Prostate Imaging Compared to Transperineal Ultrasound-guided biopsy for significant prostate cancer Risk Evaluation (PICTURE): a prospective cohort validating study assessing Prostate HistoScanning

Lucy A. M. Simmons[1,2] * MD(Res)

Abi Kanthabalan[1,2] * MBBS

Manit Arya[2] FRCS (Urol)

Tim Briggs[4] FRCS (Urol)

Susan C. Charman[5,6] MSc

Alex Freeman[3] FRCPath

James Gelister[4] FRCS (Urol)

Charles Jameson[3] FRCPath

Neil McCartan[1,2]

Caroline M. Moore[1,2] FRCS (Uol)

Jan van der Muelen[5,6] PhD

Mark Emberton[1,2]§ FRCS (Urol)

Hashim U. Ahmed[1,2]§ FRCS (Urol)

* Joint first authors (these authors have made an equal contribution to this study and manuscript)

§ Joint senior authors (these authors have made an equal contribution to this study and manuscript)

1. Division of Surgery and Interventional Science, University College London, Faculty of Medical Sciences, London, United Kingdom,

2. Department of Urology, UCLH NHS Foundation Trust, London, UK

3. Department of Pathology, UCLH NHS Foundation Trust London, United Kingdom

4. Department of Urology, The Royal Free London NHS Foundation Trust, London, UK

5. Department of Health Services Research and Policy, London School of Hygiene and Tropical Medicine, London, United Kingdom

6. Clinical Effectiveness Unit, The Royal College of Surgeons of England, London, UK

Corresponding Author

Professor Hashim Uddin Ahmed

Professor and Chair

Division of Surgery

Department of Surgery and Cancer

Imperial College London

Email: hashim.ahmed@imperial.ac.uk

Key Words: Prostate Cancer, Imaging, HistoScanning

Abbreviations:

mpMRI- Multiparametric Magnetic Resonance Imaging

PHS- Prostate HistoScanning

TRUS biopsy- Transrectal ultrasound guided biopsy

TPM- Transperineal Template Mapping biopsy

PSA- Prostate specific antigen

NPV- Negative predictive value

PPV- Positive predictive value

Abstract

Background: Men with negative prostate biopsies or those diagnosed with low risk or low volume intermediate risk prostate cancers often require a second prostate biopsy prior to a treatment decision. Prostate HistoScanning (PHS) is an ultrasound imaging test that might inform prostate biopsy in such men.

Methods: PICTURE was a prospective, paired-cohort validating trial to assess the diagnostic accuracy of imaging in men requiring a further biopsy (clinicaltrials.gov, NCT01492270) (11/Jan/2012-29/01/2014). We enrolled 330 men who had undergone a prior TRUS biopsy but where diagnostic uncertainty remained. All eligible men underwent PHS and transperineal template prostate mapping (TTPM) biopsy (reference standard). Men were blinded to the imaging results until after undergoing TTPM biopsies. We primarily assessed the ability of Prostate HistoScanning to rule-out clinically significant prostate (negative predictive value [NPV] and sensitivity) for a target histological condition of Gleason >/=4+3 and/or a cancer core length (MCCL) ≥6mm. We also assessed the role of visually-estimated PHS-targeted biopsies.

Results: Of 330 men enrolled, 249 underwent both PHS and TTPM-biopsy. Mean (SD) age was 62 (7) years, median (IQR) PSA 6.8 (4.98-9.50) ng/ml, median (IQR) number of previous biopsies 1 (1-2) and mean (SD) gland size 37 (15.5) ml. One-hundred and forty-six (59%) had no clinically significant cancer. PHS classified 174 (70%) as suspicious. Sensitivity was 70.3% (95%CI 59.8-79.5) and NPV 41.3% (95%CI 27.0-56.8). Specificity and positive predictive value (PPV) were 14.7% (95% CI 9.1-22.0) and 36.8% (95% CI 29.6-44.4), respectively. 213/220 had PHS suspicious areas targeted with targeting sensitivity 13.6% (95%CI 7.3-22.6), specificity 97.6% (95%CI 93.1-99.5), NPV 61.6% (95%CI 54.5-68.4) and PPV 80.0% (95%CI 51.9-95.7).

Conclusions

Prostate HistoScanning is not a useful test in men seeking risk stratification following initial prostate biopsy.

Funding: PICTURE received funding from the US National Institute of Health (primary award 1R01CA135089; sub-award via Riverside Research Institute NYO.G00351P.011741.12) and an unrestricted research grant from Advanced Medical Diagnostics SA.

Introduction

Transrectal ultrasound guided (TRUS) biopsy is the current verification test for men suspected of having prostate cancer and is estimated to miss-classify disease in approximately 30-50% of cases (1). Men with a negative biopsy or those with low volume prostate cancer seeking accurate risk stratification prior to a treatment decision are often advised or choose to have a further biopsy.

Prostate-HistoScanning is an ultrasound-based imaging modality that in early trials showed promise in detecting and ruling-out clinically significant prostate cancer. However, studies assessing its diagnostic performance were often retrospective, not blinded to pathology or based on either TRUS-biopsy or whole-mount pathology from radical prostatectomy (2, 3). The former is inaccurate and the latter introduces a selection biases as men need to have a positive TRUS biopsy and then choose to have surgery rather than active surveillance or radiotherapy. Thus, men undergoing radical prostatectomy are not representative of the population of men in which the imaging modalities may be applied (4). The Prostate Imaging Compared to Transperineal Ultrasound-guided biopsy for significant prostate cancer Risk Evaluation (PICTURE trial) (5) was designed to overcome methodological limitations with the literature at the time of conception.

The PICTURE trial was a prospective, paired cohort validating trial, representing level 1b evidence for diagnostic test evaluation (6) . It aimed to assess the diagnostic accuracy of imaging in men who required further biopsies for diagnosis and/or accurate risk stratification of prostate cancer. (5). Our reference standard was Transperineal Template Prostate Mapping (TTPM) biopsies, which are both accurate and avoid many of the biases described. TTPM biopsies can be applied to all men under evaluation, overcome random error of TRUS-biopsies by sampling the whole prostate and fix the systematic error by sampling every 5mm (7, 8). In this paper, we report the on the ability of Prostate-HistoScanning to detect and rule-out clinically significant prostate cancer.

Methods

The PICTURE trial is a single-centre, prospective, ethics committee approved, registered diagnostic paired cohort validating study reported to STARD (9). The full details of our protocol have been published (10). Ethics committee approval for the study was granted by London City Road and Hampstead National Research Ethics Committee (reference 11/LO/1657) and the trial was registered with ClinicalTrials.gov (NCT01492270) on 6th December 2011. The study opened to recruitment on 11th January 2012 and completed recruitment on 29th January 2014. We have concurrently submitted the outcomes of another imaging test that was undergoing validation, multi-parametric MRI, as part of a separate manuscript.

Eligibility: Men who had undergone prior TRUS biopsy but where clinical suspicion remained that either prostate cancer had been missed or incorrectly classified were eligible for the study (10).

Index test – Prostate-HistoScanning: All eligible men underwent the Prostate-HistoScanning carried out with the BK 8818 end-fire probe. Prostate-HistoScanning was prospectively analysed by experienced reporters using Prostate-HistoScanning software version 2.1 with a report pro forma designed for the trial. Prostate-HistoScanning reporting is a semi-automated procedure. The analyst first defines the prostate contours and the software then divides the prostate equidistantly between the base and apical points into base, middle and apex sections, which are further subdivided to left and right. Within the prostate volume the ultrasound voxels are analysed to deem the likelihood of containing malignant tissue using computer assisted imaging processing algorithms (11, 12).

Areas deemed suspicious are demonstrated in red overlay to the greyscale ultrasound image. The reporting software used in PICTURE then delineated the three largest Prostate-HistoScanning suspicious areas (Supplementary Figure 1). To date Prostate-HistoScanning has no way of approximating cancer grade, and therefore significance of lesions at Prostate-HistoScanning was based on size criteria (13,14). Lesions over 1.3cc were deemed significant and further analysis was performed to suspicious volumes over 0.5cc. Prior to the TPM biopsies, men with Prostate-HistoScanning lesions had targeted biopsies taken that were visually directed to the largest lesion.

Assessment of variability: 70 men from the study had repeat PHS scans performed to allow for test-retest reliability. Men also underwent a further scan performed with the BK 8848 side-fire probe, PHS software for this probe was not commercially available.

Reference test: All men then underwent the reference test - transperineal template mapping biopsy (TPM) – which was performed according to a set protocol by trained urologists regardless of the imaging findings and the preceding targeted biopsies. The procedure was carried out under general or spinal anaesthesia with antibiotic prophylaxis and patients in the lithotomy position. A urethral catheter was inserted in order to visualize and avoid traversing the urethral lumen with biopsy needles. 5mm sampling was obtained using 17G biopsy core needles inserted via a brachytherapy grid fixed on a stepper. In most prostates, two biopsies at each grid point were required in order to sample the full cranio-caudal length of the gland. At the end of the procedure the catheter was usually removed unless gross haematuria or swelling was noted which would increase risk of post-procedure retention of urine. All biopsies were reported by expert 2 uro-pathologists of greater than 20 years experience each. All negative biopsies were double-reported for quality control. The histological reporting in our institution follows the classic scheme of interpreting the Gleason grading, the one used before the International Society of Urological Pathology 2005 guidelines (15). In other words, Gleason scoring was based on the most frequent pattern and not the highest grade detected on histological analysis. Further, the cancer core length was reported as the actual amount of cancer seen in each core without counting the intervening areas of benign glands (16).

Target condition: Disease significance was defined by criteria for disease significance that we have previously developed and validated for use with TTPM biopsies (17). UCL definition one, upon which we based our primary outcome, incorporated a maximum cancer core length (MCCL) involvement of 6mm or greater in any one location (representing the length of cancer which was able to detect 95% of lesions that were 0.5ml or greater in volume) or the presence of dominant/primary Gleason pattern 4 or greater (i.e., Gleason >/=4+3).

Sample size calculation: The sample size calculation was performed for the primary objective of calculating the negative predictive value (NPV) of PHS, using a precision-based estimate (18-20). Targeting an NPV of 90% for UCL definition one disease, for a 95% confidence interval, with a confidence width 10%, the number of patients needed with a negative test was 139. Assuming a prevalence of 38% for UCL definition one disease in the population of interest based on prior data at our centre (21), and assuming the performance characteristics of PHS equate to sensitivity and specificity of approximately 70%, a sample size of 316 patients would allow for 139 patients with a negative test. As the prevalence of men without clinically significant disease was not precisely known in the PICTURE study, an interim analysis at 114 recruited men permitted an adjustment in recruitment to ensure that at least 139 men with a negative reference test were available for analysis.

Statistics: Clinical validity was evaluated on a whole-gland basis using each patient as the unit of assessment. Sensitivity, specificity, positive and negative predictive values were calculated for all eligible men with binomial 95% confidence intervals. Cancer significance at Prostate-HistoScanning was defined as a suspicious area with volume 1.3ml or greater or secondarily 0.5ml or greater (13, 14). The histological target condition was defined as UCL definition the reference TTPM biopsy. Overall accuracy was assessed using area under receiver operating characteristic (AUROC) curves. Inter-observer variability was assessed using weighted kappa values as well as AUROC curves. STATA version 3.0 software was used with any tests of significance using p=0.05 as the threshold for statistical significance. Bland Altman was used to compare Prostate-HistoScanning volumes between the two time points and the two types of probes.

Results

Patient Demographics:

Three-hundred and thirty men were enrolled. After 110 men were withdrawn (Figure 1), 220 were available for primary analysis. Men eligible for analysis had mean (SD) age 62 (7) years, median (IQR) PSA 6.6 (4.98-9.50) ng/ml and median (IQR) number of previous biopsies 1 (1-2) and mean (SD) gland size 37ml (15.5) (Table 1). 91 (41.4%) men had UCL definition 1 significant cancer on biopsy.

Mean (SD) volume for Prostate-HistoScanning suspicious areas was 3.4 (SD 2.27) ml, with the largest suspicious area in any one prostate measuring mean 2.7 (SD 2.3) ml. Prostate-HistoScanning tested positive with a suspicious area of 1.3ml or greater.

Primary Outcome:

In ruling-out clinically significant prostate, sensitivity was 70.3% (95% CI 59.8-79.5) with negative predictive value 41.3% (95%CI 27.0-56.8). Specificity and positive predictive value were 14.7% (95% CI 9.1-22.0) and 36.8% (95% CI 29.6-44.4), respectively. Overall accuracy assessed by area under the receiver operating characteristic (AUROC) curve was 0.43 (0.37-0.48) (Table 3).

Secondary Outcomes

First, when the target condition was changed to ≥0.5cc cancer volumes sensitivity and negative predictive value were 93.4% (95% CI 86.2-97.5) and 14.3 (0.4-57.9), respectively. Specificity was 0.8% (95% CI 0.00-4.2) and positive predictive value 39.9 (33.3-46.8), respectively (Table 3). Overall accuracy assessed by area under the receiver operating characteristic (AUROC) curve was 0.47 (0.44-0.50).

Second, 213 of 220 men had Prostate HistoScanning lesions targeted. Sensitivity was 13.6% (95%CI 7.3-22.6), specificity 97.6% (95%CI 93.1-99.5), NPV 61.6% (95%CI 54.5-68.4) and PPV 80.0% (95%CI 51.9-95.7). Of the 213 men targeted based on the Prostate HistoScanning, 49 (23%) showed concordance with the TTPM-biopsy result. 76 (36%) were incorrectly classified as benign or insignificant when they actually harboured clinically significance disease at TTPM-biopsy. Three (1.4%) were found to have significant disease at Prostate HistoScanning targeted biopsies that were incorrectly classified as insignificant at TTPM-biopsy.

Second, when assessing inter-test variability, we scanned 70 men with the 8818 probe at consent and prior to TTPM-biopsy. Predicted suspicious area volumes (Supplementary Figure 2) between the two time-points showed a mean difference -0.5cc with lower and upper limits of agreement of -4.99 and 4.89, respectively. The largest suspicious area

showed a mean difference 0.07cc between the two time-points with lower and upper limits of agreement of -5.42 to 5.57, respectively (Supplementary Figure 3). Further, mean gland volumes were 39.43cc (SD 1.73) and 41.7cc (SD 1.82), at the first and second time-point. Supplementary Figure 4 shows a Bland Altman plot showing limits of agreement for gland volume with the mean difference between the two time points of 2.3 (lower and upper limits of agreement -9.0 and 13.6 respectively).

Third, when assessing inter-probe variability, we scanned 201 men with the 8818 and then the 8848 at baseline. Mean difference between suspicious area volumes was -2.07cc, with lower and upper limits of agreement of -8.90 and 4.76 (figure 5a), respectively. The main area of suspicious had mean differences between the two probes of 2.18cc, with lower and upper limits of agreement of -9.40 to 5.04, respectively (Supplementary Figure 5).

Fourth, there were no serious adverse events resulting from PHS. Serious adverse events resulting from TTPM biopsy occurred in 9 (3.6%) men. Adverse events were captured in 236 patients in a median of 38±56 days after biopsy. Haematuria and poor urine flow were reported respectively in 220 (93.2%) and 108 (45.8%). Urinary retention was diagnosed in 56 (23.7%). Urinary tract infection was diagnosed in 23 (9.8%) and perineal skin infection in 8 (3.4%). Rectal and perineal pain, and perineal bruising were reported in 59 (25.1%), 95 (40.3%), and 136 (57.6%), respectively. De novo erectile dysfunction occurred in 20.8%. Most were temporary with 2 requiring oral medication.

Discussion

In summary, our PICTURE trial shows that Prostate-HistoScanning has poor accuracy in men requiring further biopsies. Further, it also has poor performance when using it as a targeting modality. We also demonstrated poor reliability of gland and lesion volume both between time points with the same probe and between the two types of probes when performing Prostate-HistoScanning.

There are some limitations to our study. First, due to technical failures with the Prostate-HistoScanning device and consequent loss of data for reporting, several men were withdrawn after consent. Second, we relied heavily on the automated reporting software

with minimal manual refinement. At the time, the device manufacturers advised a manual 'refinement' the predicted suspicious areas to remove areas thought to contain poor ultrasound data. This was a highly subjective measure that we felt was not easily reproducible and standardised by all users of the technology. Third, our targeting technique was one based on visual estimation as an image-fusion platform for real-time Prostate-HistoScanning to live ultrasound was not available. Real-time targeting might have enhanced the localisation of the target and reduced targeting errors. Fourth, Prostate-HistoScanning as an ultrasound modality is known to be user dependent. Although all scans in our study were acquired by trained clinicians signed off for competency in the technique there may be some element of variability.

There has been a degree of controversy surrounding Prostate-HistoScanning in the literature (22), with initial proof of concept study results for the technology demonstrating very high performance characteristics with sensitivity and specificity of 100% and 82% for the detection of 0.5cc lesions, respectively. (11, 12) Further studies showed sensitivities ranging from 37- 90% and specificity 33-71% (23-25). Most of these were small single centre studies. The largest study of Prostate-HistoScanning to date was a retrospective series by Schiffman et al (26) looking at the volume of cancer at Prostate-HistoScanning and RP which showed very little correlation of size of Prostate-HistoScanning and radical prostatectomy lesions. The same group assessed the ability of Prostate-HistoScanning to predict positive biopsy (27), and found, as we did in the PICTURE study, that PHS has a rate of false positives which artificially inflated sensitivity.

To date PICTURE is the largest prospective study of Prostate-HistoScanning in a cohort of men requiring further biopsies, against an accurate reference test, TTPM-biopsy, which was able to minimise biases. Most other cohorts have been small, retrospective, not blinded and used either TRUS-biopsy or whole-mount histology with the inaccuracy and biases these introduce.

Prostate-HistoScanning although demonstrating reasonable sensitivity (70%) in this cohort of men, showed poor specificity, PPV and NPV. For a test to be useful in the prostate cancer pathway it needs to not only accurately detect clinically significant cancer but also rule-out

clinically significant cancer. The specificity and negative predictive value in this cohort were poor at 14.7% and 41.3%, respectively. The overall accuracy seemed at best to be no better than chance.

The Prostate-HistoScanning reliability work in PICTURE showed that the outputs of Prostate-HistoScanning, both in terms of gland size and lesion volume, were not stable between two time points using the same probe. Agreement between scans at different time points was seen to be poor with a mean difference of 2.3cc, but limits of agreement for gland size varying wildly from -9 to 13.6. The high variability in both the volume of the prostate gland and the volume of prostate lesions seen using Prostate-HistoScanning in this cohort deem the test unreliable as well as inaccurate.

Conclusion

The Prostate-HistoScanning results from the PICTURE study have shown that this imaging test has a poor ability to discriminate benign from malignant tissue. Prostate-HistoScanning has also not shown any promise in the guidance of prostate biopsy. These poor performance characteristics mean it has no place in the prostate cancer diagnostic pathway.

Acknowledgements

We would like to thank the men who participated in this study.

Other sources of funding and potential conflicts of interest

Prof. Mark Emberton's research is supported by core funding from the NIHR UCH/UCL Biomedical Research Centre. He is an NIHR Senior Investigator.

Prof. Hashim Ahmed receives funding from the Wellcome Trust, MRC and Prostate Cancer UK. Professor Ahmed's research is supported by core funding from the NIHR UCH/UCL Biomedical Research Centre.

Prof. Hashim Ahmed receives funding from Sonacare Medical, Sophiris and Trod Medical for other trials. Travel allowance was previously provided from Sonacare Inc. He is a paid consultant to Sophiris for trial-related work.

Professor Mark Emberton has stock interest in Nuada Medical Ltd. He is also a consultant to Steba Biotech and GSK. He receives travel funding from Sanofi Aventis, Astellas, GSK and Sonacare**.** He previously received trial funding or resources from GSK, Steba Biotech and Angiodynamics and currently receives funding for trials from Sonacare Inc, Sophiris Inc and Trod Medical.

Dr Alex Freeman has stock interest in Nuada Medical Ltd.

All others have no conflicts of interest.

Authorship

Study concept and initial design: Simmons, Moore, Emberton, Ahmed.

Study design and statistical analysis: Ahmed, Moore, Emberton, Charman, van der Meulen

Acquisition of data and Data analysis and interpretation: Arya, Ahmed, Briggs, Charman, Emberton, Freeman, Gelister, Jameson, Kanthabalan, McCartan, Moore, Simmons, van der Meulen,

Guarantor of data: Hashim Ahmed

References

1. Shaw GL, Thomas BC, Dawson SN, Srivastava G, Vowler SL, Gnanapragasam VJ, et al. Identification of pathologically insignificant prostate cancer is not accurate in unscreened men. Br J Cancer. 2014;110(10):2405-11.
2. Valerio M, Willis S, van der Meulen J, Emberton M, Ahmed HU. Methodological considerations in assessing the utility of imaging in early prostate cancer. Curr Opin Urol. 2015;25(6):536-42.
3. Valerio M, El-Shater Bosaily A, Emberton M, Ahmed HU. Defining the level of evidence for technology adoption in the localized prostate cancer pathway. Urol Oncol. 2014;32(6):924-30.
4. Barzell WE, Melamed MR, Cathcart P, Moore CM, Ahmed HU, Emberton M. Identifying candidates for active surveillance: an evaluation of the repeat biopsy strategy for men with favorable risk prostate cancer. J Urol. 2012;188(3):762-7.
5. Simmons LA, Ahmed HU, Moore CM, Punwani S, Freeman A, Hu Y, et al. The PICTURE study -- prostate imaging (multi-parametric MRI and Prostate HistoScanning) compared to transperineal ultrasound guided biopsy for significant prostate cancer risk evaluation. Contemp Clin Trials. 2014;37(1):69-83.
6. medicine OCfE-b. Levels of Evidence. 2009.
7. Crawford ED, Rove KO, Barqawi AB, Maroni PD, Werahera PN, Baer CA, et al. Clinical-pathologic correlation between transperineal mapping biopsies of the prostate and three-dimensional reconstruction of prostatectomy specimens. Prostate. 2013;73(7):778-87.
8. Lecornet E, Ahmed HU, Hu Y, Moore CM, Nevoux P, Barratt D, et al. The accuracy of different biopsy strategies for the detection of clinically important prostate cancer: a computer simulation. J Urol. 2012;188(3):974-80.
9. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. Ann Intern Med. 2003;138(1):40-4.
10. Simmons LA, Ahmed HU, Moore CM, Punwani S, Freeman A, Hu Y, et al. The PICTURE study - Prostate Imaging (multi-parametric MRI and Prostate HistoScanning) Compared to Transperineal Ultrasound guided biopsy for significant prostate cancer Risk Evaluation. Contemp Clin Trials. 2014;37(1):69-83.
11. Braeckman J, Autier P, Garbar C, Marichal MP, Soviany C, Nir R, et al. Computer-aided ultrasonography (HistoScanning): a novel technology for locating and characterizing prostate cancer. BJU Int. 2008;101(3):293-8.
12. Braeckman J, Autier P, Soviany C, Nir R, Nir D, Michielsen D, et al. The accuracy of transrectal ultrasonography supplemented with computer-aided ultrasonography for detecting small prostate cancers. BJU Int. 2008;102(11):1560-5.
13. Wolters T, Roobol MJ, van Leeuwen PJ, van den Bergh RC, Hoedemaeker RF, van Leenders GJ, et al. A critical analysis of the tumor volume threshold for clinically insignificant prostate cancer using a data set of a randomized screening trial. J Urol. 2011;185(1):121-5.
14. Stamey TA, Freiha FS, McNeal JE, Redwine EA, Whittemore AS, Schmid HP. Localized prostate cancer. Relationship of tumor volume to clinical significance for treatment of prostate cancer. Cancer. 1993;71(3 Suppl):933-8.

15. Epstein JI, Allsbrook WC, Amin MB, Egevad LL. The 2005 international society of urological pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma. Am J Surg Pathol. 2005;29(9):1228-42.
16. Karram S, Trock BJ, Netto GJ, Epstein JI. Should intervening benign tissue be included in the measurement of discontinuous foci of cancer on prostate needle biopsy? Correlation with radical prostatectomy findings. Am J Surg Pathol. 2011;35(9):1351-5.
17. Ahmed HU, Hu Y, Carter T, Arumainayagam N, Lecornet E, Freeman A, et al. Characterizing clinically significant prostate cancer using template prostate mapping biopsy. J Urol. 2011;186(2):458-64.
18. Flahault A, Cadilhac M, Thomas G. Sample size calculation should be performed for design accuracy in diagnostic test studies. J Clin Epidemiol. 2005;58(8):859-62.
19. Salmi LR, Michel P. Sample size for diagnostic accuracy studies. J Clin Epidemiol. 2004;57(8):869-70; author reply 71-2.
20. Knottnerus JA, Muris JW. Assessment of the accuracy of diagnostic tests: the cross-sectional study. J Clin Epidemiol. 2003;56(11):1118-28.
21. Valerio M, Anele C, Bott SR, Charman SC, van der Meulen J, El-Mahallawi H, et al. The prevalence of clinically significant prostate cancer according to commonly used histological thresholds in men undergoing template prostate mapping biopsies. J Urol. 2015.
22. Schiffmann J, Manka L, Boehm K, Leyh-Bannurah SR, Karakiewicz PI, Graefen M, et al. Controversial evidence for the use of HistoScanning in the detection of prostate cancer. World J Urol. 2015.
23. Simmons LA, Autier P, Zat'ura F, Braeckman J, Peltier A, Romic I, et al. Detection, localisation and characterisation of prostate cancer by prostate HistoScanning(). BJU Int. 2012;110(1):28-35.
24. Javed S, Chadwick E, Edwards AA, Beveridge S, Laing R, Bott S, et al. Does prostate HistoScanning play a role in detecting prostate cancer in routine clinical practice? Results from three independent studies. BJU Int. 2013.
25. Macek P, Barret E, Sanchez-Salas R, Galiano M, Rozet F, Ahallal Y, et al. Prostate histoscanning in clinically localized biopsy proven prostate cancer: an accuracy study. J Endourol. 2014;28(3):371-6.
26. Schiffmann J, Fischer J, Tennstedt P, Beyer B, Bohm K, Michl U, et al. Comparison of prostate cancer volume measured by HistoScanning and final histopathological results. World J Urol. 2014;32(4):939-44.
27. Schiffmann J, Tennstedt P, Fischer J, Tian Z, Beyer B, Boehm K, et al. Does HistoScanning predict positive results in prostate biopsy? A retrospective analysis of 1,188 sextants of the prostate. World J Urol. 2014;32(4):925-30.

Tables and figures:

Table 1: Patient demographics

	All men n=330	**Men eligible for analysis n=220**
Characteristic	Median (IQR)	Median (mean, SD, range)
Age, years	63 (42-83)	62.1 (61.7, 7.2, 41.7-83.2)
PSA concentration at consent, ng/ml	7.4 (0.7-58.05)	6.6 (7.7, 4.1, 0.9-30.03)
	Mean (SD)	Mean (SD)
Number of previous biopsies	1.49, (0.79)	1.4 (0.7)
Number of previous positive biopsies	0.79 (0.65)	0.9 (0.6)
PHS Prostate volume, cc	43.1 (21.28)	37 (15.5)

Table 2: Template biopsy disease distribution for men in PHS analysis n=220

	Mean (SD)	
Total number of cores	48.69 (12.3)	
Number of cancer cores	6.88 (5.95)	
MCCL (mm)	4.65 (3.59)	
	For men in PHS analysis n=220	
Gleason grade n (%)	Number	%
Benign	28	12.73
3+3	63	28.64
3+4	100	45.45
4+3	26	11.82
>/= 4+4	3	1.36

Table 3: PHS performance characteristics for Definition 1 disease

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	AUC (95% CI)
Prostate-HistoScanning largest suspicious area ≥1.3ml	70.3 (59.8-79.5)	14.7 (9.1-22.0)	36.8 (29.6-44.4)	41.3 (27.0-56.8)	0.82 (0.71-0.96)	2.01 (1.20-3.40)	0.43 (0.37-0.48)
Prostate-HistoScanning largest suspicious area ≥0.5ml	93.4 (86.2-97.5)	0.8 (0.0-4.2)	39.9 (33.3-46.8)	14.3 (0.4-57.9)	0.94 (0.89-1.00)	8.51 (1.04-69.5)	0.47 (0.44-0.50)

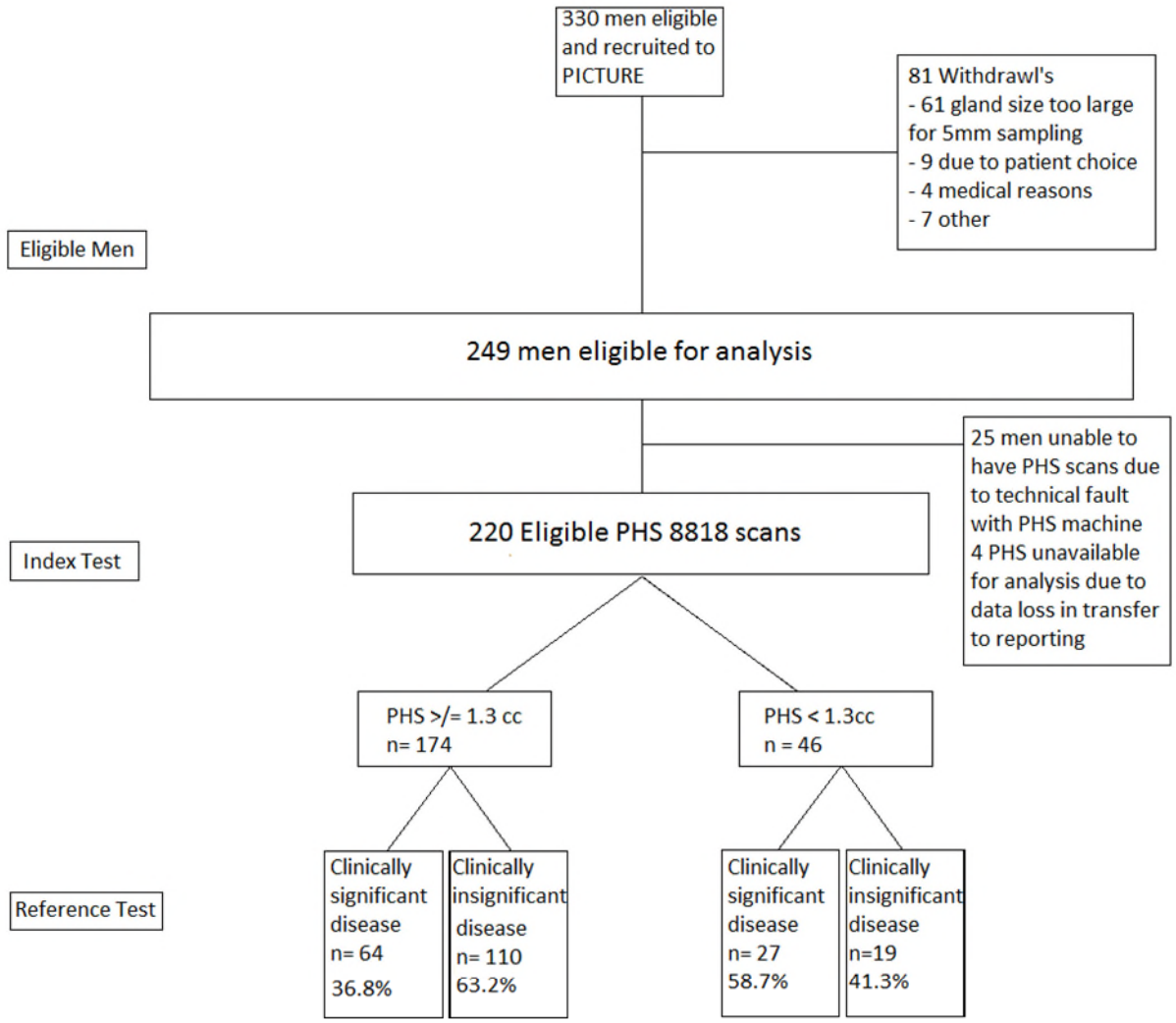
Table 4: Prostate-HistoScanning Targeted results

		Template Mapping biopsies				
		Benign	**Insignificant**	**Definition 2**	**Definition 1**	**Totals**
Prostate-HistoScanning **targeted biopsies**	**Benign**	26	32	44	58	**160**
	Insignificant	0	4	8	9	**21**
	Definition 2	0	0	8	9	**17**
	Definition 1	1	0	2	12	**15**
	Totals	**27**	**36**	**62**	**88**	**213**

Significant disease missed by TPM

Significant disease missed by targeting

Figure 1: PICTURE Prostate-HistoScanning **STARD compliant flowchart**



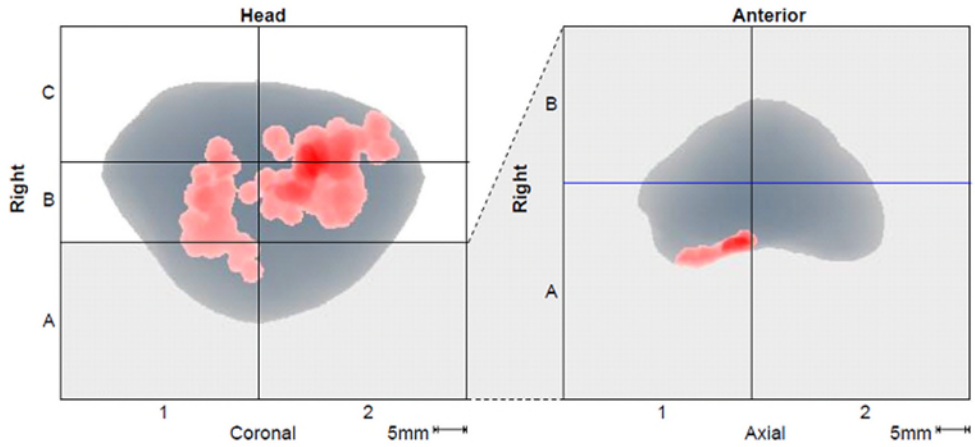
Supplementary Figure 1: Example of a Prostate-HistoScanning report in a man with prior negative TRUS-biopsy and an on-going clinical suspicion of prostate cancer requiring further biopsies (PSA 5.8ng/ml). TPM found G3+4 disease MCCL 6mm Definition 1 disease (concordant with both PHS targeted biopsy and MRI)

Statistics

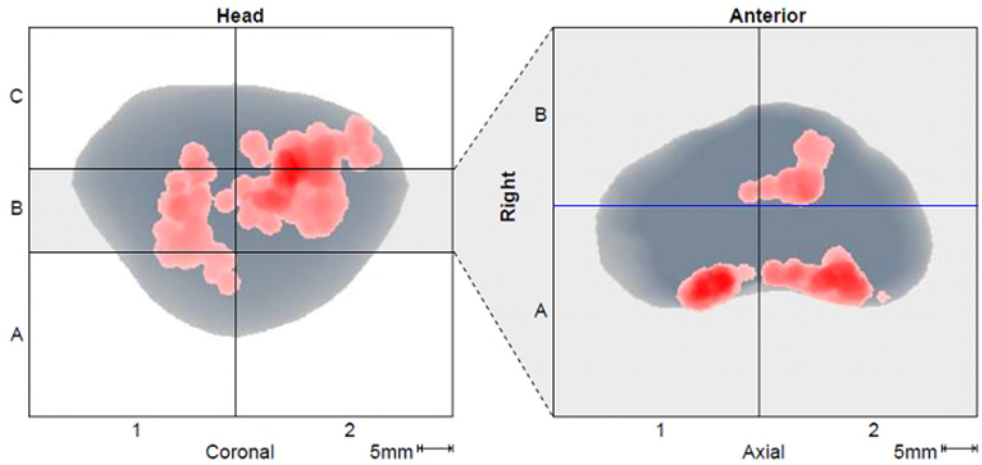
Total Volume	22.00 (cm³)	**HistoScanning Signals**	2.29 (cm³)
Connection 2	0.69 (cm³)	**Connection 3**	0.37 (cm³)
Connection 4	0.32 (cm³)	**Total Connection Volume**	1.38 (cm³)

Apex Mid Base Projections

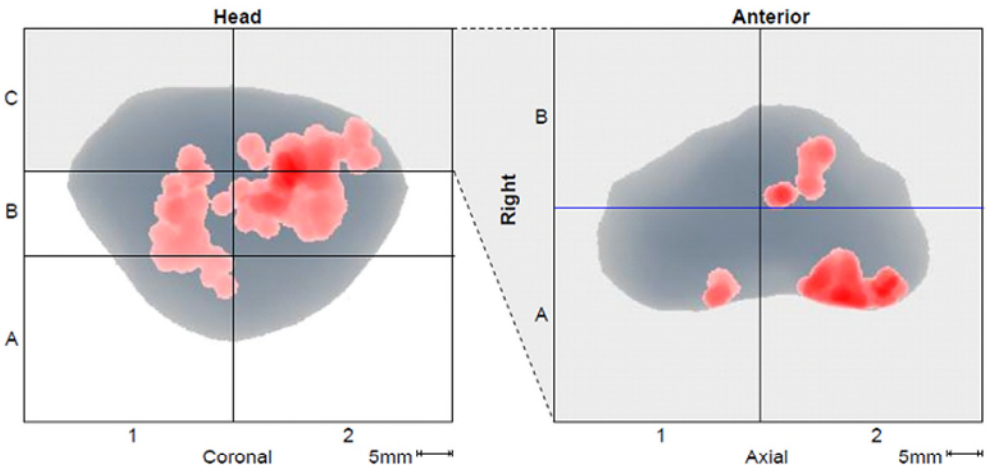
APEX



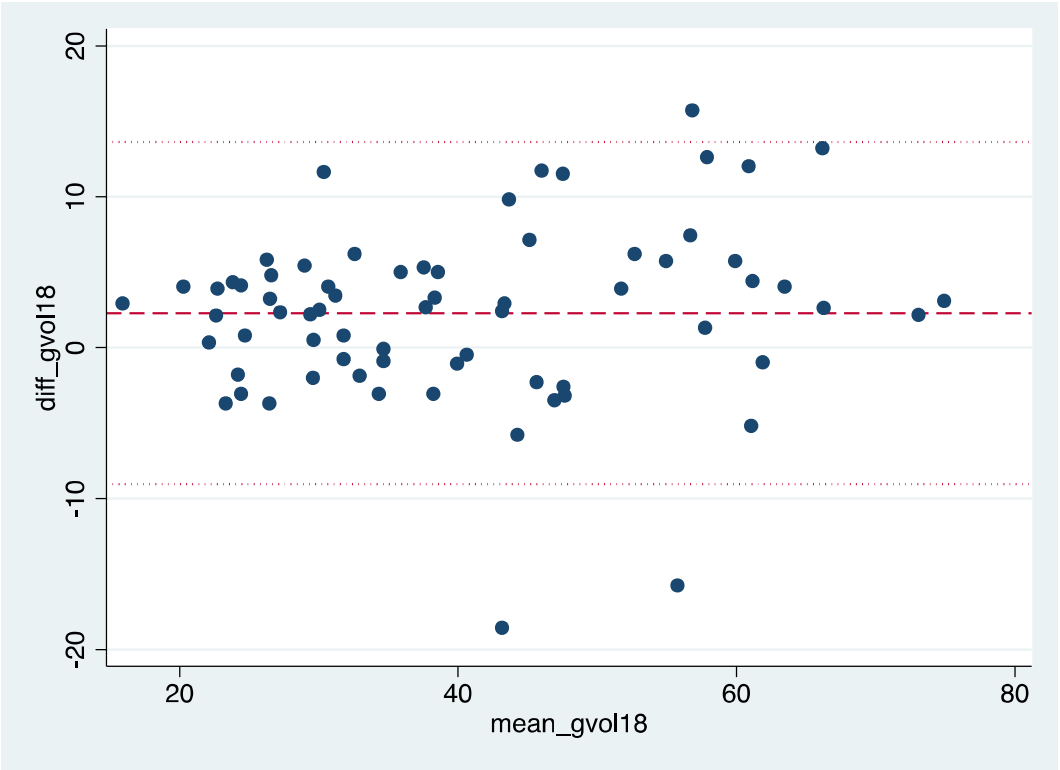
MID



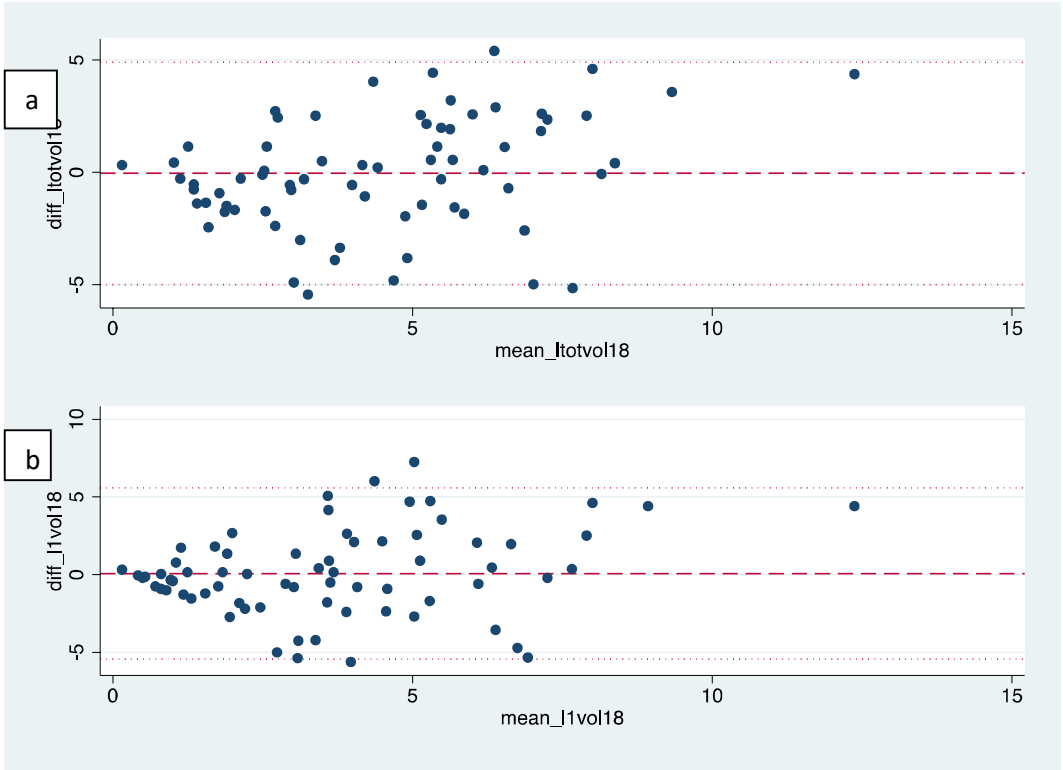
BASE



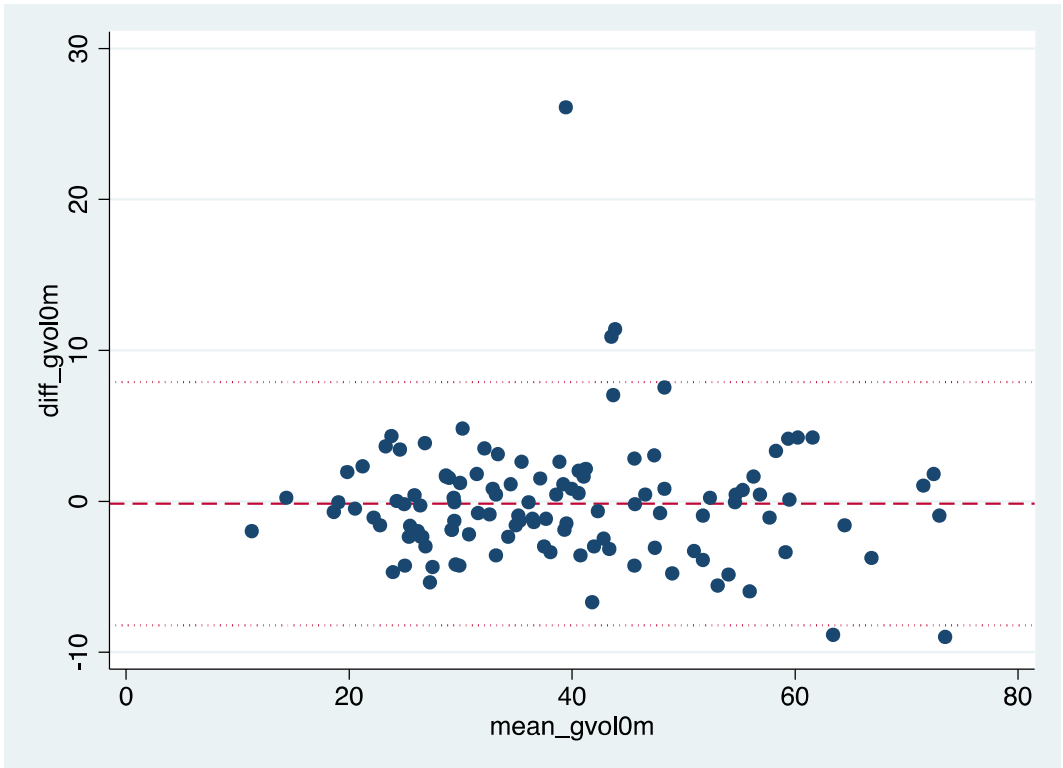
Supplementary Figure 2: Bland Altman plot of 8818 gland volume at consent and prior to TPM



Supplementary Figure 3: Bland Altman plot of (a) 8818 total suspicious area volume (totvol) and (b) main suspicious area volume (l1vol) volume at consent and prior to TTPM -biopsy



Supplementary Figure 4: Comparing probes 8818 & 8848 at baseline for Total Prostate gland volume



Supplementary Figure 5: Comparing probes 8818 & 8848 at baseline-

a) total suspicious area volume (totvol0m), b) Main suspicious area volume (l1Vol0m)