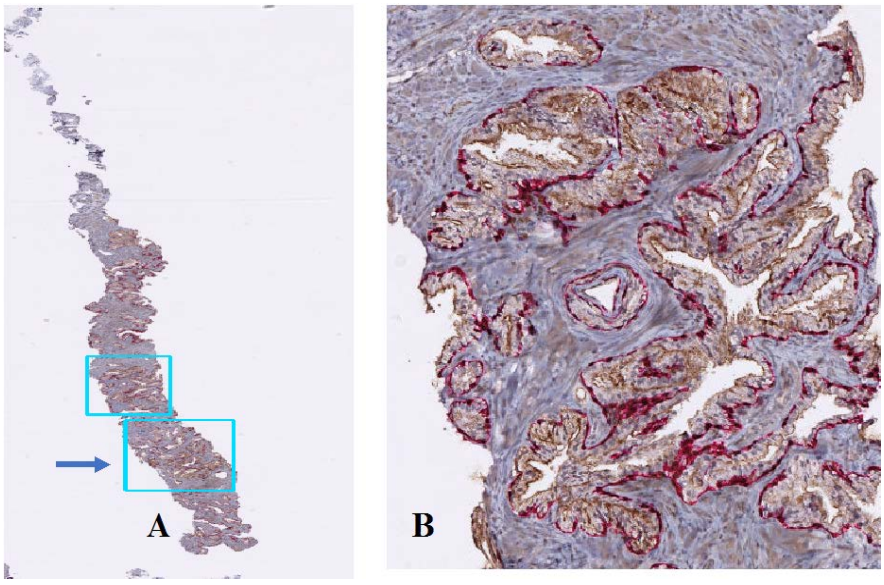

Figure 3. Prostate biopsy analysed by Cellda with two blue frames (A) indicating the presence of LGPIN (arrow) in these two areas, as demonstrated at higher magnification (B).

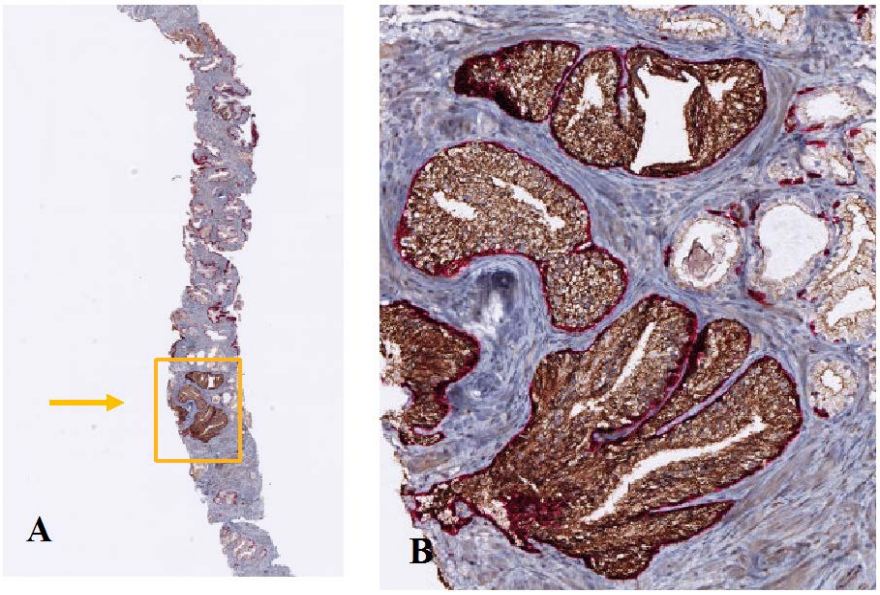

Figure 4. Picture of a prostate biopsy (A), analysed by Cellda containing a yellow frame (arrow) indicating the presence of HGPIN as demonstrated at higher magnification (B).

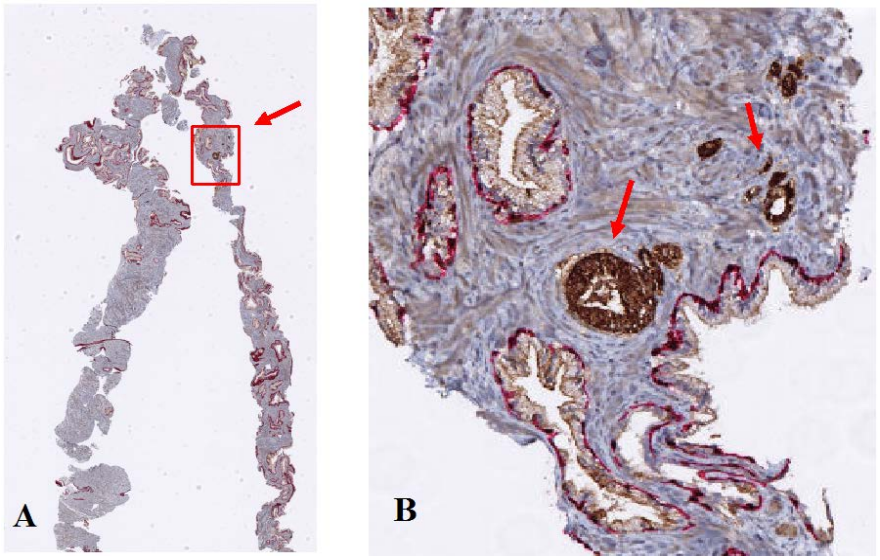

Figure 5. Two prostate biopsies (A) analysed by Cellda with a red frame in the right biopsy, indicating prostate cancer. At higher magnification it is morphologically considered as a borderline case (red arrows) between HGPIN and early invasive cancer (B).

Of the 14 men with a visual microscopic diagnosis of non-malignant disease and a diagnosis of cancer on the basis of computer analysis, 11 showed HGPIN, in some cases extensive, with from 1 to 18 areas with yellow (HGPIN) frames in addition to the red frames indicating cancer. Three of these men had a previous or later diagnosis of adenocarcinoma. Biopsies from two men contained areas with blue frames indicating LGPIN, and one man with a malignant diagnosis was without pre-malignant changes in conjunction with a malignant diagnosis. This further, underlines that most of these men were on the borderline between

pre-malignant and malignant disease.

The results on the individual level shown in **Table 1** indicate a 92.1% rate of agreement between the two methods and a kappa value of 0.823. The sensitivity was 99.2 and the specificity was 0.80.

The discrepancy was mainly due to the higher number of cancers recognised by the Cellda analysis (**Figure 5(A)** and **Figure 5(B)**), as also indicated in **Table 1**. One case was recorded after visual microscopy as cancer Gleason 3 + 3 occurring in one biopsy in a small focus of 0.7 mm in diameter. By Cellda analysis, this was considered as a case with LGPIN, but the small cancer focus was not identified.

5. Discussions

Prostate biopsies still constitute the ultimate basis for the diagnosis of prostatic adenocarcinoma, although other visual methods such as ultrasonography and data tomography are used as adjuncts. Non-microscopy methods have too low a specificity for a secure diagnosis of prostate cancer [5].

During the past decade, scanning microscopy and visual analysis of digital images on a data screen have become more commonly used as a diagnostic method in pathology departments, and the method is beginning to replace ordinary visual microscopy. This trend facilitates the application of computer techniques for analysis of digitised microscopic tissue sections. In the long run, the computer method will probably gradually relieve the pressure on pathologists and reduce their workload [9].

In line with this trend, a number of recent scientific publications have investigated computer methods mainly based on deep learning and artificial intelligence (AI), including studies of prostate biopsies [11] [12] [13]. The investigations are usually performed on haematoxylin-eosin-stained prostate sections. The focus is often on the goal of grading prostate cancer according to Gleason, in order to obtain results that are less time consuming and more reproducible than those obtained with visual microscopy [14]-[19]. It is well known that agreement between pathologists in the assessment of biopsies and Gleason grading is less than optimal. It has also been suggested that an AI system could improve sensitivity by detecting adenocarcinoma foci that would otherwise be accidentally overlooked [3] [4].

This study used a computer method based on classical image analysis, and the tissue sections were not haematoxylin-eosin-stained but stained with a triple antibody stain (AMACRA, p62 and CK5). This is because antibody staining gives sharper and stronger colour identification of the different tissue components. It is well known that AMACRA antibody staining is negative in normal prostate glands and positive in the presence of HGPIN and adenocarcinoma. Meanwhile, the myoepithelial cells in the periphery of the glandular structures stain with p63 in the nucleus and CK5 in the cytoplasm—features that are of importance in the evaluation of prostate tissue structures [20] [21] [22] [23].

The most prominent problem with the present automatic computer analysis as performed with Cellda was the labelling of tissue artifacts, which were mostly due to folding of the tissue sections or by overstaining caused by variations in tissue thickness. This labelling of artifacts occurred in 10.4% of the "indications" (red frames) produced by the computer program.

On the biopsy level, the computer program identified 63.7% of biopsies as non-malignant, and the corresponding figure for visual microscopy was 69.6%. The discrepancy was only 5.9%, indicating good correlation. Cancer was identified by the computer program in 36.3% of biopsies and by visual microscopy in 30.4%. The discrepancy was minor and was caused by the occurrence of artifacts in red frame areas. The computer program identified 5.9% more biopsies with cancer in comparison with visual microscopy. This discrepancy can be explained by the computer program identifying early adenocarcinoma or borderline cases with HGPIN and focal loss of myoepithelial cells as suggestive of infiltrating adenocarcinoma but with insufficient evidence of indisputable invasive adenocarcinoma.

In antibody-stained sections the HGPIN lesions were easily recognised, showing dark brown staining of glandular cells and red staining of the surrounding myoepithelial cells. This observation is of some significance, since men with HGPIN, preferably of multiple origin, are at increased risk of developing adenocarcinoma compared with men with only normal biopsies [24] [25]. In accordance, 25% of biopsies regarded as non-malignant after visual analysis showed HGPIN alterations after computer analysis and after exclusion of the 14 males with borderline alterations.

This investigation is to our knowledge the first to describe automatic computer analysis of prostate biopsies stained with a triple antibody stain. It is possible that automatic scanning of immune-stained prostate tissue, followed by digital computer analysis of the images, could be used as a screening method in the future. This method would allow normal prostate images to be sorted out from those showing premalignant and malignant alterations [18]. The normal biopsies could thus be set aside from those passed on for visual microscopic examination by a specialist in surgical pathology, considerably reducing the workload for pathologists.

It might also be possible to introduce computer analysis as a tool in the diagnosis and Gleason grading of prostate adenocarcinoma [14]-[19]. The advantage would be that the well-known problem with variation between diagnoses obtained by different pathologists would be reduced [2] [3]. The method would also be expected to be considerably faster and more cost effective than the present visual procedure, especially given the lack of specialists in surgical pathology.

The computer program is undergoing a process of refinement. One of the main objects of concern is the identification of artifacts by the computer program and refining the handling of the tissue biopsies, by more careful sectioning, to avoid the occurrence of tissue artifacts. In addition, by adding new informa-

tion to the computer program it may even prove possible to Gleason-grade cancerous biopsies.

Acknowledgements

The authors (EW and SN) are shareholders of MM18 Medical AB. Financial support has been received from EIT Health, EU, Uppsala University Innovation, ALMI and the National Board of Health and Welfare in Sweden. The support with the development of the computer program by Tadas Marcinkevicius is appreciated. The computer program, Cellda is patent pending. The study was approved by the local ethics committee, Dnr. 2020-03586.

All authors made equal contributions to the study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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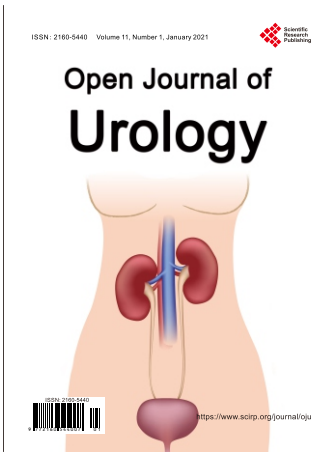

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