BJU Int 2022 doi:10.1111/bju.15938

BJUI
BJU International

Original Article

Confocal laser microscopy for assessment of surgical margins during radical prostatectomy

Diederik J.H. Baas[1,2], Willem Vreuls[3], J.P. Michiel Sedelaar[2,4], Henricus J.E.J. Vrijhof[2,5], Robert J. Hoekstra[2,5], Saskia F. Zomer[3], Geert J.L.H. van Leenders[6], Jean-Paul A. van Basten[1,2] and Diederik M. Somford[1,2]

[1]*Department of Urology, Canisius Wilhelmina Hospital, Nijmegen,* [2]*Prosper Prostate Cancer Clinics, Nijmegen, Eindhoven,* [3]*Department of Pathology, Canisius Wilhelmina Hospital,* [4]*Department of Urology, Radboudumc, Nijmegen,* [5]*Department of Urology, Catharina Hospital, Eindhoven, and* [6]*Department of Pathology, Erasmus MC Cancer Institute, University Medical Centre, Rotterdam, The Netherlands*

Objective

To evaluate the feasibility of confocal laser microscopy (CLM) for intraoperative margin assessment as faster alternative to neurovascular structure-adjacent frozen-section examination (NeuroSAFE) during robot-assisted radical prostatectomy (RARP).

Patients and Methods

Surgical margins were assessed during 50 RARP procedures in patients scheduled for NeuroSAFE. Posterolateral sections were cut and imaged with CLM and further processed to conform with the NeuroSAFE protocol. Secondary resection (SR) was performed in case a positive surgical margin (PSM) was observed with NeuroSAFE. Afterwards, the CLM images were non-blinded assessed for the presence of PSMs. The accuracy of both NeuroSAFE and CLM was compared with conventional histopathology. Agreement for detection of PSMs between NeuroSAFE and CLM was evaluated with Cohen's kappa coefficient. Procedure times were compared with a Wilcoxon signed-ranks test.

Results

In total, 96 posterolateral sections of RP specimens were evaluated for the presence of PSMs. CLM identified 15 (16%) PSMs and NeuroSAFE identified 14 (15%) PSMs. CLM had a calculated sensitivity, specificity, positive predictive value and negative predictive value of 86%, 96%, 80% and 98% respectively for the detection of PSMs compared to definite pathology. After SR, residual tumour was found in six of 13 cases (46%), which were all identified by both techniques. There was a substantial level of agreement between CLM and NeuroSAFE ($\kappa = 0.80$). The median procedure time for CLM was significantly shorter compared to NeuroSAFE (8 vs 50 min, $P < 0.001$). The main limitation of this study was the non-blinded assessment of the CLM images.

Conclusions

Compared to NeuroSAFE, CLM is a promising technique for intraoperative margin assessment and is able to reduce the time of intraoperative margin assessment.

Keywords

confocal laser microscopy, digital imaging, frozen section, NeuroSAFE, prostate cancer, radical prostatectomy, surgical margins, #PCSM, #ProstateCancer, #uroonc

Introduction

Robot-assisted radical prostatectomy (RARP) in patients with localised prostate cancer aims for radical removal of the tumour whilst minimising injury to surrounding tissue. Despite being an oncological effective treatment, long-term side-effects like urinary incontinence (3–16%) and erectile dysfunction (20–90%) are frequently reported after RARP [1–3]. Preservation of the neurovascular bundles (NVBs) in patients undergoing RARP may spare erectile function and is therefore recommended when erectile preservation is desired [4]. However, nerve-sparing surgery (NSS) implies an increased risk of positive surgical margins (PSMs) [5]. The presence and extent of a PSM is associated with a higher risk of biochemical recurrence (BCR) [6–9].

Several novel intraoperative imaging techniques based on cellular imaging for both in vivo and ex vivo surgical margin

assessment have been evaluated in the past, but implementation in clinical practice has been limited due to technical challenges, lack of evidence, and cost-effectiveness [10]. Frozen section can be used for intraoperative surgical margin assessment, but its value remains controversial. Adaptation has therefore been varying and limited due to the lack of prospective randomised trials [11]. In some high-volume expert-centres, eligible patients are offered intraoperative neurovascular structure-adjacent frozen-section examination (NeuroSAFE) during RARP, to minimise the risk of a PSM in NSS. This procedure was first described by Eichelberg et al. [12]. Since then, its reproducibility and performance has been described in multiple series [11,13–17]. Recently published perioperative outcomes of the prospective randomised controlled trial (NeuroSAFEPROOF; ClinicalTrials.gov Identifier: NCT03317990) reported an excellent histopathological concordance between NeuroSAFE and definite histopathology, with a sensitivity of 100% and specificity of 92.7% [18]. However, the reported mean duration of RARP with NeuroSAFE was 72 min longer compared to RARP without NeuroSAFE. This demonstrates that NeuroSAFE is an accurate, but time-consuming, laborious, and costly procedure, restricting its use in clinical practice. New in vivo imaging techniques for intraoperative surgical margin assessment could serve as faster and less laborious alternatives to NeuroSAFE.

The concept of whole surface imaging has been investigated with the use of fluorescence confocal laser microscopy (CLM). CLM is an optical technique that generates high-resolution digital images of ex vivo tissue whilst preserving the tissue for further histopathological examination. It has been previously demonstrated to be useful for diagnosing prostate cancer on prostatic biopsies [19]. Recently, Rocco et al. [20] performed a prospective study in 24 patients, evaluating CLM of posterolateral shavings from the prostate for intraoperative margin assessment. They found the use of CLM to be reliable, faster, and less expensive compared to conventional histopathology. Furthermore, the use of digital images instead of conventional frozen sections is suitable for remote assessment. A comparison with NeuroSAFE was not made.

The aim of this study was to evaluate if CLM is a feasible technique for intraoperative surgical margin assessment during RARP in a direct comparison with NeuroSAFE.

Patients and Methods

Between May and October 2021, 50 patients scheduled for RARP with bilateral NeuroSAFE were included for subsequent evaluation with CLM. The indication for NeuroSAFE was based on the patient-reported preoperative quality of erectile function and tumour characteristics. Patients were discussed in a surgical tumour board meeting at which the indication for NeuroSAFE was confirmed. Both techniques were compared with conventional histopathological examination of the tissue. The local scientific review committee gave approval for this study (CWZ 031-2021).

Surgical Procedure

Bilateral NSS RARP was performed by one of five experienced urologists (M.S., E.V., R.H., J.vB., D.S.) in a single centre. Following resection of the prostate, two thin slices of ~2 × 1 cm from the left and right posterolateral side were sectioned by the operating urologist as described by Beyer et al. [15]. This technique was adapted to avoid the use of ink, which interferes with the fluorescent agent that is used prior to the acquisition of the CLM images. A suture was placed at the intraprostatic side of the slice to maintain anatomical orientation.

Confocal Laser Microscopy Imaging Procedure

The digital CLM images were acquired using the Histolog® Scanner, manufactured and provided for use during this study by SamanTree Medical SA, Lausanne, Switzerland. The Histolog scanner is a Conformité Européene (CE) certified scanning device for in vitro diagnostics with a wide-field-of-view confocal laser scanning microscope, designed for scanning large biological specimens in a clinical setting. Tissue fluorescence is excited by a laser at the wavelength of 488 nm and fluorescence emission is collected at a wavelength >500 nm. The fluorescence image provide seamless images without additional post-processing.

After arrival in the pathology laboratory, the tissue slices are processed by a pathology technician with prior experience with the NeuroSAFE procedure, who received additional training on specimen handling and operation of the Histolog Scanner. The slices were immersed in a fluorescent agent (Histolog Dip, SamanTree Medical SA) for 10 s and afterwards rinsed in 0.9% saline. Starting with the left side, the slice was placed on the scanner tray allowing to image 48 × 36 mm in one field of view with the external side facing down (*en face*), the internal side with the suture facing upwards (Fig. 1). A small bag of flour was placed on the slice to ensure full contact of the slice with the scanning surface. A low-resolution preview image was acquired in ~7 s to make a technical judgement by the trained pathology technician on the completeness of the image, presence of excess fluid, and absence of air bubbles. If necessary, the slice was repositioned and a high-resolution digital image of 2 µm lateral resolution and 30 µm depth was acquired within 50 s. The same procedure was done with the tissue slice of the right side. The total procedure time was recorded for each case. The CLM start time was defined as arrival of the specimen at the pathology laboratory and end time was defined as acquisition

Fig. 1 Utilisation of the Histolog® scanner. A preview image is generated to ensure correct imaging of the surface.

of the last high-resolution CLM image. The reporting time of the CLM images was not included in the procedure time. The slices were then further processed for the NeuroSAFE procedure.

Neurovascular Structure-Adjacent Frozen-Section Examination Procedure

The left and right slices were inked, using separate colours for NVB resection margin and prostatic side, cut in 4 mm cross-lamellar slices and snap frozen to −35°C using PrestoCHILL (Milestone Medical). The 5-µm thick sections were cut and stained with haematoxylin and eosin (H&E). These frozen-section slices were assessed by the pathologist on call, who was unaware of the result of the CLM images. NeuroSAFE was considered positive if tumour was found in the inked external surface (tumour-on-ink). The operating urologist was consulted to discuss the NeuroSAFE outcome (negative or positive, and if positive to which extent in mm and Gleason grading in the PSM). In case of a positive NeuroSAFE outcome, a secondary resection (SR) of the complete NVB, which is the current standard of care in our institution, was performed. Histological assessment of the resected NVB was not part of the intraoperative NeuroSAFE procedure and was performed as a conventional histopathological procedure. Therefore, no further resections based upon the definitive histopathological result of the SR were executed. NeuroSAFE procedure time was recorded and started when the CLM procedure was finished. End time was defined as the presentation of the last slide to the pathologist for evaluation, including re-cuts if necessary.

Histopathological Examination

After completion of the NeuroSAFE procedure, the frozen tissues were formalin fixed and paraffin embedded for routine diagnosis and to seek concordance with the NeuroSAFE and CLM outcomes. The specimen was formalin fixed for 24 h and totally embedded for histopathological examination. The NVBs were cut from apex to base in 4-mm thick slices in a similar manner, although if the first levels of slices were negative, they were step sectioned in levels of 250 µm. Definite histopathology was rated positive when tumour-on-ink was observed.

Evaluation of CLM Images

After acquisition of the CLM images of all patients, the digital high-resolution images were reviewed in a non-blinded fashion by a single uropathologist (W.V.). When evaluating the CLM images of all NeuroSAFE-positive patients, the assessing pathologist had the pathology report and H&E slides at his disposal. Subsequently, the CLM images of all NeuroSAFE-negative patients were reviewed. Images were scored ‘positive’ if tumour was present at the lateral side of the sectioned slices and were scored ‘negative’ when tumour was absent.

Statistical Analysis

Descriptive statistics were used to calculate medians, interquartile ranges (IQRs) for non-normally distributed data. Contingency tables were used to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the assessment of PSM with CLM and NeuroSAFE compared to definite pathology. The level of agreement between CLM and NeuroSAFE was assessed with Cohen’s kappa coefficient. The duration of the two procedures was compared with a Wilcoxon signed-ranks test. The Statistical Package for the Social Sciences (SPSS®) version 27.0 (IBM Corp., Armonk, NY, USA) was used for all tests.

Results

Patient and Tumour Characteristics

In total, 50 patients were evaluated. One patient was excluded from analysis as he received neoadjuvant hormonal treatment. In two patients, the CLM images of one side could not be assessed due to improper scanning and extensive cauterisation effects respectively and were therefore excluded. The

contralateral sides of these patients remained in the analysis. This resulted in 49 patients, with 96 sides (47 left, 49 right) included for analysis. Patient and tumour characteristics are listed in Table 1.

Neurovascular Structure-Adjacent Frozen-Section Examination and Secondary Resections

Of the 96 sides, 14 (15%) had PSMs on NeuroSAFE evaluation and were rated NeuroSAFE positive. In one patient there was a 1-mm PSM of International Society of Urological Pathology (ISUP) Grade Group 1 in an intraprostatic incision after which SR was omitted at the surgeon's discretion. SR was performed in the remaining 13 cases of which six cases had remaining tumour in the NVB (46%). Of those six cases, all PSMs were ≥7 mm. The NeuroSAFE procedure was not affected by the prior tissue preparation for CLM image acquisition. The calculated sensitivity was 93%, specificity 99%, PPV 93% and NPV 99% on a per side basis for the detection of PSM with NeuroSAFE compared with definite pathology (Table 2).

Confocal Laser Microscopy

In total, 15 PSMs were seen on the CLM images (CLM positive, 16%), of which 12 margins were concordantly positive on definite pathology (Table 3). The calculated sensitivity was 86%, specificity 96%, PPV 80% and NPV 98% for the detection of an intraoperative PSM with CLM. There was a substantial level of agreement between CLM and NeuroSAFE ($\kappa = 0.80$) for the detection of a PSM on definite pathology (Table S1). Of note, the PSMs in six cases with remaining tumour in the NVB at SR were observed with CLM as well as NeuroSAFE (Table S2). In two cases a PSM was identified with NeuroSAFE, but not with CLM. In both cases, histopathological examination of the NVB after SR did not reveal any residual tumour. Three cases were NeuroSAFE negative (and SR was omitted), but rated CLM positive. Also, definite pathology of these cases remained negative. Figure 2 shows an example of a whole surface CLM image of case 4 with a PSM on CLM, but negative margin on NeuroSAFE and definite pathology. Figure 3 shows the magnification of the PSM from Fig. 2.

Table 1 Patients' and tumour characteristics.

Characteristic	Value
Number of patients	49
Age, years, median (IQR)	66 (60.5–69.0)
Prostate volume, mL, median (IQR)	42.5 (30.5–55.5)
PSA level at diagnosis, µg/L, median (IQR)	6.5 (5.6–10.7)
cT stage, *n* (%)	
1c	27 (55)
2a	17 (35)
2b	2 (4)
NR	3 (6)
Highest ISUP Grade Group from biopsies, *n* (%)	
1	8 (16)
2	28 (57)
3	9 (18)
4	4 (8)
EAU risk group, *n* (%)	
Low	5 (10)
Intermediate	38 (78)
High	6 (12)
ISUP Grade Group of RP specimen, *n* (%)	
1	3 (6)
2	28 (57)
3	13 (27)
4	2 (4)
5	3 (6)
pT stage, *n* (%)	
pT2	28 (57)
pT3a	19 (39)
pT3b	2 (4)

cT, clinical T; EAU, European Association of Urology; NR, not reported; pT, pathological T.

Table 2 Concordance of NeuroSAFE and definite histopathology.

	Definite pathology positive	Definite pathology negative	Total
NeuroSAFE positive	13	1	14
NeuroSAFE negative	1	81	82
Total	14	82	96

Table 3 Concordance of CLM and conventional histopathology.

	Definite pathology positive	Definite pathology negative	Total
CLM positive	12	3	15
CLM negative	2	79	81
Total	14	82	96

Procedure Times

Compared to the procedural time of NeuroSAFE (median [IQR] 50 [45–59] min), the CLM procedures was significantly shorter (median [IQR] 8 [5–20] min, $Z = -6.094$, $P < 0.001$).

Discussion

We evaluated the feasibility of CLM for ex vivo, intraoperative assessment of surgical margins with RARP. We found CLM to be a feasible technique with significantly shorter procedure times compared to NeuroSAFE. Both techniques identified all relevant PSM, defined as the cases that had residual tumour in the NVB on SR. Of note, both techniques had a high NPV for PSM at definite pathology, which is the most relevant diagnostic performance parameter for oncological safe NVB preservation.

Fig. 2 Whole-surface CLM image of the left prostatic slice of case 4. The red area was scored as a PSM on CLM, negative margin on NeuroSAFE and definite pathology (false positive).

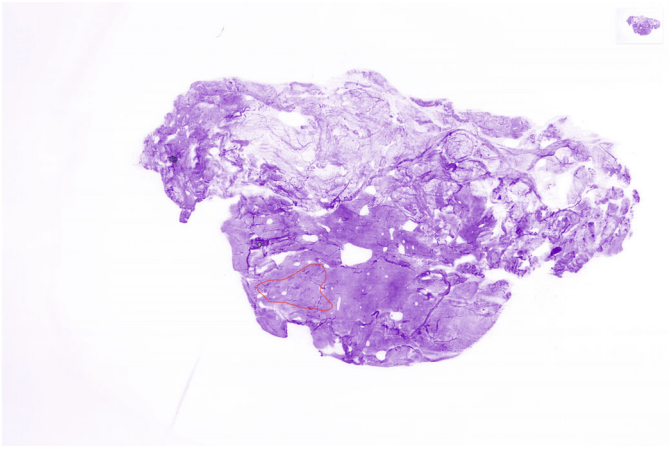
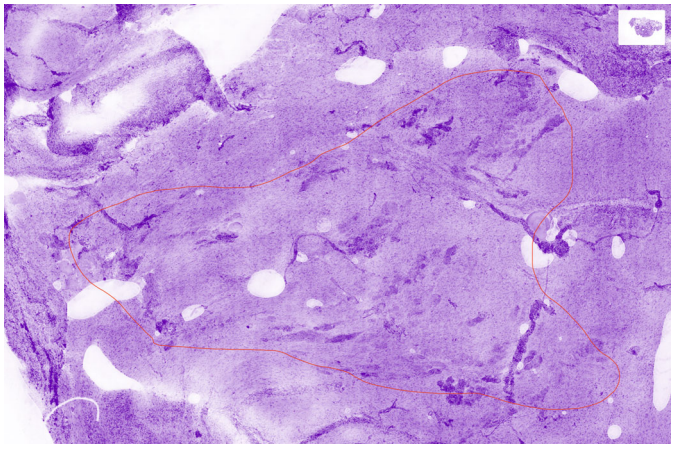


Fig. 3 High magnification of the same CLM image (Fig. 2) of a prostatic slice with false PSM (red).



Rocco et al. [20] were the first to describe CLM for intraoperative margin assessment during RP with some differences compared to our study. They evaluated CLM of shaved prostate tissue (Mohs shaving) and verified the outcomes with conventional histopathology. If a PSM was detected with CLM, their step-wise approach facilitated a focal SR of the NVB performed based on the location of the PSM. This is in contrast to our study, in which the NeuroSAFE outcome was used for clinical decision-making and led to SR of the complete NVB in case of PSM. Besides the theoretical advantage of partial resections, Mohs shavings have a flat surface, which overcomes the issue of irregular prostatic tissue that could affect full scanning of the area as noted by Dinneen et al. [21]. This was observed in our study as well and overcome by using a counterweight. Promising 1-year follow-up results for BCR-free survival, reported continence and erectile recovery were reported, which was not the subject of our study.

Following a SR, final pathology reveals residual tumour in only a limited number of NVBs, with incidences ranging from 23% to 42.4% in recent studies [13,14,16,17,22]. In our series we found a somewhat higher rate (46%) of positive SRs.

Current evidence suggests that small (<3 mm) PSMs and ISUP Grade Group 1 score extending into the PSM poses similar risk of BCR as for those patients with negative surgical margins. This might influence the decision to perform a SR in case of a focal positive NeuroSAFE margin with low-grade tumour [23,24]. An algorithm to be used when a PSM is identified by NeuroSAFE, or other imaging techniques might therefore be an important contribution in clinical decision-making. In patients with a PSM on NeuroSAFE where a SR was performed, we found that all PSMs of ≤5 mm were negative on SR. This strengthens the concept that small PSMs might be irrelevant. Future research should focus on the long-term oncological safety of omitting a SR in those cases.

The main strength of this study is the comprehensive description of a novel technique compared to NeuroSAFE in a large group of patients. Several limitations of our study were recognised. The most important being the non-blinded reading of the CLM images as self-training of the pathologist was necessary. This was unavoidable as prior experience with or training sets for the assessment of CLM images were lacking. Bertoni et al. [25] demonstrated that untrained pathologists are very able to assess CLM images of prostatic biopsies, with a sensitivity and specificity ≥86% and 97% respectively after re-evaluating after 90 days. Although a different scanner was used in their study and their goal was not intraoperative surgical margin assessment, a short learning curve was observed for the assessment of CLM images.

Further research, with trained pathologists blinded to NeuroSAFE results establishing inter-observer agreement, is therefore needed to establish the accuracy of CLM imaging compared to NeuroSAFE and conventional histopathology. Furthermore, we suggest that besides evaluation of the posterolateral sides, performance of CLM in other prostatic areas, such as the base and apex, could be assessed in future research, as current procedures only evaluate the posterolateral sides of the prostate.

Compared to NeuroSAFE, the main advantage of CLM is the significant shorter procedure time, reducing the total surgery time and subsequently leading to cost reduction. The most important difference between both techniques, is the orientation of the tissue and margin assessment. With the NeuroSAFE margins assessed transversally, whilst with CLM

the assessment is done *en face* or whole surface. Instead of dedicated infrastructure with two technicians and a pathologist on-site for NeuroSAFE, CLM requires a single device, one technician and a (remote) pathologist. Whole-surface scanning offers the possibility to precisely locate a focal PSM, whilst orientation of prostatic tissue is maintained, facilitating a tailored SR. Furthermore, the circumferential measurement of the malignant regions instead of the length currently used in transverse sections, could aid in the development of an algorithm using the real extent of the PSM for clinical decision-making. This was not within the scope of our trial, but we recommend this to be incorporated in future research. Compared to NeuroSAFE, CLM maintains tissue integrity that allows for conventional histopathology assessments of the whole-mount specimen afterwards.

It is important to consider that the impact of intraoperative surgical margin assessment on long-term functional and oncological outcomes is still under debate. Currently the randomised controlled NeuroSAFEPROOF trial is ongoing but has so far only reported on perioperative outcomes [18], and long-term functional and oncological outcomes are awaited to finally establish the added value of NeuroSAFE on those domains. Although not the aim of this study, we described the diagnostic accuracy of NeuroSAFE compared to final histopathology. We found a somewhat lower sensitivity and higher specificity of NeuroSAFE compared with the results from the NeuroSAFEPROOF trial (93% vs 100% and 99% vs 93%). Currently NeuroSAFE has been implemented in several high-volume expert centres in Europe. However, awaiting long-term functional and oncological outcomes, many low- and intermediate-volume centres have not been willing or able to build up the necessary infrastructure for NeuroSAFE. In the meantime, an alternative such as CLM might reduce the costs in centres currently using NeuroSAFE and increase the availability of intraoperative surgical margin assessment for patients with prostate cancer undergoing RARP in non-NeuroSAFE centres.

Conclusion

Confocal laser microscopy is a promising technique for intraoperative surgical margin assessment in patients undergoing RARP, with potential logistical and clinical benefits compared to NeuroSAFE. Further research should focus on validating CLM's accuracy, developing algorithms for intraoperative clinical decision-making, and long-term functional and oncological outcomes of techniques for intraoperative surgical margin assessment.

Disclosure of Interest

Dr. Baas reports: the Histolog Scanner® and utensils were provided for use during this trial by SamanTree Medical SA, Lausanne, Switzerland. Dr. Vreuls has nothing to disclose. Dr. Sedelaar has nothing to disclose. Dr. Vrijhof reports personal fees from Teleflex outside the submitted work. Dr. Hoekstra has nothing to disclose. Dr. van Leenders has nothing to disclose. Dr. van Basten reports the following role: President of the Dutch endo-urological society. Dr. Somford reports the following roles: Chair Dutch Uro-Oncology Working Group, Chair Clinical Audit Board – Dutch Prostate Cancer Registry, Member Dutsch Prostate Cancer Guidelines.

Funding

The Histolog Scanner® and utensils were provided for use during this trial by SamanTree Medical SA, Lausanne, Switzerland.

Data Availability Statement

Diederik Baas had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1 **Arroyo C, Martini A, Wang J, Tewari AK.** Anatomical, surgical and technical factors influencing continence after radical prostatectomy. *Ther Adv Urol* 2019; 11: 175628721881378

2 **Tal R, Alphs HH, Krebs P, Nelson CJ, Mulhall JP.** Erectile function recovery rate after radical prostatectomy: a meta-analysis. *J Sex Med* 2009; 6: 2538–46

3 **Vernooij RWM, Cremers RGHM, Jansen H et al.** Urinary incontinence and erectile dysfunction in patients with localized or locally advanced prostate cancer: a nationwide observational study. *Urol Oncol Semin Orig Investig* 2020; 38: 735.e17–735.e25

4 **Mottet N, van den Bergh RCN, Briers E et al.** EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer—2020 update. Part 1: screening, diagnosis, and local treatment with curative intent. *Eur Urol* 2021; 79: 243–62

5 **Soeterik TFW, van Melick HHE, Dijksman LM, Stomps S, Witjes JA, van Basten JPA.** Nerve sparing during robot-assisted radical prostatectomy increases the risk of ipsilateral positive surgical margins. *J Urol* 2020; 204: 91–5

6 **John A, John H, Catterwell R, Selth LA, Callaghan MO.** Primary Gleason grade and Gleason grade group at positive surgical margins: a systematic review and meta-analysis. *BJU Int* 2021; 127: 13–22

7 **Zhang L, Wu B, Zha Z et al.** Surgical margin status and its impact on prostate cancer prognosis after radical prostatectomy: a meta-analysis. *World J Urol* 2018; 36: 1803–15

8 **Sooriakumaran P, Ploumidis A, Nyberg T et al.** The impact of length and location of positive margins in predicting biochemical recurrence after robot-assisted radical prostatectomy with a minimum follow-up of 5 years. *BJU Int* 2015; 115: 106–13

9 **Somford DM, van Oort IM, Cosyns JP, Witjes JA, Kiemeney LALM, Tombal B.** Prognostic relevance of number and bilaterality of positive surgical margins after radical prostatectomy. *World J Urol* 2012; 30: 105–10

10 **olde Heuvel J, de Wit-van der Veen BJ, DMV H et al.** State-of-the-art intraoperative imaging technologies for prostate margin assessment: a systematic review. *Eur Urol Focus* 2021; 7: 733–41

11 **Dinneen EP, Van Der Slot M, Adasonla K et al.** Intraoperative frozen section for margin evaluation during radical prostatectomy: a systematic review. *Eur Urol Focus* 2020; 6: 664–73

12 **Eichelberg C**, **Erbersdobler A**, **Haese A et al.** Frozen section for the management of intraoperatively detected palpable tumor lesions during nerve-sparing scheduled radical prostatectomy. *Eur Urol* 2006; 49: 1011–8
13 **Slot MA**, **Bakker MA**, **Klaver S et al.** Intraoperative assessment and reporting of radical prostatectomy specimens to guide nerve-sparing surgery in prostate cancer patients (NeuroSAFE). *Histopathology* 2020; 77: 539–47
14 **Mirmilstein G**, **Rai BP**, **Gbolahan O et al.** The neurovascular structure-adjacent frozen-section examination (NeuroSAFE) approach to nerve sparing in robot-assisted laparoscopic radical prostatectomy in a British setting – a prospective observational comparative study. *BJU Int* 2018; 121: 854–62
15 **Beyer B**, **Schlomm T**, **Tennstedt P et al.** A feasible and time-efficient adaptation of NeuroSAFE for da Vinci robot-assisted radical prostatectomy. *Eur Urol* 2014; 66: 138–44
16 **Schlomm T**, **Tennstedt P**, **Huxhold C et al.** Neurovascular structure-adjacent frozen-section examination (NeuroSAFE) increases nerve-sparing frequency and reduces positive surgical margins in open and robot-assisted laparoscopic radical prostatectomy: experience after 11 069 consecutive patients. *Eur Urol* 2012; 62: 333–40
17 **Noël J**, **Spencer NH**, **Lodia S et al.** Neurovascular structure-adjacent frozen-section examination robotic-assisted radical prostatectomy: outcomes from 500 consecutive cases in the UK. *J Robot Surg* 2021; 16: 951–6
18 **Dinneen E**, **Haider A**, **Grierson J et al.** NeuroSAFE frozen section during robot-assisted radical prostatectomy: peri-operative and histopathological outcomes from the NeuroSAFE PROOF feasibility randomized controlled trial. *BJU Int* 2021; 127: 676–86
19 **Rocco B**, **Sighinolfi MC**, **Sandri M et al.** Digital biopsy with fluorescence confocal microscope for effective real-time diagnosis of prostate cancer: a prospective, comparative study. *Eur Urol Oncol* 2021; 4: 784–91
20 **Rocco B**, **Sarchi L**, **Assumma S et al.** Digital frozen sections with fluorescence confocal microscopy during robot-assisted radical prostatectomy: surgical technique. *Eur Urol* 2021; 80: 724–9
21 **Dinneen E**, **Oxley J**, **Shaw G et al.** Re: Bernardo Rocco, Luca Sarchi, Simone Assumma, et al. Digital frozen sections with fluorescence confocal microscopy during robot-assisted radical prostatectomy: surgical technique. *Eur Urol* 2021; 80: e120–1
22 **Fromont G**, **Baumert H**, **Cathelineau X**, **Rozet F**, **Validire P**, **Vallancien G**. Intraoperative frozen section analysis during nerve sparing laparoscopic radical prostatectomy: Feasibility study. *J Urol* 2003; 170: 1843–6
23 **Dev HS**, **Wiklund P**, **Patel V et al.** Surgical margin length and location affect recurrence rates after robotic prostatectomy. *Urol Oncol Semin Orig Investig* 2015; 33: 109.e7–13
24 **Preisser F**, **Coxilha G**, **Heinze A et al.** Impact of positive surgical margin length and Gleason grade at the margin on biochemical recurrence in patients with organ-confined prostate cancer. *Prostate* 2019; 79: 1832–6
25 **Bertoni L**, **Puliatti S**, **Reggiani Bonetti L et al.** Ex vivo fluorescence confocal microscopy: prostatic and periprostatic tissues atlas and evaluation of the learning curve. *Virchows Arch* 2020; 476: 511–20

Correspondence: Diederik J.H. Baas, Prosper Prostate Cancer Clinics, Department of Urology, Canisius Wilhelmina Hospital, Weg door Jonkerbos 100, 6532 SZ Nijmegen, The Netherlands.

e-mail: d.baas@cwz.nl

Abbreviations: BCR, biochemical recurrence; CLM, confocal laser microscopy; IQR, interquartile range; ISUP, International Society of Urological Pathology; NeuroSAFE, neurovascular structure-adjacent frozen-section examination; NPV, negative predictive value; NSS, nerve-sparing surgery; NVB, neurovascular bundle; PPV, positive predictive value; PSM, positive surgical margin; (RA)RP, robot-assisted radical prostatectomy; SR, secondary resection.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Agreement of CLM and NeuroSAFE for the detection of PSM.

Table S2. List of cases with PSM at NeuroSAFE, CLM, or definite pathology.