BJU Int 2021; **127:** 676–686 doi:10.1111/bju.15256

Original Article

BJUI
BJU International

NeuroSAFE frozen section during robot-assisted radical prostatectomy: peri-operative and histopathological outcomes from the NeuroSAFE PROOF feasibility randomized controlled trial

Eoin Dinneen[1,2], Aiman Haider[3], Jack Grierson[1], Alex Freeman[3], Jon Oxley[4], Tim Briggs[2], Senthil Nathan[2], Norman R. Williams[1], Chris Brew-Graves[5], Raj Persad[6], Jon Aning[6], Charles Jameson[3], Marzena Ratynska[3], Imen Ben-Salha[3], Rhys Ball[3], Rosie Clow[2], Clare Allen[2], Daniel Heffernan-Ho[2], John Kelly[1,2] and Greg Shaw[1,2]

[1]Division of Surgery and Interventional Science, University College London, London, [2]Department of Urology, Westmoreland Street Hospital, University College Hospital London, London, [3]Department of Histopathology, University College Hospital London, London, [4]Department of Histopathology, North Bristol Hospitals Trust, Southmead Hospital, Bristol, [5]Division of Medicine, University College London, London, and [6]Department of Urology, North Bristol Hospitals Trust, Southmead Hospital, Bristol, UK

Objectives

To report on the methods, peri-operative outcomes and histopathological concordance between frozen and final section from the NeuroSAFE PROOF feasibility study (NCT03317990).

Patients and Methods

Between May 2018 and March 2019, 49 patients at two UK centres underwent robot-assisted radical prostatectomy (RARP). Twenty-five patient were randomized to NeuroSAFE RARP (intervention arm) and 24 to standard RARP (control arm). Frozen section was compared to final paraffin section margin assessment in the 25 patients in the NeuroSAFE arm. Operation timings and complications were collected prospectively in both arms.

Results

Fifty neurovascular bundles (NVBs) from 25 patients in the NeuroSAFE arm were analysed. When analysed by each pathological section (n = 250, average five per side), we noted a sensitivity of 100%, a specificity of 99.2%, and an area under the curve (AUC) of 0.994 (95% confidence interval [CI] 0.985 to 1; $P \leq 0.001$). On an NVB basis (n = 50), sensitivity was 100%, specificity was 92.7%, and the AUC was 0.963 (95% CI 0.914 to 1; $P \leq 0.001$). NeuroSAFE RARP lasted a mean of 3 h 16 min (knife to skin to off table, 95% CI 3 h 2 min–3 h 30 min) compared to 2 h 4 min (95% CI 2 h 2 min–2 h 25 min; $P \leq 0.001$) for standard RARP. There was no morbidity associated with the additional length of operating time on in the NeuroSAFE arm.

Conclusion

This feasibility study demonstrates the safety, reproducibility and excellent histopathological concordance of the NeuroSAFE technique in the NeuroSAFE PROOF trial. Although the technique increases the duration of RARP, this does not cause short-term harm. Confirmation of feasibility has led to the opening of the fully powered NeuroSAFE PROOF randomized controlled trial, which is currently under way at four sites in the UK.

Keywords

NeuroSAFE, frozen section, robotic prostatectomy, prostate cancer, margins, nerve-sparing, #PCSM, #ProstateCancer, #uroonc

Patient summary

What is the paper about? When the nerves next to the prostate are removed during prostate cancer surgery men suffer side effects such as impotence and incontinence. Sometimes these nerves do not have to be removed. The NeuroSAFE technique checks the edge of the prostate under the microscope during surgery to see if it is safe to protect the nerves. This paper investigates the feasibility of studying the NeuroSAFE technique in men with prostate cancer.

What does it mean for patients? This feasibility study was designed to see if men would accept being randomised in a clinical trial to standard radical prostatectomy or radical prostatectomy with the NeuroSAFE technique. Men in two UK hospitals agreed to be randomised and so the full trial, evaluating the role of the NeuroSAFE technique in the recovery from prostate cancer surgery, is now underway at four UK hospitals.

Introduction

Erectile dysfunction is a common and significant side effect of radical prostatectomy (RP). Nerve'sparing (NS) RP improves recovery of erectile function [1–3]; however, the quality of NS is often balanced against the oncological risks associated with positive surgical margins (PSMs) [4,5]. Clinical suspicion of extraprostatic extension of prostate cancer is a relative contraindication of NS RP [6] as it confers an increased risk of PSMs; however, accurate prediction of prostate cancer stage T3 (i.e. extraprostatic extension) is difficult, requiring methods such as multiparametric MRI (mpMRI) [7], nomograms [8] and DRE). The NeuroSAFE technique is a standardized approach that uses intra-operative frozen section (IFS) analysis of the postero-lateral neurovascular structure adjacent prostate margin during RP to assess margin status after NS in real time [9]. If the frozen section demonstrates the surgical margins to be positive, a wider dissection of the neurovascular bundle (NVB) may be performed. In this way, the NeuroSAFE technique seeks to promote optimal NS to maximize the opportunity for functional recovery without jeopardizing oncological safety.

Frozen section is the current 'gold standard' for providing intra-operative margin feedback during RP [10]. However, a recent systematic review performed by our group identified no prospectively performed studies, considerable variation in the technical application of IFS, and a wide range in the thoroughness of IFS technical description [11]. The same review found that the NeuroSAFE technique is the only IFS approach that has been disseminated across and reported by more than a single institution [12–14].

The NeuroSAFE PROOF feasibility study is the first randomized controlled trial (RCT) of IFS in RP. This pragmatic, single-blind, multicentre trial compares outcomes in patients randomly allocated to robot-assisted RP (RARP), with NS performed with the assistance of the NeuroSAFE technique vs RARP performed without (i.e. as per the current standard of care). An essential element of reliable IFS pathology is excellent concordance with the final pathological assessment and, in the case of the NeuroSAFE technique, margin status. Discrepancies between IFS and the final pathology diagnosis (i.e. the reference test) have the potential to negatively alter treatment decisions and adversely affect patient care [15]. Detailed technical descriptions and evaluation are essential for the interventions studied within an RCT to be considered robust and for the intervention to be reliably reproduced in wider clinical practice [16,17].

The aims of the present study were to (1) provide a detailed description of the NeuroSAFE technique as implemented in the NeuroSAFE PROOF study, including reporting concordance between margin diagnosis on frozen section and final section, (2) provide a detailed description of the pathological handling of secondarily resected neurovascular tissue including oncological outcomes of patients who had a PSM on IFS and (3) report on peri-operative outcomes from the NeuroSAFE PROOF feasibility study.

Materials and Methods

NeuroSAFE PROOF Feasibility Study

The NeuroSAFE PROOF feasibility study (NCT03317990) was a prospective, multi-site, 1:1 randomized controlled feasibility study [18]. Patients with localized operable prostate cancer (defined as absence of metastases on staging scans and absence of obvious T3b disease on imaging), who had undergone pre-biopsy mpMRI, who had been discussed at a regional multidisciplinary team meeting and who had selected RARP as their primary treatment, were deemed eligible. Eligibility criteria included good baseline erectile function (defined as International Index of Erectile Function-5 score >21) as has been used previously [19–21]. Recruited patient were randomized to RARP with NS according to the standard-of-care decision-making process (control arm) or RARP with NS

according to a decision-making process that included the NeuroSAFE technique (intervention arm). The 'standard of care decision-making process' (control arm) included available information from the prostate biopsy, the preoperative mpMRI and the DRE under anaesthetic. The primary outcome of the study was satisfactory recruitment of 50 men in order to prove feasibility of the full-scale NeuroSAFE PROOF RCT. Detailed peri-operative and histopathological data were included as secondary outcomes in order to help determine power calculations for the subsequent study.

Ethical approval was acquired prior to study commencement (Regional Ethics Committee reference 17/LO/1978). All patients provided written informed consent and were randomized using an established, centralized, online randomization service [22]. All tissue was handled in accordance with the Helsinki Declaration [23].

NeuroSAFE RARP

The intervention was performed in accordance with previously described methods, as developed at the Martini Klinik, Hamburg, Germany [9]. The surgeon starts their dissection of the NVBs with a bilateral NS RP (partial, inter- or intrafascial [3], as per the surgeon's discretion) after which they are able to verify the safety of their NS strategy as the neurovascular structure adjacent margin is inspected to check for PSMs by IFS. The prostate is removed from the body through an enlarged umbilical incision as soon as it has been detached from the surrounding structures using the Alexis Laparoscopic System (Applied Medical, Rancho Santa Margarita, CA, USA). Pneumoperitoneum was re-established once the specimen had been removed in order to continue with the remaining steps of the RARP.

If a PSM is noted on the IFS, secondary resection (SR) may be performed. One of two types of SR was performed by the surgeon. 'Partial SR' was defined as an excision of a focus of Denonvilliers'/periprostatic fascia based on the surgeon's best effort to cognitively localize the focus of prostate cancer that had given rise to the PSM, also taking into account which section was reported as having a PSM and calculating distance from the apex. 'Full SR' was defined as the entire en bloc excision of the ipsilateral NVB from base to apex [18]. The decision to perform full or partial SR was at the discretion of the surgeon based on the length and grade of the PSM on the NeuroSAFE frozen section. If IFS demonstrated a negative surgical margin, the ipsilateral NVB was left intact. Detailed timings for operation duration and complications were collected for all patients.

NeuroSAFE Technique

Immediately after removal of the prostate, the postero-lateral neurovascular structure adjacent surfaces were painted in theatre by the console surgeon (Fig. 1A). The inked prostate was then transported to the histopathology department where the entire inked margin was cleaved by a straight blade. This postero-lateral portion of prostate with ink on the outer margin surface was further divided by perpendicular cuts at intervals of 5 mm from apex to base. A minimum of four and a maximum of seven pieces from the postero-lateral prostate on each side were submitted for freezing in this manner according to the size of the prostate. Each piece of prostate tissue was then embedded into optimal cutting temperature compound on a cryostamp, and frozen (Fig. 1B). The frozen prostate tissue was then transferred to the cryostat for sectioning at

Fig. 1 Images showing the performance of intra-operative frozen section as per the NeuroSAFE technique. (**A**) Ink stains the left (yellow) and right (green) neurovascular structure adjacent prostate margin, respectively. (**B**) After cleaving the right side and slicing perpendicularly a 5-mm piece of prostate tissue sits on the cryostat before freezing. (**C**) Once embedded in optimal cutting temperature compound and frozen, 5-μm sections are prepared on the microtome before staining.

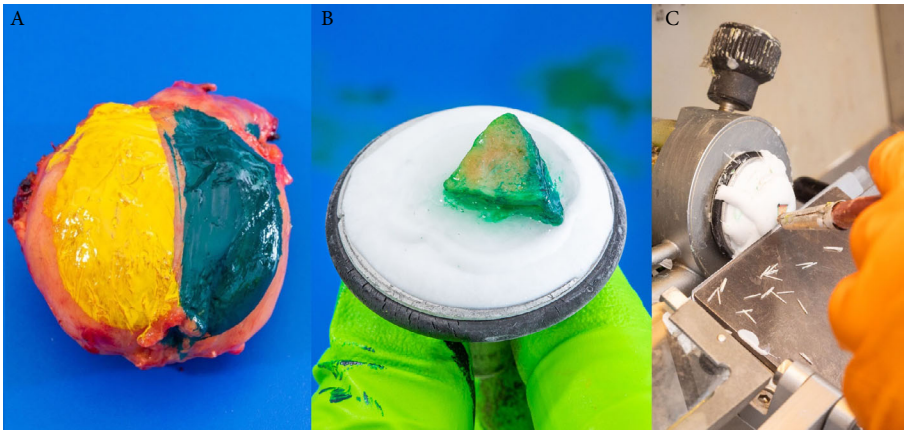
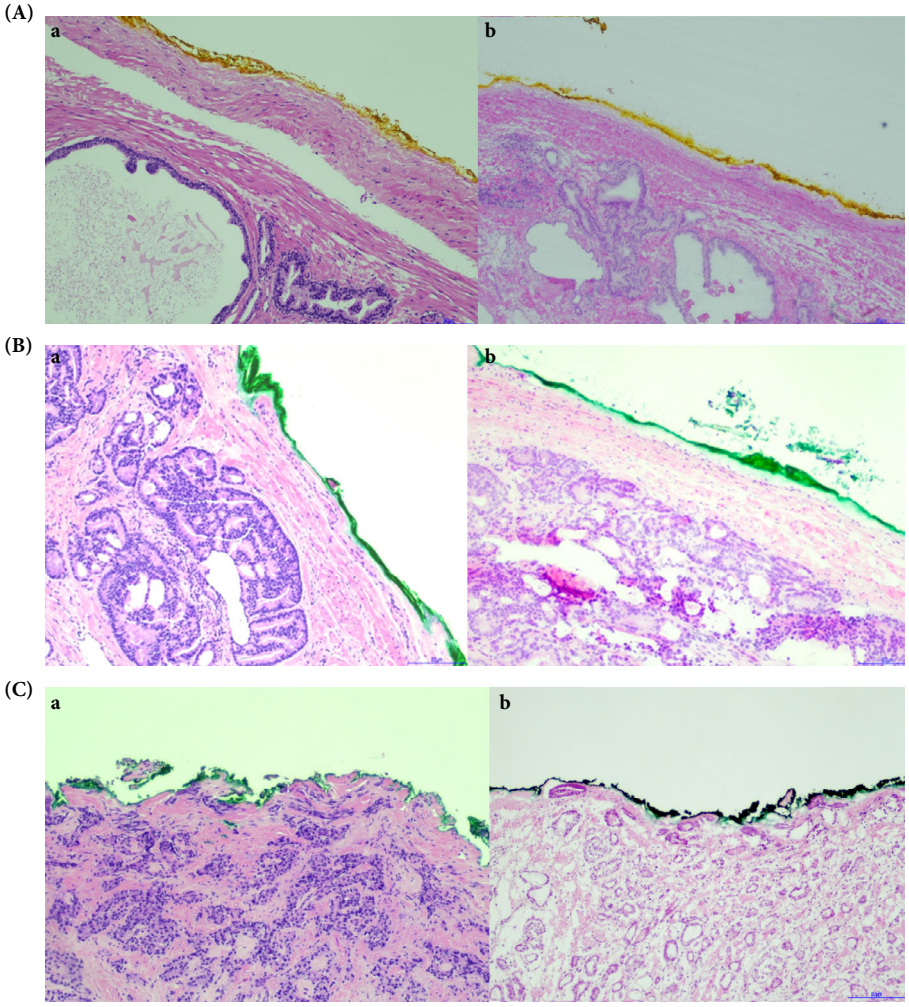


Fig. 2 (**A**) (a) Formalin-fixed paraffin-embedded (final) section of margin CLEAR ×10 magnification. (b) intra-operative frozen section (IFS) of corresponding CLEAR margin section also at ×10 magnification. (**B**) (a) Final section of NARROWLY CLEAR margin ×10 magnification. (b) IFS section of corresponding NARROWLY CLEAR margin section at x10 magnification. (**C**) (a) Final section of POSITIVE margin ×10 magnification. (b) IFS section of corresponding POSITIVE margin section at ×10 magnification.



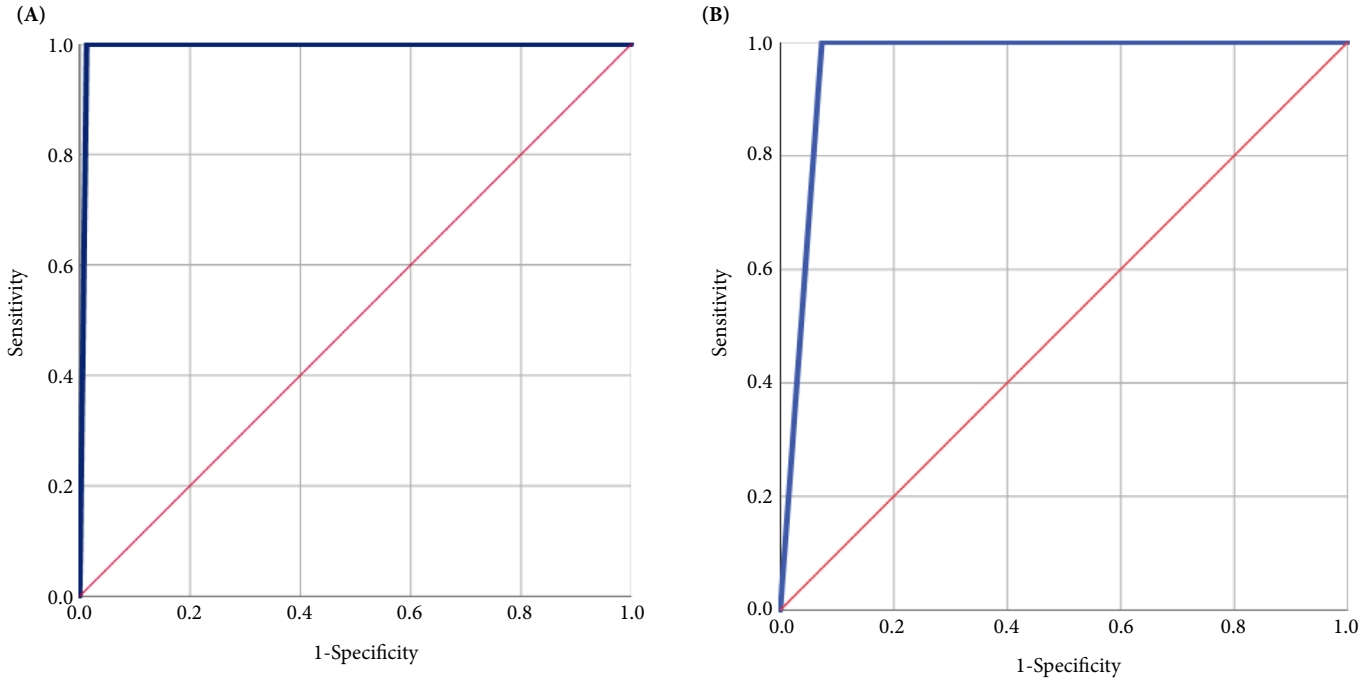
a tissue thickness of 5 μm before staining with haematoxylin and eosin (Fig. 1C).

Stained frozen sections were examined in the histopathology department by a dedicated uropathology consultant (A.F., A.H., I.B.-S., M.R., J.O. or R.B.). A PSM was defined as a single neoplastic gland present at the inked margin. Negative margins were reported as 'clear' if tumour was not seen or was >0.5 mm from the inked margin, or 'narrowly clear' if tumour was seen <0.5 mm away from the ink (Fig. 2A–C). Frozen section diagnoses were recorded prospectively on the day of surgery. Once the reporting pathologist had given a frozen section margin assessment, each piece of prostate tissue was defrosted in warm water and placed individually in formalin for subsequent paraffin embedding. Careful attention to the orientation of each piece in the processing cassette was given to ensure that the final slides examined the same surface. All prostatectomy specimens were processed in accordance with the standard procedures recommended by the International Society of Urological Pathology (ISUP) [24] and reported as per the standards outlined by the Royal College of Pathologists (June 2016) [25].

Secondary Resection

The SR NVB (when performed) was received in formalin after the completion of the operation and processed with the rest of the main prostatectomy specimen. Further IFS was never performed on the SR NVB. When the entire NVB was submitted en bloc following full SR, the specimen was annotated by the surgeon with clips and sutures to orientate the prostate adjacent side, the resection margin and apical end. When a partial SR was carried out, orientation of the

Fig. 3 Receiver-operating characteristic curves that describe the relationship between the sensitivity and specificity of frozen section and final section (**A**) per each section (n = 250), area under the curve (AUC) 0.994, and (**B**) per neurovascular bundle (n = 50), AUC 0.963.



tissue was not performed. All tissue was formalin-fixed, painted and sectioned at 5-mm intervals. Where possible the specimen was embedded from apex to base in separate cassettes. If no tumour was identified in the SR specimen, adjacent levels were taken and examined.

Concordance Assessment

After formalin fixation, paraffin embedding, haematoxylin and eosin staining, each frozen section margin was re-assessed by one of three reporting pathologists (A.F., A.H. or J.O.). This assessment of the postero-lateral margin was considered the reference test for the purposes of the concordance study. The margin was classified again as clear, narrowly clear or positive (Figs 2, 3 and 4).

Statistical Analysis

All analyses were performed using SPSS (version 26). Baseline clinical and pathological characteristics are presented as descriptive statistics. Proportions were compared using the two-tailed t-test for continuous variables and the chi-squared test for categorical variables. For frozen section (diagnostic test) compared to paraffin-embedded section (reference test), 2 × 2 contingency tables were used to present the results and calculate the diagnostic accuracy estimates with 95% CIs. The 2 × 2 contingency tables are presented, with the unit of assessment being both each single section of margin submitted and each neurovascular structure adjacent laterality (two NVB sides per patient). The diagnostic performance was also evaluated using the receiver-operating characteristic (ROC) curve and the area under the curve (AUC) to describe the relationship between the sensitivity and specificity of the test. P values < 0.05 were taken to indicate statistical significance.

Results

Forty-nine patients underwent RARP at two UK NHS participating centres (University College Hospital London and North Bristol Trust) between 29 May 2018 and 25 March 2019 (see CONSORT Diagram in Appendix S1). Twenty-five men underwent NeuroSAFE RARP and 24 underwent standard RARP according to randomization. One patient had treatment deferred as a result of cardiological comorbidities identified as part of his preoperative assessments and was withdrawn from the trial. Baseline clinical and pathological characteristics of the patients included in the study are shown in Table 1.

Pathological Concordance for the NeuroSAFE Technique

From 25 patients undergoing NeuroSAFE RARP, 250 postero-lateral neurovascular structure adjacent prostate margin sections were submitted for IFS. Table 2A shows the

Table 1 Clinical and pathological characteristics of the NeuroSAFE (intervention) and standard (control) arms.

Variable	NeuroSAFE	Standard	P*
Number of patients	25	24	
Mean (range) age, years	57 (51–66)	55.9 (44–63)	0.66
Mean (range) preoperative PSA, ng/mL	10.4 (1.2–39.2)	9.5 (4–35)	0.99
MRI stage, *n* (%)			
2	5 (20)	7 (29)	0.52
3	20 (80)	17 (71)	
Biopsy ISUP, *n* (%)			
1	1 (4)	3 (13)	0.5
2	19 (76)	17 (70)	
3	5 (20)	3 (13)	
4	0	1 (4)	
5	0	0	
Mean (range) prostate weight, g	43.9 (21–75)	42.2 (15–59)	0.71
LND performed, *n* (%)	6 (24)	5 (21)	0.78
Pathological stage, *n* (%)			
2a/2b	2 (8)	0	0.25
2c	13 (52)	16 (67)	
3a	7 (28)	7 (28)	
3b	3 (13)	1 (4)	
Mean (range) tumour volume, mL	5.0 (0.25–22.27)	4.7 (0.7–12.89)	0.17
Final ISUP grade, *n* (%)			
1	2 (8)	0	0.27
2	17 (68)	21 (83)	
3	5 (20)	3 (13)	
4	0	0	
5	1 (4)	0	
Mean (range) operation length, h:min†	3:16 (2:05–4:20)	2:13 (1:30–3:06)	<0.001
First 5 cases	3:20 (3:05–3:30)	2:15 (1:30–2:35)	
Last 5 cases	3:07 (2:40–3:30)	2:06 (1:45–2:45)	
Clavien–Dindo complications grade > 1	1	2	

*ISUP, International Society of Urological Pathology; LND, lymph node dissection. *Statistical tests; two sample t-test for means, Fisher's exact test for proportions. Analysis performed using SPSS version 26. †Missing data for two patients, both in the NeuroSAFE arm.*

Table 2A Concordance between frozen section and final section per slide generated.

	Frozen section-negative	Frozen section-positive	Total
Final section-negative	234	3	237
Final section-positive	0	13	13
Total	234	16	250

Sensitivity = 100%, specificity = 98.7%, positive predictive value = 97.1%, negative predictive value = 100%.

Table 2B Concordance between frozen section and final section per neurovascular bundle.

	Frozen section-negative	Frozen section-positive	Total
Final section-negative	38	3	41
Final section-positive	0	9	9
Total	38	12	50

Sensitivity = 100%, specificity = 92.7%, positive predictive value = 75%, negative predictive value = 100%.

concordance between the frozen section and the final section assessment for each individual section. For the purposes of this analysis, the diagnosis of 'clear' and 'narrowly clear' were considered together. Frozen section sensitivity was 100%, specificity 98.7%, positive predictive value 97.1%, and negative predictive value 100% ($P < 0.001$). The ROC curve is shown in Fig. 3, with an AUC of 0.994 (95% CI 0.985 to 1; $P < 0.001$).

In analyses considering frozen section results with the 50 NVBs as the denominator (two per NeuroSAFE patient), frozen section had a sensitivity of 100%, a specificity of 92.7%, a positive predictive value of 75% and a negative predictive value of 100% ($P < 0.001$; Table 2B). The ROC curve is shown in Fig. 4, with an AUC of 0.963 (95% CI 0.914 to 1; $P < 0.001$). There were three instances when a PSM on intra-operative frozen section was subsequently considered narrowly clear on final section assessment.

Secondary Resection

In total, 11/25 patients had PSMs identified by the NeuroSAFE technique. Bilateral PSMs were identified in one patient. Seven out of 11 patients underwent SR (Table 3). Of the seven patients who had SR, four underwent partial and three patients full SR of the ipsilateral NVB. Cancer was found in the SR in three out of seven patients on final

Table 3 Summary of histological and oncological outcomes for patients in the NeuroSAFE arm who had a positive surgical margin on intra-operative frozen section.

Patient*	IFS margin: number, length	Final margin†	SR performed	SR +ve cancer	PSA	Biopsy ISUP	Tumour biopsy	DRE	Path ISUP	pT	pN	RP failure
1	Single, 3 mm	P	Partial	No	5.8	2	Bilateral	T2	2	3a	NX	N
2	Multiple, 7 mm	P	Full	Yes	11	2	Bilateral	T3	2	3a	NX	Y
3	Multiple, 5 mm	P	Full	No	7.1	2	Left	T1	2	2c	NX	Y
4	Single, 2 mm	P	Partial	No	8	2	Bilateral	T2	2	3a	NX	Y
5	Single, <1 mm	C	Nil	n/a	4	2	Bilateral	T1	2	2c	NX	N
5 (other side)	Single, <1 mm	P	Nil	n/a	4	2	Bilateral	T1	2	2c	NX	N
6	Multiple (each only 1 mm) 2 mm	P	Nil	n/a	14.3	3	Bilateral	T3	3	3b	N0	Y
7	Single, 0.5 mm	C	Nil	n/a	1.2	2	Left	T2	2	2a	NX	N
8	Multiple, 4 mm	P	Partial	Yes	7.5	3	Bilateral	T3	2	3a	NX	Y
9	Single, <1 mm	C	Partial	No	8	2	Bilateral	T1	2	3a	NX	Y
10	Single, 1 mm	P	Full	Yes	6.7	2	Left	T2	2	2c	NX	N
11	Single, 1 mm	P	Nil	n/a	9.4	2	Right	T1	2	2c	N0	N

*C, clear; IFS, intra-operative frozen section; ISUP, International Society of Urological Pathology; N, no; P, positive; RP, radical prostatectomy; SR, secondary resection; Y, yes. RP failure defined as adjuvant therapy or PSA > 0.2 ng/mL at follow-up of 12 months. *Patient numbers arbitrary not chronological in this table to preserve anonymity. †Final margin at the corresponding final section only.*

histological examination (two full SR and one partial SR). Of the four patients who underwent partial SR, one had cancer present in the SR specimen and three had RP failure (defined as requiring adjuvant therapy or a PSA of >0.2 ng/mL) at 12-month follow-up. Of the three patients who underwent full SR, two out of the three had cancer present in the SR specimen and two went on to have RP failure at 12-month follow-up.

Four patients (patients 5, 6, 7 and 11 in Table 3) had five PSMs on IFS, and no SR was performed because of short length of Gleason Grade Group 1 cancer at the PSM. In all three patients where there was a single PSM of < 1 mm, no RP failure was noted at 12-month follow-up. Moreover, three patients men (patients 5, 7 and 9 Table 3) had a single PSM of < 1 mm in length, all three of which were converted to 'clear' on final section analysis.

Four men (patients 2, 3, 6 and 8 in Table 3) who had multiple section PSMs during IFS had RP failure regardless of whether they had SR. Of these four patients, two had full SR (of whom one had cancer observed within it), one man had a partial resection with cancer observed within it, and the last had no SR.

Operation Timings and Peri-operative Outcomes

The mean length of NeuroSAFE RARP was 3 h 16 min (95% CI 3 h 2 min to 3 h 30 min) compared to 2 h 14 min (95% CI 2 h 2 min to 2 h 25 min; $P < 0.001$) in the standard RARP arm. Incorporating the additional time in the operating room and the pathology laboratory expense incurred by performing the NeuroSAFE technique, the estimated additional cost of the procedure was £1000.

There were no serious adverse events in the study. All patients were discharged home the day after surgery. There were three complications of Clavien–Dindo grade >1 in two patients (one NeuroSAFE and one standard arm), including one readmission within 90 days. One patient in the NeuroSAFE arm developed bladder neck stenosis and required bladder neck dilatation under general anaesthetic (Clavien–Dindo grade 3b). One patient in the standard arm was readmitted to hospital with urosepsis and treated with antibiotics (Clavien–Dindo grade 2). The same patient had a urinary leak seen on cystogram and required a prolonged period with a urinary catheter (Clavien–Dindo grade 3a). There were no postoperative complications recorded in the intervention arm on account of the additional time spent under general anaesthesia.

Discussion

A recent systematic review found no prospectively performed studies on IFS or the NeuroSAFE technique [11]. Our feasibility study, which is the first prospective and

Table 4 Previously published studies on the NeuroSAFE (*or neurovascular bundle-focused intra-operative frozen section) technique including details on pathological stage and presence of cancer in the secondary resection specimen, where given.

	Year	*n*	pT2 (%)	pT3a (%)	pT3b (%)	SR +ve cancer (%)
Schlomm et al. [12]	2012	11 069	70	20	10	23
Hatzichristodoulou et al. [30]*	2016	458	81	11.6	6.8	16.1
Mirmilstein et al. [13]	2017	277	83	14	3	42.4
Preisser et al. [14]	2019	346	60	40*	U	NA
Fossa et al. [31]	2020	407	57	42*	U	NA
NeuroSAFE PROOF feasibility study	2020	50	63	28	9	42

*NA, information not available in manuscript; SR, secondary resection; U, unknown;. *Value for pT3a and pT3b not provided separately.*

randomized study of its kind, shows that the technique is safe, that the results of the frozen section are reproducible on final section analysis, and that the technique can be carried out in more than one institution as part of the same trial. Additional detailed analysis of oncological outcomes up to 1 year after surgery in men who had PSMs on IFS has allowed alteration of the NeuroSAFE technique that is now being employed in the full NeuroSAFE PROOF trial.

In our feasibility study, the NeuroSAFE technique contributed an additional hour to the length of RARP. This is considerably longer than the Martini Klinik experience, where Beyer et al. [26] noted that, although the results of the NeuroSAFE took 35 min until communication to the surgeon, the additional step only added 4 min to the length of the operation. Possible explanations for this discrepancy include greater pathology laboratory capacity at the Martini Klinik where there are five cryostats in the laboratory and the fact that, in the present study, lymph node dissection (LND) was only performed in 11/49 patients (22%) [26]. If the proportion of patients in both arms undergoing LND was higher, the difference in the length of RARP between arms may have lessened as the procedure would take longer on average in the standard arm. It should also be noted that, in both our participating study centres, the pathology laboratories were in different buildings from the operating room and were equipped with two cryostats each. van der Slot et al. [27] describe a reduction in the duration of NeuroSAFE RARP with increasing experience. This finding is also familiar to our group, as system familiarity and planning, transfer to the laboratory and technical experience all shorten times. Despite this, within the number of NeuroSAFE RARPs performed in the intervention arm during the feasibility study ($n = 25$) the difference between the mean duration of the first five cases (3 h 20 min) and the last five cases (3 h 7 min) did not meet statistical significance ($P = 0.49$; Table 1).

In spite of the additional time taken to perform the NeuroSAFE technique, the completion of the feasibility study at two NHS UK hospitals shows that the technique is feasible and that it can be performed at units that have access to routinely available frozen section equipment (cryostat, cryostamp, staining apparatus). Since the completion of the feasibility study, two further centres (Sheffield University Teaching Hospitals NHS Trust and Glasgow and Clyde NHS Trust) have also joined the NeuroSAFE PROOF RCT. Importantly, our experience shows that there were no intra-operative or immediate postoperative complications for patients associated with the longer time spent under general anaesthesia.

To date, only two other groups who use the NeuroSAFE technique have published their frozen to final section concordance. Our results, showing excellent diagnostic accuracy and agreement between IFS and final section, are consistent with these two aforementioned retrospective case–control studies. At the Martini Klinik, Hamburg, Germany, where the technique was developed, Schlomm et al. [12] noted that frozen section had a sensitivity of 93.5% and a specificity of 98.8% when compared to final section. Similarly, Mirmilstein et al. [13] reported a sensitivity of 90% and a specificity of 97.4% when comparing frozen to final section at the neurovascular structure adjacent margin only. It is noteworthy that in the present study no PSM was missed by IFS that was subsequently found on final section. For these reasons, IFS may still be deemed the 'gold standard' for real-time resection margin assessment during uro-oncological surgery [10]. The accuracy of the NeuroSAFE technique can be increased by reducing the slice thickness at the postero-lateral margin to 3 mm (from 5 mm), but we have demonstrated excellent concordance with our current process and so it remains unchanged in the protocol in order to manage resources well with no compromise on detail. The emergence of promising fast digital imaging techniques [28] and the ongoing development of new platforms to incorporate preoperative mpMRI information intra-operatively via augmented reality also hold considerable promise for future strategies to reduce the risk of PSMs [29].

Our finding of cancer in 42% of the SR tissue submitted is at the upper end of what has previously been reported in the IFS in the RP literature (0–42.4%) [11,14,30,31]. This may be because our patients represent a higher risk group

than those in previous studies on the NeuroSAFE technique (Table 4). This is the first study in the literature that gives a detailed account of individual patient oncological outcomes according to the type of SR performed. Our analysis has led to the development of our protocol for the response to PSM on IFS within the full NeuroSAFE PROOF RCT. Firstly, our protocol now instructs that only full SR is performed. Secondly, the indications for full SR are now: (1) any PSM on multiple sections on a side; (2) any Gleason grade 4 or grade 5 adenocarcinoma at the margin; and (3) any single section PSM >2 mm of Gleason Grade Group 1.

The impact of PSM on cancer control outcomes is not necessarily an all-or-none phenomenon, i.e. grade, length and patient tumour characteristics independent of margin status all impact long-term oncological outcomes and the likelihood of RP failure [32]. Various studies, including our feasibility data, have informed the development of our approach to the positive NeuroSAFE as described above. van der Slot et al. [27] found that in patients with <1 mm of Gleason pattern 3 at the margin, adenocarcinoma was never seen in the SR specimen. Moreover, the presence of Gleason pattern 3 only at the surgical margin has been associated with a decreased risk of biochemical recurrence [33,34], and several studies have demonstrated that men with ≤3 mm PSM length on final prostatectomy specimen have similar biochemical recurrence-free survival as compared to those with negative surgical margin status [4,35,36]. Conversely, Choi et al. [37] found that Gleason pattern 4 and higher on IFS was predictive of biochemical recurrence. We have presented 1-year oncological outcomes to help further explain the rationale behind the development of the protocol for the NeuroSAFE technique within the full NeuroSAFE PROOF RCT. We do not present long-term outcomes for all patients, as the full trial is ongoing, and we do not want to compromise the conduct of this definitive study.

Our feasibility study has some considerable strengths. It is the first report to have collected information on the NeuroSAFE technique that is either prospective or randomized. It also represents a dual-site, multi-surgeon and multi-pathologist experience, and therefore, it is a realistic account of the use of the technique in the wider clinical setting.

The study also has some limitations. Firstly, we cannot yet draw conclusions about oncological outcomes because this was a small study, which was powered only to demonstrate the feasibility of conducting a larger study. Secondly, we have not reported on the grade of the adenocarcinoma contributing to the PSM on IFS. Similarly, we did not record whether PSM on IFS may have been considered capsular incision, or whether there were benign non-cancer intracapsular margins. These features have been incorporated into the reporting of the NeuroSAFE technique in the definitive study as we continue to learn from our experience.

In conclusion, the present study demonstrates the safety, reproducibility and excellent histopathological concordance of the NeuroSAFE technique in the NeuroSAFE PROOF trial. Although the technique increases the duration of RARP, this does not cause short-term harm to patients. These results have been used to aid the development of the full NeuroSAFE PROOF RCT, which will, in turn, provide further long-awaited information as to whether NeuroSAFE RARP can improve outcomes for men undergoing surgery for prostate cancer cure.

Acknowledgements

The NeuroSAFE PROOF feasibility trial was funded by the National Institute for Health Research (NIHR) Research for Patient Benefit (RfPB) programme (PB-PG-1216-20013) and the Rosetrees Foundation. Eoin Dinneen is funded by the NIHR RfPB. We are grateful to the participants, principal investigators, research nurses, clinicians involved in providing care, regional cancer service coordinators, data managers, and other site staff responsible for setting up, recruiting participants, and collecting data for the trial. We are grateful for the trial oversight provided by the sponsor and members of the trial steering committee, Jack Cuzick (Chair), Queen Mary's University of London, Alastair Lamb, Oxford, and Abay Mulatu, Coventry.

Conflict of Interest

The authors have no personal conflicts of interest to disclose. Within NeuroSAFE PROOF, laparoscopic ports are supplied by Applied Medical. Applied Medical had no role in the design, analysis, or collection of the data, in writing of the manuscript, or in the decision to submit the manuscript for publication.

References

1 **Ficarra V, Sooriakumaran P, Novara G et al.** Systematic review of methods for reporting combined outcomes after radical prostatectomy and proposal of a novel system: the survival, continence, and potency (SCP) classification. *Eur Urol* 2012; 61: 541–8

2 **Sooriakumaran P, Pini G, Nyberg T et al.** Erectile function and oncologic outcomes following open retropubic and robot-assisted radical prostatectomy: results from the laparoscopic prostatectomy robot open trial. *Eur Urol* 2018; 73: 618–27

3 **Tewari AK, Srivastava A, Huang MW et al.** Anatomical grades of nerve sparing: a risk-stratified approach to neural-hammock sparing during robot-assisted radical prostatectomy (RARP). *BJU Int* 2011; 108(6 Pt 2): 984–92

4 **Dev HS, Wiklund P, Patel V et al.** Surgical margin length and location affect recurrence rates after robotic prostatectomy. *Urol Oncol* 2015; 33: 109. e7–13

5 **Philippou Y**, **Harriss E**, **Davies L et al.** Prostatic capsular incision during radical prostatectomy has important oncological implications: a systematic review and meta-analysis. *BJU Int* 2018; 124: 554–66

6 **Mottet N**, **van den Bergh RCN**, **Briers E et al.** EAU - EANM - ESTRO - ESUR -SIOG guidelines on Prostate Cancer. 2019:49. Available at: https://uroweb.org/wp-content/uploads/EAU-EANM-ESTRO-ESUR-SIOG-Guidelines-on-Prostate-Cancer-2020v4.pdf.

7 **de Rooij M**, **Hamoen EH**, **Witjes JA**, **Barentsz JO**, **Rovers MM**. Accuracy of magnetic resonance imaging for local staging of prostate cancer: a diagnostic meta-analysis. *Eur Urol* 2016; 70: 233–45

8 **Rocco B**, **Sighinolfi MC**, **Sandri M et al.** Is extraprostatic extension of cancer predictable? A review of predictive tools and an external validation based on a large and a single center cohort of prostate cancer patients. *Urology* 2019; 129: 8–20

9 **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: 1011–6

10 **Eissa A**, **Zoeir A**, **Sighinolfi MC et al.** "Real-time" assessment of surgical margins during radical prostatectomy: state-of-the-art. *Clin Genitourin Cancer* 2020; 18: 95-104

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 **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

13 **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

14 **Preisser F**, **Theissen L**, **Wild P et al.** Implementation of Intraoperative Frozen Section During Radical Prostatectomy: Short-term Results from a German Tertiary-care Center. *European Urology Focus*. 2019. https://doi.org/10.1016/j.euf.2019.03.007.

15 **Sams SB**, **Wisell JA**. Discordance between intraoperative consultation by frozen section and final diagnosis. *Int J Surg Pathol* 2017; 25: 41–50

16 **Johnson BS**, **Fladie I**, **Jellison S**, **McMurray M**, **Narayan V**, **Vassar M**. Quality of intervention reporting in randomized clinical trials published in urology journals. *Eur Urol* 2020; 77: 296–8

17 **Jacquier I**, **Boutron I**, **Moher D**, **Roy C**, **Ravaud P**. The reporting of randomized clinical trials using a surgical intervention is in need of immediate improvement: a systematic review. *Ann Surg* 2006; 244: 677–83

18 **Dinneen E**, **Haider A**, **Allen C et al.** NeuroSAFE robot-assisted laparoscopic prostatectomy versus standard robot-assisted laparoscopic prostatectomy for men with localised prostate cancer (NeuroSAFE PROOF): protocol for a randomised controlled feasibility study. *BMJ Open* 2019; 9: e028132

19 **Ficarra V**, **Novara G**, **Ahlering TE et al.** Systematic review and meta-analysis of studies reporting potency rates after robot-assisted radical prostatectomy. *Eur Urol* 2012; 62: 418–30

20 **Carlucci JR**, **Nabizada-Pace F**, **Samadi DB**. Robot-assisted laparoscopic radical prostatectomy: technique and outcomes of 700 cases. *Int J Biomed Sci* 2009; 5: 201–8

21 **Murphy DG**, **Kerger M**, **Crowe H**, **Peters JS**, **Costello AJ**. Operative details and oncological and functional outcome of robotic-assisted laparoscopic radical prostatectomy: 400 cases with a minimum of 12 months follow-up. *Eur Urol* 2009; 55: 1358–66

22 **Sealed Envelope Ltd**. Comprehensive randomisation service [online], 2020. Available at: https://www.sealedenvelope.com. Accessed July 1, 2020.

23 **World Medical Association**. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Bull World Health Organ*. 2001; 79: 373–4

24 **Samaratunga H**, **Montironi R**, **True L et al.** International Society of Urological Pathology (ISUP) Consensus Conference on Handling and Staging of Radical Prostatectomy Specimens. Working group 1: specimen handling. *Mod Pathol* 2011; 24: 6–15.

25 **Royal College of Pathologists**. *Standard and Datasets for Reporting Cancers. Dataset for Histopatholgy Reports for Prostatic Carcinoma*. London: Royal College of Pathologists, 2016

26 **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

27 **van der Slot MA**, **den 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

28 **Olde Heuvel J**, **de Wit-van der Veen BJ**, **Huizing DMV et al.** State-of-the-art intraoperative imaging technologies for prostate margin assessment: A systematic review. *European Urology Focus*. 2020. https://doi.org/10.1016/j.euf.2020.02.004

29 **Porpiglia F**, **Checcucci E**, **Amparore D et al.** Three-dimensional elastic augmented-reality robot-assisted radical prostatectomy using hyperaccuracy three-dimensional reconstruction technology: a step further in the identification of capsular involvement. *Eur Urol* 2019; 76: 505–14

30 **Hatzichristodoulou G**, **Wagenpfeil S**, **Weirich G et al.** Intraoperative frozen section monitoring during nerve-sparing radical prostatectomy: evaluation of partial secondary resection of neurovascular bundles and its effect on oncologic and functional outcome. *World J Urol* 2016; 34: 229–36

31 **Fossa SD**, **Beyer B**, **Dahl AA et al.** Improved patient-reported functional outcomes after nerve-sparing radical prostatectomy by using NeuroSAFE technique. *Scand J Urol* 2019; 53: 385–91

32 **Abdollah F**, **Moschini M**, **Sood A et al.** When should a positive surgical margin ring a bell? An analysis of a multi-institutional robot-assisted laparoscopic radical prostatectomy database. *J Endourol* 2016; 30: 201–7

33 **Kates M**, **Sopko NA**, **Han M**, **Partin AW**, **Epstein JI**. Importance of reporting the gleason score at the positive surgical margin site: analysis of 4,082 consecutive radical prostatectomy cases. *J Urol* 2016; 195: 337–42

34 **Brimo F**, **Partin AW**, **Epstein JI**. Tumor grade at margins of resection in radical prostatectomy specimens is an independent predictor of prognosis. *Urology* 2010; 76: 1206–9

35 **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

36 **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

37 **Kim CS**, **Choi SY**, **Chi BH et al.** Does intraoperative frozen section really predict significant positive surgical margins after robot-assisted laparoscopic prostatectomy? A retrospective study. *Asian Journal of Andrology*. 2020. https://doi.org/10.4103/aja.aja_16_20.

Correspondence: Eoin Dinneen, Division of Surgery and Interventional Science, University College London, Charles Bell House, 43-45 Foley Street, Fitzrovia, London, W1W 7TY, UK.

e-mail: eoin.dinneen@nhs.net

Abbreviations: AUC, area under the curve; IFS, intra-operative frozen section; LND, lymph node dissection; mpMRI, multiparametric MRI; NS, nerve-sparing; NVB, neurovascular bundle; PSM, positive surgical margin; RARP, robot-assisted radical prostatectomy; RCT, randomized controlled trial; ROC, receiver-operating characteristic; RP, radical prostatectomy; SR, secondary resection.

Supporting Information

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

Appendix S1. CONSORT Flow Diagram.