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# Intraoperative assessment and reporting of radical prostatectomy specimens to guide nerve-sparing surgery in prostate cancer patients (NeuroSAFE)

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**Abstract**

**Aims** Radical prostatectomy for prostate cancer is frequently complicated by urinary incontinence and erectile dysfunction. Nerve-sparing surgery reduces the risk of post-operative complications and can be optimized using intraoperative frozen sections of the adjacent neurovascular structure (NeuroSAFE). The aim of this study was to evaluate the pathological outcomes of the NeuroSAFE technique and to develop a comprehensive algorithm for intraoperative clinical decision-making.

**Methods and Results** Between September 2018 and May 2019, 491 NeuroSAFE procedures were performed in 258 patients undergoing radical prostatectomy; 74/491 (15.1%) NeuroSAFEs had a positive surgical margin. Compared to the corresponding paraffin sections, NeuroSAFE had a positive and negative predictive value of 85.1% and 95.4%, respectively. In 72.2% secondary neurovascular bundle resections prompted by a positive margin at NeuroSAFE, no tumour was present. These cases more often had a positive surgical margin of ≤1 mm (48.7% versus 20.0%; p=0.001) and only one positive slide (69.2% vs 33.3%; p=0.008). None of the nine patients with Gleason pattern 3 at the surgical margin, positive margin length ≤1 mm and one positive slide had tumour in the secondary resection.

**Conclusions** This study provides a systematic reporting template for pathological intraoperative NeuroSAFE evaluation, supporting intraoperative clinical decision-making and comparison between prostate cancer operation centres.

**Keywords**: prostate cancer, frozen section, prostatectomy, surgical margin, NeuroSAFE

**Introduction**

Radical prostatectomy (RP) is one of the main treatment modalities for men with localized prostate cancer. While RP was initially mostly performed for low- to intermediate-risk disease, patients with high-risk cancer are increasingly offered RP in Europe and North America (1, 2). Despite its efficacy in oncological disease control, RP is complicated by urinary incontinence and erectile dysfunction in 3-16% and 20-90% of men, respectively (3, 4). Surgical preservation of neurovascular bundles adjacent to the prostate, urologists' experience and centralization in high-volume expert centres can all contribute to reduction of complication rates (5-9). Clinical suspicion of extra-prostatic expansion is a relative contraindication for nerve-sparing surgery, limiting its potential beneficial effects in this high-risk subgroup of prostate cancer patients (10-12).

Standardized intraoperative frozen section (IFS) assessment of surgical margins during RP according to the NeuroSAFE technique has shown to significantly increase nerve-sparing surgery without negatively affecting oncological outcome (9, 13-15). For this purpose, urologists initially perform a bilateral nerve-sparing RP, after which prostate tissue adjacent to the neurovascular bundles, which are still *in situ*, is removed from the specimen and submitted for detailed pathological intraoperative evaluation. If adenocarcinoma does not reach into the surgical margin, the ipsilateral nerve-bundle remains intact; in case of a positive IFS margin the adjacent neurovascular bundle is then removed. NeuroSAFE is increasingly being offered to prostate cancer patients in Europe (13, 15, 16). Implementation of the NeuroSAFE methodology requires standardization of pathologic evaluation and reporting as well as development of clinical algorithms for subsequent surgical decision-making (17).

Since September 2018, seven medical centres in The Netherlands collaborate within the Anser Prostate Network, in which all RPs are performed with NeuroSAFE in one high-volume operation clinic. The aim of this study is to report pathological outcomes of the NeuroSAFE technique, and to develop a comprehensive algorithm for pathologic reporting and intraoperative clinical decision-making.

## Materials and methods

### *Study population*

Patients undergoing RP for prostate cancer in the Anser Prostate operation Clinic, Maasstad Hospital, Rotterdam, The Netherlands between September 2018 and May 2019 were included. Intraoperative assessment of surgical margin status according to the NeuroSAFE methodology was offered to the vast majority of patients. NeuroSAFE was not applied uni- or bilaterally in case of clinical T3 disease, fibrotic adhesions for instance due to previous operations, or patient anxiety. The study was approved by the local ethical committee (METC-2019-0352).

### *NeuroSAFE procedure*

The NeuroSAFE procedure was performed as described by Schlomm et al (13). Initially, each patient underwent a bilaterally nerve-sparing RP. After removal of the prostate, the urologist cleaved the posterolateral sides being adjacent to the neurovascular bundles from apex to base; the neurovascular bundles themselves remained *in situ* during this procedure. The cleaved posterolateral prostate tissues were inked at the apical, outer and inner surfaces for orientation. The right and left inked prostate tissues were submitted for IFS assessment at the pathology department.

In case IFS assessment did not reveal tumour in the surgical margin, the operation was finished leaving the neurovascular bundles intact. If tumour was identified within the IFS surgical margin, a partial or total secondary resection of the ipsilateral neurovascular bundle was performed. A partial secondary resection was performed only if the surgeon was able to precisely identify the anatomical area directly adjacent to the positive surgical margin and the margin was positive in one or at most two adjacent slides. The location was determined as the slice number counted from the marked apex.

From September 2018 to February 2019, a secondary resection of the neurovascular bundle was performed in all cases with a positive IFS margin. After February 2019 a secondary resection was performed only if a positive surgical margin was present in more than one slide, had a cumulative

length of >1 mm, or contained Gleason pattern 4 or 5 tumour. In secondary bundle resections, the non-prostate side i.e. the external surface representing the definitive surgical margin was inked by the urologist for orientation.

*Frozen section analysis*

After gross reporting, the inked prostate tissue was transversely cut into 5-mm sections, resulting in 7 to 10 slices per side, which were oriented from apex to base. Standard 5 micron HE frozen sections were prepared from the prostate slices. Five pathologists with interest in genitourinary pathology reported all IFS. A positive surgical margin was defined as at least one malignant gland abutting the inked margin. In case of a positive surgical margin, the urologist was informed on the number and location of the positive slides, the cumulative positive surgical margin length, and the Gleason pattern at the margin. After IFS evaluation remaining tissue was thawed, formalin-fixed and embedded for preparation of standard HE slides.

*Pathological analysis*

After formalin-fixation, residual RP specimens were transversely sectioned into 4-mm slices from apex to base, and entirely submitted for diagnostic purposes together with the IFS and formalin-fixed paraffin-embedded NeuroSAFE slices. In case of a secondary neurovascular excision, the tissue was transversely cut into 2-mm sections. At microscopic evaluation, the following parameters were recorded: Gleason score and Grade group according to the WHO 2016 guidelines, pT-stage (AJCC 8$^{th}$ edition), and surgical margin status. Pathological stage T3a was defined as presence of prostate cancer cells between or at the level of peri-prostatic fat tissue. A positive margin at IFS without a secondary resection and no extra-prostatic extension was defined as pT2. Patients with a positive surgical margin at IFS but negative outer surface margin at secondary resection, were considered to have a negative surgical margin at definitive reporting. Patients with a surgical margin being

negative at IFS but positive on corresponding paraffin section, were considered to have a positive surgical margin.

*Statistical analysis*

Median cumulative lengths of positive surgical margins were compared with the Mann-Whitney test. Categorical Grade groups, positive surgical margin length groups, and number of positive slides were compared with the Chi-square test. The Spearman coefficient was used to determine the association between pathology IFS and operation duration in relation to time. Statistical analyses were performed with IBM SPSS version 24. A p-value of ≤0.05 was considered statistically significant.

**Results**

*Patient characteristics*

Between September 2018 and May 2019, 276 men underwent a robot-assisted RP of whom 258 with NeuroSAFE procedure (Table 1). The median age of the 258 patients was 67.0 years (Interquartile range (IQR) 63.0-71.0) and median pre-operative Prostate Specific Antigen (PSA) level was 9.4 ng/ml (IQR 6.4-12.7). Fifty men (19.4%) had a pre-operative biopsy Grade group (GG) 1, 101 (39.1%) had GG2, 62 (24.0%) GG3, 27 (10.5%) GG4, 17 (6.6%) GG5, and 1 (0.4%) was unknown. NeuroSAFE was performed bilaterally in 233 (90.3%) and unilaterally in 25 (9.7%) patients, resulting in a total of 491 IFS analyses. If NeuroSAFE was performed at one side, the other side was operated non-nerve sparing.

*Intra-operative frozen sections and corresponding paraffin slides*

Of the 491 NeuroSAFE samples, 417 (84.9%) had a negative IFS surgical margin and 74 (15.1%) were positive (Figure 1). Corresponding paraffin sections of the IFS slides with negative surgical margin revealed similar surgical margin status in 398/417 samples (95.4%; negative predictive value), but were positive in 19 (4.6%) cases (Figure 2). Re-evaluation of the original IFS slides confirmed a negative IFS surgical margin in all 19 cases, indicating the positive margin at the paraffin sections resulted from deeper cutting of the tissue block. These 19 discrepant cases had a median cumulative positive surgical margin length of 0.2 mm (IQR 0.1-0.4) at the corresponding paraffin section; 17/19 (89.5%) had a positive surgical margin length of ≤1 mm, and 2 (10.5%) between 1 and 3 mm (Table 2).

Corresponding paraffin sections of the 74 samples with positive IFS surgical margin revealed a concordant positive margin in 63 samples (85.1%; positive predictive value) and were negative in 11 (14.9%) cases (Figure 2). Re-evaluation of the original IFS slides confirmed a positive surgical margin in 9/11 (81.8%) patients, again indicating discrepancy was caused by inherent 250-300 µm deeper sectioning of the paraffin block. However, in two samples re-evaluation disclosed that the artificial margin at the prostate slice edge which was close to the true margin was erroneously called

positive; one of these patients underwent a secondary resection. Therefore, the overall sensitivity and specificity of the IFS compared to the corresponding paraffin section was 76.8% (63/82) and 97.3% (398/409), respectively.

The median interval from registration to reporting by the pathology department was 43 minutes (IQR 39-50) and decreased over time (Spearman rho -0.26; p<0.001). The median pathology time of the first 100 NeuroSAFE procedures was 46 minutes (IQR 40-53) compared to 41 minutes (IQR 37-48) of the last 100 (p=0.004). For unilateral NeuroSAFE the median time was 40 minutes (IQR 31-46) compared to 44 minutes (IQR 39-50) for bilateral procedures (p=0.003).

*Secondary neurovascular bundle resections*

In 61/491 (12.4%) NeuroSAFE procedures a secondary neurovascular bundle resection was performed. Fifty-five (90.2%) of these were prompted by a positive IFS surgical margin, while 6 (9.8%) were performed in spite of a negative IFS margin because of strong clinical suspicion of extensive disease during operation (Figure 1). Two out of these 6 IFS negative cases had a positive margin at the corresponding paraffin slides, of which in one sample tumour was present in the secondary resection; in the other five secondary resections no tumour was found. Fifty-five out of 74 (74.3%) samples with positive IFS surgical margin underwent a secondary resection. While it was omitted in 19 (25.7%) cases as the margin was only minimally affected by Gleason pattern 3 disease. The two false-positive IFS cases, of which one had undergone a secondary resection, were excluded from further analysis.

In 15/54 (27.8%) secondary resections, adenocarcinoma was present within the neurovascular bundle tissue, while 39/54 (72.2%) cases did not contain tumour. Tumour was present in 5/25 (20.0%) partial and in 10/29 (34.5%) total secondary resections. Compared to cases with tumour present in the secondary resection, those without tumour more often had a positive IFS surgical margin of ≤1 mm (48.7% *versus* 20.0%; p=0.001) and only one single surgical margin positive IFS slide (69.2% *versus* 33.3%; p=0.008) (Table 2). In none of the nine NeuroSAFE specimens with a positive surgical margin of ≤1 mm and Gleason pattern 3 in one IFS section, adenocarcinoma was present in the secondary resection. In 51/54 (94.4%) samples the definitive neurovascular bundle

surgical margin converted to negative by secondary resection, while two patients had positive surgical margins at secondary resections. Of these, one patient had a unilateral positive surgical margin at a total secondary resection and one had bilateral positive surgical margins at both partial secondary resections.

*Definitive radical prostatectomy findings*

At final pathological evaluation of the RP specimen, 26 (10.1%) patients had GG1, 115 (44.6%) GG2, 88 (34.1%) GG3, 12 (4.7%) GG4 and 17 (6.6%) had GG5 disease (Table 3). In total 140 (54.3%) patients had pT2, 79 (30.6%) pT3a and 39 (15.1%) pT3b. After analysis of corresponding paraffin NeuroSAFE sections, RP specimens and secondary neurovascular bundle resections, 89/258 (34.5%) men had positive surgical margins at final pathology reporting. The margin was positive at the apical, basal or anterolateral non-NeuroSAFE side in 54 (60.7%) patients, at the posterolateral NeuroSAFE side in 18 (20.2%) men, and at both NeuroSAFE and non-NeuroSAFE sides in 17 (19.1%). A final positive margin at a NeuroSAFE side was prompted by: a) presence of a positive surgical margin in corresponding paraffin sections of negative IFS (n=15), b) minutely positive IFS sections without secondary resection (n=16), c) positive paraffin margin in negative IFS (a) on one side and omission of secondary resection (b) at the contralateral side (n=2), and d) positive margin in the secondary neurovascular bundle resection (n=2). In 49/64 patients with positive IFS margin, a secondary resection had been performed, which led to conversion to a definitive negative margin at the ipsilateral neurovascular bundle side in 47 (95.9%) men, while one had a positive margin in the secondary resection.

The median surgery duration was 194 minutes and decreased over time (Spearman rho -0.18; p=0.005). No significant difference was found between unilateral or bilateral NeuroSAFE procedures. The median time of the first 100 NeuroSAFE procedures was 203 minutes (IQR 175-229) compared to 189 minutes (IQR 163-212) for the last 100 patients (p=0.03).

**Discussion**

Extensive IFS analysis according to the NeuroSAFE procedure enables more frequent nerve-sparing surgery without having negative impact on oncological outcome (13). In the current study, 74/491 (15.1%) NeuroSAFE specimens from 64 patients had a positive surgical margin. In 39/54 (72.2%) secondary resections performed for a positive NeuroSAFE, no remaining tumour was present in the neurovascular bundle. The positive IFS margin length was significantly smaller in these cases and more often present in only one slide. No tumour was present in secondary resections in case the IFS margin was positive in one section with a length of ≤1 mm and Gleason pattern 3. These results indicate that secondary neurovascular bundle resection might be omitted in cases with limited low-grade disease at the positive IFS surgical.

Our 85.1% positive and 95.4% negative predictive value of NeuroSAFE analysis is well in line with those reported by others (13, 15, 18, 19). Also, our frequency of 27.8% tumour in the secondary neurovascular bundle resections corresponds well with the proportion of 23% reported by Schlomm et al. in 1368 cases, and of 33% reported by Fromont et al. in 24 resections (13, 20). In contrast, Mirmilstein et al. found a higher frequency of 42.4% tumours in the secondary resections among 33 resections (15). Our sensitivity of 76.8% is lower than that reported by Schlomm et al. (93.5%) and Mirmilstein et al. (90%), but higher than Tsuboi et al. (62%), while our 97.3% specificity was in line with previous studies (13, 15, 21). Since the frequency of tumour in the secondary resections was comparable to other studies, our lower sensitivity might be explained by differences in local work-up of corresponding FFPE blocks, or use of more strict criteria for calling a positive surgical margin (13, 20).

If no tumour was found in the secondary resection, the positive margin length at IFS was mostly small. Although positive surgical margin status is not equivalent to biochemical recurrence, recurrence rates do increase with incremental cumulative length and tumour grade in the surgical margin (22-26). Several studies found that men with ≤3 mm positive surgical margin length had similar biochemical recurrence-free survival as compared to those with negative surgical margins

(22, 26, 27). Furthermore, presence of Gleason pattern 3 in the surgical margin has been associated with decreased risk of recurrence (28, 29).

Introduction of NeuroSAFE requires standardization of work-up, evaluation, reporting and clinical decision-making. In our study, none of the patients with a positive intraoperative surgical margin in one tissue section, length of ≤1 mm and Gleason pattern 3 into the ink, had tumour in the secondary resections. Based on the above-mentioned RP studies and our preliminary data, our group decided to abstain from secondary neurovascular bundle resection in cases meeting these criteria. Intraoperative reporting of positive NeuroSAFE procedure in our centre therefore routinely includes the following parameters: location as determined by distance from the apex, number of positive slides, cumulative length and Gleason pattern in the margin. This synoptic reporting allows for standardization of subsequent intraoperative decision-making and serves quality assurance purposes.

Extensive IFS techniques are increasingly applied in European prostate cancer operation centres. Other groups have shown that NeuroSAFE resulted in increased nerve-sparing surgery, while randomized controlled trials in relation to functional outcome are still ongoing (13, 15, 26, 30). Despite its putative positive effects on functional outcome, the NeuroSAFE technique requires logistic adaptations in pathology laboratories for processing and reporting of 10 to 20 frozen sections per RP. In our centre this was achieved by a team of 3 technicians who simultaneously prepared frozen sections at 2 cryostat stations. After the first 100 NeuroSAFE procedures, the median time for NeuroSAFE processing and reporting decreased from 46 to 41 minutes. This time is comparable with that of Beyer et al. who reported an average NeuroSAFE pathology time of 35 minutes in more than 1000 patients, indicating optimization is still possible at larger numbers of procedures (14).

This is the first detailed study on pathological evaluation of IFS according to the NeuroSAFE technique and might serve as a guidance for centres introducing this procedure. However, the number of patients was relatively limited, and the change of protocol with regard to intraoperative decision-making might have caused a bias. Furthermore, follow-up was too short for analysis of oncological or functional outcome in our cohort.

In conclusion, this study provides guidance for reporting and clinical decision-making for intraoperative NeuroSAFE procedures. In men with minute positive surgical margins of ≤1 mm in one section with Gleason pattern 3, secondary nerve-bundle resection might be omitted, leading to maximisation of nerve-sparing prostate cancer operations.

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Van der Slot, den Bakker, Kliffen, Goemaere, Budel, Hamoen, Klaver, Busstra, Gan, Roobol performed the research.

Van Leenders, den Bakker, Bangma, Roobol designed the research study.

Helleman, Roobol, Bangma contributed essential tools

Van der Slot, den Bakker, van Leenders analysed the data

Van der Slot, van Leenders wrote the paper

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**Table 1**. Pre-operative patient characteristics.

| **Parameters** | **NeuroSAFE Patients** |
|---|---|
| Number of patients | 258 |
| Age, years, median (IQR) | 67.0 (63.0-71.0) |
| Preoperative PSA (ng/ml), median (IQR) | 9.4 (6.4-12.7) |
| Preoperative Grade group (GG) | |
| GG 1 | 50 (19.4%) |
| GG 2 | 101 (39.1%) |
| GG 3 | 62 (24.0%) |
| GG 4 | 27 (10.5%) |
| GG 5 | 17 (6.6%) |
| Unknown | 1 (0.4%) |
| Total biopsy number, median (IQR) | 10.0 (8.0-11.0) |
| Positive biopsy number, median (IQR) | 4.0 (2.0-6.0) |
| D'Amico risk stratification | |
| Low | 30 (11.6%) |
| Intermediate | 159 (61.6%) |
| High | 68 (26.4%) |
| Unknown | 1 (0.4%) |

**Table 2**. Pathological characteristics of intraoperative NeuroSAFE in relation to corresponding paraffin sections and secondary neurovascular bundle resections.

| | **Frozen and paraffin sections** | | | **Secondary resection** | | |
|---|---|---|---|---|---|---|
| Parameter | **positive/ positive*** | **negative/ positive*** | **p-value** | **tumour** | **no tumour** | **p-value** |
| NeuroSAFE number | 63 | 19 | | 15 | 39 | |
| Margin length, continuous** | 1.1 mm (0.4-2.4) | 0.2 mm (0.1-0.4) | <0.001 | 1.6 mm (1.1-2.2) | 1.2 mm (0.3-3.1) | 0.524 |
| Margin length, categorical | | | 0.012 | | | 0.002 |
| ≤1 mm | 30 (47.6%) | 17 (89.4%) | | 3 (20.0%) | 19 (48.7%) | |
| 1-2 mm | 16 (25.4%) | 1 (5.3%) | | 8 (53.3%) | 6 (15.4%) | |
| 2-3 mm | 6 (9.5%) | 1 (5.3%) | | 2 (13.3%) | 4 (10.3%) | |
| >3 mm | 11 (17.5%) | 0 | | 2 (13.3%) | 10 (25.6%) | |
| Margin Grade group | | | 0.864 | | | 0.025 |
| 1 | 32 (50.8%) | 11 (57.9%) | | 10 (66.7%) | 16 (41.0%) | |
| 2 | 10 (15.9%) | 2 (10.5%) | | 1 (6.7%) | 8 (20.5%) | |
| 3 | 7 (11.1%) | 1 (5.3%) | | 4 (26.7%) | 2 (5.1%) | |
| 4 | 10 (15.9%) | 3 (15.8%) | | 0 | 10 (25.6%) | |
| 5 | 4 (6.3%) | 2 (10.5%) | | 0 | 3 (7.7%) | |
| Number of positive slides | | | 0.023 | | | 0.004 |
| 1 | 39 (61.9%) | 18 (94.7%) | | 5 (33.3%) | 27 (69.2%) | |
| 2 | 17 (27.0%) | 1 (5.3%) | | 5 (33.3%) | 10 (25.6%) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ≥3 | 7 (11.1%) | 0 | | 5 (33.3%) | 2 (5.1%) | |

* Margin of the frozen section / Margin of the corresponding paraffin section

** Median positive surgical margin length in mm (IQR).

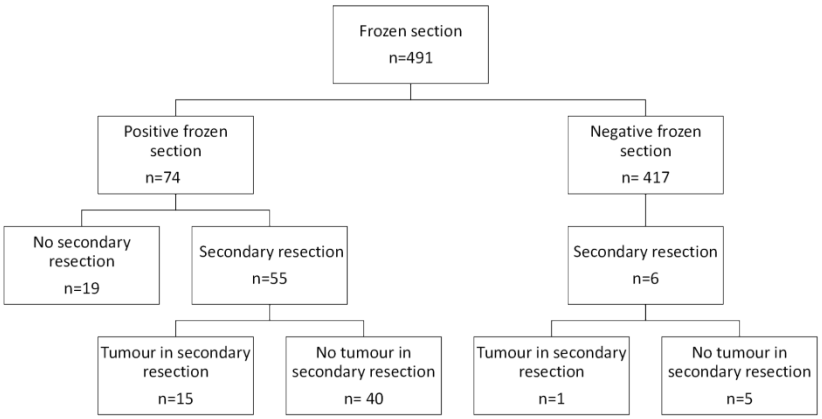
**Table 3**. Final pathological characteristics after radical prostatectomy.

| **Parameters** | **NeuroSAFE Patients** | **Uni- or bilateral positive frozen section(s)** |
|---|---|---|
| Number of patients | 258 | 64 (24.8%) |
| Grade group (GG) | | |
| GG 1 | 26 (10.1%) | 3 (11.5%) |
| GG 2 | 115 (44.6%) | 34 (29.6%) |
| GG 3 | 88 (34.1%) | 22 (25.0%) |
| GG 4 | 12 (4.7%) | 1 (8.3%) |
| GG 5 | 17 (6.6%) | 4 (23.5%) |
| Tumour stage (pT) | | |
| pT2 | 140 (54.3%) | 31 (22.1%) |
| pT3a | 79 (30.6%) | 22 (27.8%) |
| pT3b | 39 (15.1%) | 11 (28.2%) |
| Positive surgical margin | | |
| pT2 | 35 (25.0%) | |
| pT3a | 33 (41.8%) | |
| pT3b | 21 (53.8%) | |

**Figure legends**

**Figure 1**. Schematic overview of NeuroSAFE procedures and secondary neurovascular bundle resections.

**Figure 2**. Frozen sections (A, C) and corresponding paraffin sections (B, D) of two NeuroSAFE slices. A, B: both frozen and corresponding paraffin section have a positive surgical margin with tumour cells abutting into the ink (concordant). C, D: frozen section was called surgical margin negative, while corresponding paraffin section revealed a positive surgical margin (discordant). HE, original magnification 200x

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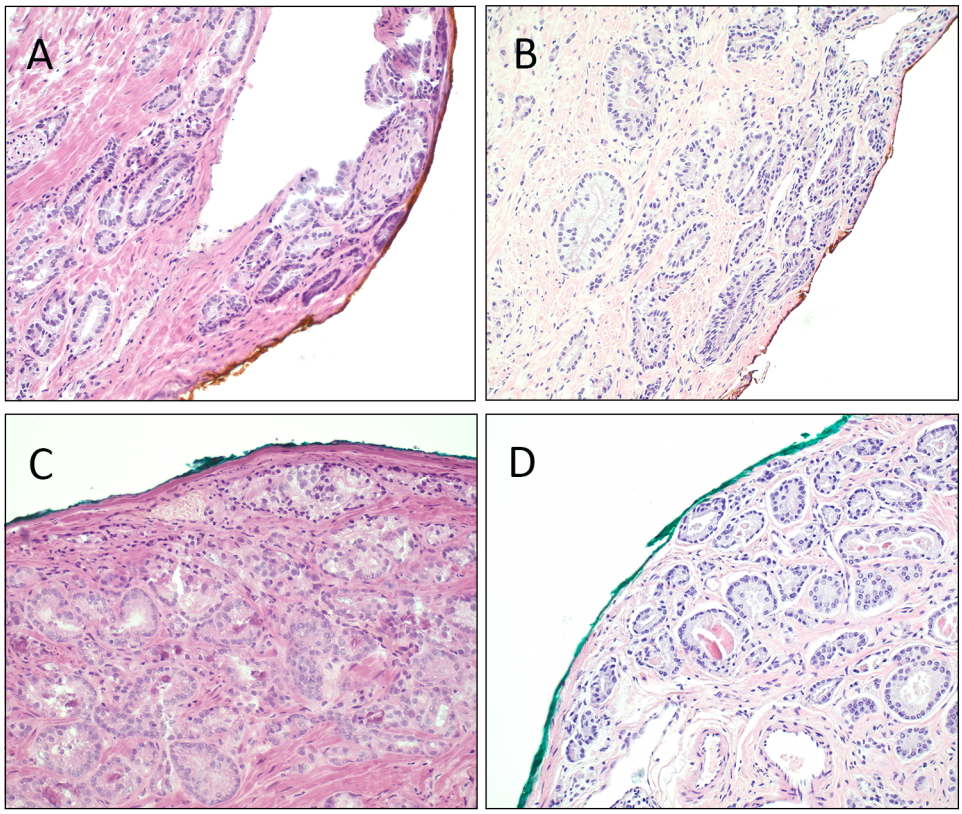


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