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SUSPICION DE CANCER DE LA PROSTATE ET IRM NEGATIVE. IMPLICATIONS DIAGNOSTIQUES ET POUR LA SURVEILLANCE ACTIVE.

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Résumé

Le dépistage du cancer de la prostate, réalisé par dosage sérique du PSA et toucher rectal, permet une diminution de la mortalité spécifique de 21% à 13 ans. Le diagnostic de lésions de volume et de grade significatifs en cas de test de dépistage suspects a été amélioré par l'IRM prostatique qui depuis la fin des années 2000 a permis de mieux détecter les lésions malignes notamment de faible volume ou d'accès difficile pour les biopsier.
Du fait d'une valeur prédictive négative (VPN) élevée entre 85% et 95% pour les cancers significatifs, l'IRM prostatique a été utilisée à la fin des années 2010 pour diminuer le sur-diagnostic de cancers cliniquement non significatifs de faibles volume et grade en évitant de biopsier les hommes en cas d'IRM non suspecte. Cette stratégie d'utilisation de l'IRM comme un test de tri entre le dépistage et la pratique des biopsies a été évaluée en association à des critères cliniques (facteurs de risque familiaux) et des marqueurs (densité du PSA) pour augmenter la VPN de l'IRM. Cette évaluation correspond au premier objectif de la thèse.

Cependant au début des années 2010, des biopsies en cartographie étaient indiquées en cas d'IRM non suspecte et entrainaient la détection d'un cancer cliniquement non significatif de faible volume et grade. Dans ces cas, la stratégie de surveillance de ces cancers a été proposée pour éviter leur sur-traitement. Cette situation concernait environ 20% des cancers détectés. La surveillance permet de différer le traitement jusqu'à progression à un stade plus élevé. L'évaluation des critères de sélection des cas à surveiller et de leur suivi correspond au deuxième objectif de la thèse.

Dans la première partie nous avons évalué les facteurs cliniques, biologiques et familiaux pouvant améliorer la VPN de l'IRM. A l'aide d'une revue systématique de la littérature avec méta-analyse, nous avons mis en évidence que la densité du PSA (PSA/volume prostatique) était le facteur prédictif améliorant la VPN de l'IRM le plus validé pour le diagnostic de cancer significatif.
Nous avons aussi validé rétrospectivement le risque de ne pas diagnostiquer un cancer significatif en d'IRM non suspecte dans cohorte de 503 patients qui avaient

été tous été biopsiés initialement et suivis sur une durée d'inclusion de 10 ans. L'analyse a montré que ce risque était de 9% au moment du diagnostic et de 4% supplémentaire lors du suivi. Ce risque initial diminuait de 9% à 2.4% en intégrant dans l'analyse au moment du diagnostic la densité de PSA, le toucher rectal ou les antécédents familiaux pour décider ou non d'une biopsie.

Dans la deuxième partie, nous avons étudié, comme critère de sélection à une surveillance active l'intérêt de l'IRM en plus des critères de cancers cliniquement non significatifs. Une étude rétrospective multicentrique, comparant 1035 patients ayant une IRM non suspecte et 1084 une IRM suspecte à l'inclusion a montré que le risque de progression tumoral et de sortie de surveillance active était diminué en cas d'IRM non suspecte à l'inclusion.
Nous avons aussi évalué les examens de suivi des patients en surveillance active avec IRM non suspecte et plus particulièrement la réalisation de biopsies de confirmation à un an. Une étude prospective incluant 2 cohortes successives de 78 et 71 patients avec et sans biopsies de confirmation à un an a comparé les taux de diagnostic de progression tumorale à 2 ans et le rôle de la cinétique du PSA pour cette prédire cette progression. La conclusion était qu'il n'y avait pas de différence de diagnostic de progression entre les 2 groupes et que la cinétique du PSA suspecte à elle seule pouvait faire indiquer une biopsie. Cette étude nous a permis de proposer de ne plus réaliser ces biopsies de confirmation en cas d'IRM non suspecte au diagnostic.

En plus des facteurs cliniques, du PSA et de l'IRM, d'autres marqueurs tissulaires sont en cours d'évaluation pour aider à prédire le risque de progression du cancer au cours de la surveillance.

Abstract

Prostate cancer screening, carried out by serum PSA and rectal examination, allows a reduction in specific mortality of 21% at 13 years. Since the end of the 2000s, prostate MRI has emerged as an important technique for characterizing and targeting the biopsy of suspected lesion as it efficiently detects clinically significant cancers.

Due to a high negative predictive value (NPV) between 85% and 95%, prostate MRI could be used to reduce the over-diagnosis of clinically insignificant cancers of low volume and grade by avoiding biopsy of men in case non-suspicious MRI since the end of the 2010s. This strategy of using MRI as a “triage test” between screening and biopsies was evaluated in association with clinical criteria (family risk factors) and markers (PSA density) to increase of MRI NPV and corresponds to the first objective of the thesis.

However, in the early 2010s, systematic biopsies were indicated in non-suspicious MRI cases and resulted in the detection of clinically insignificant cancer of low volume and grade. In these cases, the strategy of active surveillance for these cancers has been proposed to avoid over-treatment. This situation concerns about 20% of cancers detected. It aims to avoid or delay the use of curative treatments without compromising the long-term survival of patients. Evaluation of the selection criteria and follow-up monitoring corresponds to the second objective of the thesis.

In the first part we evaluated the clinical, biological and familial factors that could improve the MRI NPV. We systematically reviewed the literature on predictive factors for clinically significant prostate cancer diagnosis after pre-biopsy non suspicious MRI in prostate cancer naïve patients. The use of PSA density was the most useful factor to identify men without clinically significant prostate.

Then, we have described the risk of clinically significant prostate cancer in a negative magnetic resonance imaging biopsy naïve population at baseline and during long-term follow-up. Analysis of a single-center retrospective cohort study of 503 patients who had been initially biopsied and followed up for a median of 4 years showed that this risk was 9% (91% NPV on MRI) at the time of diagnosis and an

additional 4% during follow-up. Performing biopsy in patients with non-suspicious MRI and PSA density> 0.15 ng/ml/ml or abnormal digital rectal examination or prostate cancer family history would have decreased from 9% to 2.4% the risk of missing clinically significant prostate cancer at baseline.

In the second part, we studied, as a selection criterion for active surveillance, the benefit of MRI in addition to the criteria of clinically insignificant cancers of low volume and grade. A retrospective multicenter study, comparing 1035 patients with non-suspicious MRI and 1084 with suspicious MRI at inclusion, showed that the risk of tumor progression and discharge from active surveillance was reduced in the non-suspicious MRI group at inclusion.
We evaluated the follow-up examinations of patients under active surveillance with non-suspicious MRI and more particularly the performance of confirmatory biopsy at one year. A prospective study including 2 successive cohorts of 78 and 71 patients with and without confirmatory biopsies at one year, compared the rates of diagnosis of tumor progression at 2 years and the role of PSA kinetics in predicting this progression. The conclusion was that there was no difference in the diagnosis of progression between the 2 groups and that abnormal PSA kinetics alone could indicate biopsy. This study allowed us to no longer perform these confirmatory biopsies in case of non-suspicious MRI at entry.

In addition to clinical factors, PSA and MRI, other tissue markers are being evaluated to help predict the risk of cancer progression during surveillance.

Introduction

Épidémiologie et histoire naturelle du cancer de la prostate

Le cancer de la prostate est le deuxième cancer le plus fréquent dans le monde avec 1.276.000 nouveaux cas diagnostiqués en 2018 (1). Cela correspond à une incidence de 29.3 cas pour 100 000 hommes et 13.5% des cancers chez l'homme (1). Au cours de sa vie, 1 homme sur 6 aura un risque de diagnostic de cancer de la prostate, la prévalence augmentant avec l'âge. On estime que 359.000 hommes dans le monde sont décédés du cancer de la prostate en 2018, soit 6,7% de tous les décès par cancer chez les hommes, ce qui en fait la 5ème cause de mortalité par cancer chez les hommes (1). Cela en fait une problématique de santé publique majeure à travers le monde.

Le cancer de la prostate localisé est une maladie hétérogène tant au niveau de sa morphologie que son comportement clinique (2). Le défi de la prise en charge des cancers de la prostate localisés est de différencier les patients porteurs de cancers cliniquement significatifs pour lesquels un traitement de la glande entière sera bénéfique justifiant certains effets secondaires, des patients porteurs de cancers à faible risque évolutif (3). En effet, certains patients sont porteurs de formes débutantes, de bas stade et de bas grade, à risque d'évolution à un stade symptomatique en une ou plusieurs décennies (4). Certains patients porteurs de ces cancers, en fonction de leur espérance de vie, ne développeront jamais aucun symptôme. Ces cancers à faible risque de progression sont appelés cancer de la prostate cliniquement non significatifs. Ils ont été définis par Stamey comme des tumeurs de score histo-pronostique de Gleason 6 de petits volumes (<0.5cc) (5).
L'incidence des cancers cliniquement non significatifs a augmenté dans les pays dans lesquels le dépistage du cancer de la prostate est réalisé. La proportion de cancers de bas risque a augmenté de 30% en 1992 à 45% en 2001 du fait de l'utilisation du dosage sérique du PSA (6). Au CHU de Lille 30% des cancers à l'incidence étaient de bas risque dans la classification de d'Amico (7).

Dépistage du cancer de la prostate

Le dépistage du cancer de prostate consiste à rechercher la maladie de manière systématique dans une population asymptomatique. Il est réalisé par dosage sérique du PSA qui associé au toucher rectal permet un diagnostic précoce de la pathologie à un stade pouvant bénéficier d'une prise en charge curative. En cas de PSA total suspect (>4ng/ml) ou de toucher rectal suspect, des biopsies prostatiques sont recommandées pour confirmer le diagnostic de cancer de prostate. Cependant ce dépistage a fait l'objet de controverses du fait des résultats de mortalités spécifiques apparemment contradictoires des études PLCO (8) (The Prostate, Lung, Colorectal and Ovarian cancer screening trial) et ERSPC (European Randomized Study of Screening for Prostate Cancer) (9). L'étude ERSPC a démontré un gain de la survie spécifique de 21% de la mortalité à 13 ans chez les patients ayant été dépistés. Quant à l'étude PLCO qui avait rapporté initialement une absence de bénéfice du dépistage sur la mortalité spécifique, elle s'est secondairement montrée biaisée à cause d'une contamination majeure du bras témoin (10). En effet, 90% des patients du bras témoins avaient réalisés un PSA ce qui était supérieur au bras dépistage.

L'autre désavantage du dépistage est le risque de sur-diagnostic de cancers non cliniquement significatifs et par conséquence un risque de sur-traitement (11). Pour mieux défendre le dépistage du cancer de la prostate, il faut développer des stratégies qui permettent de diminuer les risques de sur-diagnostic et de sur-traitement.

L'IRM de prostate

La détection de lésions de volume et de grade significatifs en cas de tests de dépistage suspects a été amélioré par l'IRM prostatique depuis le début des années 2000 (12).

Grâce aux nouvelles IRM à 1.5 puis 3 Tesla et au développement du concept de l'IRM multiparamétrique comportant d'abord les séquences T2 et dynamiques puis les séquences de diffusion (13), plusieurs équipes européennes dont le CHU de Lille et nord-américaines ont décrit la sémiologie pour la détection des cancers de la prostate. Avec l'équipe d'imagerie génito-urinaire, de pathologie et d'urologie du CHU de Lille, la première publication concernant la performance de l'IRM pour la détection des cancers de la prostate a été publié en 2006 (14). Les connaissances sur la morphométrie des cancers et leur mode d'extension intra-prostatique a considérablement progressé grâce aux résultats de l'IRM qui détectait tous les cancers significatifs, et cela indépendamment de leur localisation et leur accessibilité à l'examen clinique et à la biopsie par voie transrectale (15–17).

L'expérience croissante des radiologues dans l'interprétation des examens, l'intérêt de la double lecture, la publication des scores de suspicion, la formation et la diffusion de ces acquis auprès de la communauté radiologique a permis à l'IRM de devenir, en France et en Europe, l'examen de référence pour l'imagerie de la prostate, remplaçant l'échographie (18–21).

En plus de la détection des cancers significatifs en cas de lésion suspecte, des études ont évalué l'intérêt de l'IRM non suspecte pour évaluer l'absence de tumeurs significatives. Dans l'étude PROMIS, les patients avaient une IRM pré-biopsique, des biopsies systématisées et dirigées ainsi que des biopsies en cartographie tous les 5mm (22). Cette étude a démontré qu'utiliser l'IRM non suspecte comme test de tri entre le PSA et les biopsies, permettrait d'éviter 27% de biopsies tout en diagnostiquant par excès 5% de cancers non cliniquement significatifs. De plus les biopsies ciblées permettaient de diagnostiquer 18% de cancer significatifs en plus des biopsies systématisées seules. En 2017, Moldovan et al. a démontré dans une méta-analyse que la valeur prédictive négative de l'IRM pour le cancers cliniquement significatifs était de 88% (23). Cette valeur diminue quand la prévalence du cancer de prostate augmente dans la population cible.

Stratégie diagnostique

Le comité de cancérologie de l'association française d'urologie (CC-AFU) en 2018-2020, recommandait la réalisation d'une IRM multiparamétrique de la prostate avant toute série biopsique (24). En cas d'IRM non suspecte, il était toujours recommandé de réaliser 12 biopsies systématisées. A la fin des années 2010, deux études prospectives multicentriques PRECISION et MRI-FIRST ont évalué le rôle de l'IRM de la prostate pour l'indication de toutes biopsies(25,26). L'étude PRECISION a comparé après randomisation, un groupe de 248 patients ayant des biopsies systématisées sans IRM à un groupe de 252 patients ayant une IRM puis des biopsies ciblées seules en cas d'IRM suspecte. Les patients ayant une IRM non suspecte n'étaient pas biopsiés. Dans le bras avec IRM pré-biopsique, le taux de détection de cancers cliniquement significatifs était significativement plus élevé (38% vs. 26%, p=0.005) et le taux de détection de cancer de la prostate cliniquement non significatifs plus faible (9% vs22%, p<0.001) (25). Cette étude validant l'apport de l'IRM avant une première série de biopsies pour le diagnostic de cancers cliniquement significatifs a également entrainé des commentaires sur le choix de ne pas biopsier les patients avec IRM non suspectes. Dans un éditorial sur l'étude PRECISION, Nzenza et al. proposait la réalisation de biopsies systématisées malgré une IRM non suspecte en cas de présence d'autres facteurs de risque de cancer significatif tels que la présence d'antécédents familiaux, une densité du PSA élevée > 0,15 ng/ml, une vélocité du PSA > 0,5ng/ml/an, la présence de mutation du gène BRCA ou d'un toucher rectal suspect (27). L'étude française MRI-First a inclut 275 patients ayant tous bénéficié d'une IRM, de biopsies systématisées et de biopsies dirigées en cas d'IRM suspecte. Le taux de détection des cancers cliniquement significatifs était supérieur avec une approche combinant biopsies systématisées et dirigées (26).

Les résultats de ces 2 études, du fait de la VPN élevée de l'IRM (85-95%), ont ouvert la voie à une stratégie diagnostique utilisant l'IRM de prostate comme test de tri entre dépistage et biopsies. Le but est de réduire le nombre de biopsies de prostate non nécessaires, et donc celui du risque de sur-diagnostic et ainsi d'améliorer l'acceptabilité du dépistage (28). La valeur élevée de la VPN de l'IRM peut être aussi améliorée si on associe à l'IRM d'autres critères cliniques, biologiques ou familiaux.

La surveillance active

En réponse au sur-traitement des cancers de la prostate cliniquement non significatifs, le principe de surveillance active a été proposé à partir de 2001 (29). La surveillance active a pour objectif d'éviter ou de différer les traitements curatifs de la prostate comme la prostatectomie totale ou la radiothérapie pour les cancers cliniquement non significatifs. Le but est d'éviter un traitement inutile en plus d'éviter leurs effets secondaires tels que l'incontinence, la dysfonction érectile ou les troubles digestifs (30). En cas de progression, tout en restant dans la fenêtre de curabilité de la maladie, un traitement curatif est proposé. La surveillance active consiste en une sélection des patients présentant des cancers à faible risque de progression, un suivi rapproché par des examens cliniques et para-cliniques réguliers afin de ne pas méconnaitre une progression de ces tumeurs. Enfin des critères de progression sont définis afin de réaliser un traitement curatif. Cette approche a été validée par plusieurs séries prospectives telles que l'étude de Klotz qui a montré, après un suivi médian de 7 ans dans une série de 450 patients une survie spécifique à 10 ans de 97.2% (31). Les critères d'inclusion des patients éligibles à la surveillance active varient d'une étude à l'autre (Tableau1) (32). Ils sont basés sur l'examen au toucher rectal, le PSA total, la densité du PSA, le score de Gleason et le volume tumoral sur les biopsies. Aucune étude prospective n'a comparé les différents critères d'inclusion en surveillance active qui ne sont donc pas à ce jour consensuels.

Tableau 1 : Protocoles de surveillance active publiés (CC-AFU) (33)

	N	Cohorte	Critères d'inclusion	Monitoring	Progression	Follow-up
University of Toronto	993	Unicentrique	GS 6 et PSA < 10 ou GS 3 + 4 et PSA < 20 et EV < 10y	TR + PSA/3 mo pdt 2 ans, puis /6 mo Biopsie à 1 an puis /3-4 ans	PSADT < 3 ans (jusqu'à 2009) Gleason 7 Clinique	6,4 ans (0,2-19,8)
UCSF	321	Unicentrique	T1-T2 PSA < 10 GS 6 < 33 % biopsies +	TR + PSA /3-6 mo Biopsie /1-2 ans	PSAV > 0,75 Gleason 7	3,6 ans
PRIAS	2494	Multicentrique	T1/T2 PSA < 10 PSAD < 0.2 GS 6 1-2 biopsies +	TR + PSA/3mo pdt 2 ans puis /6mo Biopsie1-4-7 ans	PSADT < 3y Gleason 7 Progresssion biopsique	1,6 ans
Göteborg	341	Unicentrique	T1 GS 6 PSA < 10*	TR + PSA /3-6mo Biopsie dans les 3 ans	PSA Gleason 7 Progresssion biopsique	6,0 ans
Beaumont Hospital	80	Unicentrique	T1 GS 6 PSA < 10 1-2 biopsies+ < 50%/biopsie	TR + PSA/3 mo 1 an puis /4 mo 2 ans puis /6 mo MRI 6 mo Biopsie1-3-6 ans	PSADT < 3y Gleason 7 Progresssion biopsique Clinique	3,1 ans
University of Miami	230	Unicentrique	T1-T2 GS 6 PSA < 10 1-2 biopsies + < 20%/biopsie	TR + PSA/3-4 mo 2 ans puis /6 mo Biopsie /1 an	Gleason 7 Progresssion biopsique	2,7 ans
Royal Marsden Hospital	471	Unicentrique	T1-T2 PSA < 15 < 50 %/biopsies GS 6 OR GS3 + 4 si > 65 ans	TR + PSA/3 mo 1 an puis /4 mo 1 an puis /6 mo Biopsie1-3-5 ans	PSAV > 1 Gleason 7 Progresssion biopsique	5,7 ans
Johns Hopkins University	769	Unicentrique	T1 PSAD < 0,15 GS 6 1-2 biopsies + < 50 %/biopsie	TR + PSA/6 mo Biopsie /1 an	Gleason 7 Progresssion biopsique	2,7 ans
REDEEM	155	Multicentrique	T1-T2 GS 6 PSA < 11 1-3 biopsies + < 50 %/biopsie	PSA/3 mo 1 an puis /6 mo TR 18 mo-3 ans Biopsie 18 mo-3 ans	Gleason 7 Progresssion biopsique	2,7 ans

* mais aussi 92 risque intermédiaire et 6 haut risque inclus
EV : espérance de vie
PSAD : PSA densité ; PSADT : PSA temps de doublement ; PSAV : PSA vélocité
GS : score Gleason
TR : toucher rectal

Objectifs de la thèse :

Dans une première partie*, l'objectif était d'évaluer la **stratégie diagnostique** visant à placer l'IRM comme test de tri entre le dépistage et la réalisation des biopsies prostatiques (étude « post-PRECISION »). Pour cela nous avons évalué les facteurs cliniques, biologiques et familiaux pouvant améliorer la VPN de l'IRM dans une population naïve de cancer de prostate. Nous avons ensuite évalué le risque de ne pas diagnostiquer un cancer cliniquement significatif au diagnostic et pendant le suivi dans le cas d'une IRM non suspecte, si des biopsies n'avaient pas été réalisées.

Dans une seconde partie*, l'objectif était d'évaluer certains critères d'imagerie et biologique de **sélection et de suivi pour la surveillance active**. Le rôle de l'IRM en plus des critères de cancers cliniquement non significatifs de faibles volume et grade a été étudié dans une série multicentrique. La cinétique du PSA a été évaluée dans le suivi des patients en surveillance active avec IRM non suspecte à l'inclusion, pour l'indication des biopsies de confirmation.

* Nous avons choisi de présenter les 4 articles de cette thèse suivant l'ordre thématique utilisé dans les recommandations en oncologie et non pas en fonction de la date de publication ou de soumission des articles. Cela a permis de regrouper les articles en fonction de leurs thématiques et de leurs impacts éventuels sur les pratiques cliniques

Stratégie diagnostique : l'IRM comme test de tri pour réduire le sur-diagnostic

Première partie – Article 1

Du fait d'une valeur prédictive négative (VPN) élevée entre 85% et 95% pour les cancers significatifs (23), l'IRM prostatique a été utilisée à la fin des années 2010 pour diminuer le sur-diagnostic de cancers cliniquement non significatifs de faibles volume et grade en évitant de biopsier les hommes en cas d'IRM non suspecte. Cette stratégie d'utilisation de l'IRM comme un test de tri entre le dépistage et la pratique des biopsies a été évaluée en association à des critères cliniques (facteurs de risque familiaux) et des marqueurs (densité du PSA) pour augmenter la VPN de l'IRM.

C'est pourquoi, nous avons dans un premier temps, recherché des facteurs cliniques, biologiques et familiaux pouvant améliorer la VPN de l'IRM en réalisant une revue systématique de la littérature avec méta-analyse. Notre étude "*Predictive Factors of Missed Clinically Significant Prostate Cancers in Men with Negative Magnetic Resonance Imaging: A Systematic Review and Meta-Analysis*" a été publiée dans la revue Journal of Urology en Juillet 2020. Nous avons mis en évidence que la densité du PSA (PSA/volume prostatique) était le facteur prédictif améliorant la VPN de l'IRM le plus validé pour le diagnostic de cancer significatif.

Predictive factors of missed clinically significant prostate cancers in men with negative MRI: a systematic review and meta-analysis.

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Keywords: clinically significant prostate cancer; negative MRI; predictive factors; risk factors; PSA density

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Context: Some guidelines recommend that men may avoid biopsy in case of a non-suspicious MRI (nMRI). MRI has a negative predictive value (NPV) of 85-95% which leads to this strategy missing 5-15% clinically significant PCa (csPCa). Patient factors and biochemical markers can be used in addition to MRI to inform biopsy decisions in order to reduce the risk of missing csPCa.

Objective: To systematically review the literature on predictive factors for csPCa diagnosis after nMRI in PCa-naïve patients.

Evidence acquisition: The Medline and Scopus databases were searched up to March 2019. **The review protocol was published in the PROSPERO database (CRD42019125549).** The clinical factors and markers studied were age, PSA, PSA isoforms, PSA density (PSAD), PCA3, prostate volume, family history, ethnicity, and risk calculators. The primary objective was to determine their predictive ability for csPCa diagnosis. Secondary objectives included meta-analysis of the NPV of nMRI when combined with these predictive factors.

Evidence synthesis: A total of 16 studies were eligible for inclusion. Few studies reported NPV of MRI combined with a marker. PSAD was the best studied and the strongest predictor of csPCa in men with nMRI. Eight studies (1015 patients) were eligible for meta-analysis of the added value of PSAD< 0.15ng/ml/ml to MRI in reducing the risk of missing csPCa. When combined with PSAD, overall MRI NPV increased from 84.4% to 90.4% in cancer naïve patients. The increase was from 82.7% to 88.7% in biopsy naïve and from 88.2% to 94.1% in previous negative biopsy sub-groups.

Conclusion: The use of PSAD<0.15ng/ml/ml in the presence of a nMRI was the most useful factor to identify men without csPCa who could avoid biopsy.

Introduction

A major consideration in prostate cancer screening and early detection is over-diagnosis and overtreatment of indolent disease. Multi-parametric MRI (MRI) of the prostate is currently transforming the prostate cancer diagnostic pathway. Several studies (1–4) have provided robust evidence that the use of MRI improves the diagnosis of clinically significant prostate cancer (csPCa). MRI has excellent negative predictive value (NPV) in excluding csPCa (5). MRI has also shown the potential to reduce the diagnosis of insignificant prostate cancer (isPCa) (1,2).
Performing MRI before any prostate biopsy is widely recommended (6–8). Results of the PRECISION trial, where prostate biopsies were avoided in case of prebiopsy negative MRI (nMRI), suggest that MRI could be used as a triage test for prostate biopsy. However, those results need to be considered in the context of the results from the MRI-first study, in which some csPCa were missed by targeted biopsy (2,3). nMRI represents 20-30% of all MRI in cancer naive populations, and early screening tends to increase this rate (9).
The advice regarding biopsy if the prebiopsy MRI is negative (PI-RADS 1-2) is controversial. Patients with nMRI may avoid prostate biopsy, but 5-15% csPCa may be missed (1,2,10). Recent guidelines state that men may safely avoid prostate biopsy if MRI is negative, especially “if PSA density (PSAD) is low, and that PSA observation is appropriate” (7), or if “clinical suspicion of prostate cancer is low, based on shared decision making with the patient” (8). Digital rectal examination (DRE) is part of the diagnostic pathway for PCa. Fifteen% and 30% of patients had an abnormal DRE in PRECISION and MRI first studies respectively (2,3). How to identify patients with nMRI and a high risk of csPCa is not yet known. Associating MRI with predictive factors may increase the NPV of MRI and reduce the number of prostate biopsies in men whose risk of csPCa is low.

The aim of this work was to systematically review the literature on predictive factors for csPCa in naive patients with nMRI.

2. Evidence acquisition

2.1. Objective

The primary objective was to systematically review the NPV of patient factors and biochemical markers as adjuncts to MRI in ruling out csPCa. Secondary objectives were to systematically evaluate the predictive factors of csPCa in cancer naive patients, biopsy naive patients (BN) and in men with previous negative biopsies (PNB) with a nMRI, and to perform a meta-analysis of the NPV of MRI combined with these predictive factors.

2.2. Data acquisition and search strategy

The review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (11). Methods of the analysis and inclusion criteria were specified in advance and documented in the protocol. The review protocol was published in the PROSPERO database (http://www.crd.york.ac.uk/ PROSPERO; registration number CRD42019125549). A literature search using the PubMed and Scopus databases was performed, covering from January 01, 2010 to March 29, 2019, to identify eligible studies evaluating

predictive factors of PCa/csPCa in patients with nMRI. The detailed search strategy is presented in Supplement 1.

2.3. Inclusion and exclusion criteria

No restriction on study type was imposed. Participants were male, adult, human patients. Included studies concentrated on men who were assessed for suspected PCa by MRI before undergoing prostate biopsy. Systematic prostate biopsies were used as reference standard, with positive or negative cases of csPCa being determined by histopathological examination. Studies enrolling biopsy naïve (BN) and prior negative biopsy (PNB) patients were included. Prebiopsy prostate MRI was considered the index test and comprised T2-weighted imaging (T2WI), and at least two functional imaging techniques (diffusion-weighted imaging [DWI], dynamic contrast-enhanced imaging [DCE], or apparent diffusion coefficient [ADC]). No results with biparametric MRI were included. Language restrictions were applied, excluding articles not written in English.

2.4. Study selection and data collection

Abstract and full-text screenings were performed by two reviewers independently (MAP and JO). Disagreement was solved by consensus. A standardized form was used to extract data on study methodology, patient characteristics, imaging protocols and main results (supplementary table S2). Any discrepancy concerning data extraction was solved by consensus. References from the included studies were manually retrieved to identify additional studies of interest.

2.5. Quality assessment of included studies

To assess the risk of bias (RoB), all included reports were independently reviewed by two reviewers using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool for diagnostic accuracy studies (12). Any discrepancy concerning the RoB assessment was solved by consensus.

2.6. Data synthesis and analysis

Clinical factors studied were age, prostate specific antigen (PSA), PSA isoforms, PSAD, prostate cancer gene 3 (PCA3), prostate volume, family history, ethnicity, and risk calculators. Predictive factors of all PCa and csPCa for patients with nMRI were extracted as notified in the studies. Outcome data regarding false negative and true negative values of MRI before prostate biopsy were recorded as reported by authors. NPV of each study was calculated from the study data if not directly stated. NPV of MRI combined with any marker were recorded as reported by authors, or calculated from the study data if not directly stated. Missing data was requested from the authors of the included papers.

A meta-analysis was undertaken to calculate the pooled NPV of MRI coupled with PSAD across the available studies. To ensure appropriate clinical homogeneity of the studies included in this meta-analysis, we selected only the studies enrolling BN patients and/or patients with a PNB, and fulfilling the following criteria that were defined a priori:

- reference standard consisting of prostate biopsy with at least 12 samples on all patients;
- MRI protocol comprising at least T2WI, DWI and DCE;
- MRI results presented as a five-level score, using a subjective Likert scale or the Prostate Imaging Reporting Data System (PI-RADS) score;
- definition of positive MRI as a score ≥3/5;
- only studies defining csPCa as GGG≥2 were selected for the meta-analysis assessing the MRI NPV for csPCa coupled with PSAD.

To combine the pooled NPVs, we firstly transformed individual NPVs to a quantity using the Freeman-Tukey variance stabilizing arcsine transformation (13), and secondly we used fixed or DerSimonian-Laird random effects models (14) to obtain the combined estimates. Random effects model were used in case of significant heterogeneity between studies. Between-studies heterogeneity in NPV was quantified by the I^2 statistic and tested by the Cochran Q test. Data were analyzed using SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA). Forest plots were created using the DistillerSR Forest Plot Generator from Evidence Partners (https://www.evidencepartners.com/resources/forest-plot-generator/). For other studies not included in the meta-analysis based on the criteria described above, a narrative synthesis of the data was performed.

3. Evidence synthesis

3.1. Quantity of evidence identified

The study selection process is depicted in the PRISMA flow diagram (figure 1). A total of 7863 abstracts were retrieved. After abstract screening and removal of duplicates, 140 articles were eligible for full text screening, of which 16 studies were eligible for inclusion in this systematic review.

3.2. Risk of bias within studies

Out of the 16 included studies, 12 were single-center, 3 were multi-center studies, and 1 did not specify. Eight studies were prospective and 8 were retrospective. RoB assessment using QUADAS-2 was performed for each of the individual studies. Overall, the RoB was heterogeneous across studies for patient selection and flow and timing, but was homogeneous with regards to the index test and reference, in which RoB was low and unclear in all studies, respectively. RoB assessment is reported in figure 2.

3.3. Characteristics of studies

3.3.1. Main characteristics

The study and patient baseline characteristics are presented in Table 1.

3.3.2. Biopsy status

The patient population consisted of BN in 6 studies, PNB in 2 studies, BN and PNB in 3 studies, BN, PNB and previous positive biopsy (PPB) in 3 studies. 2 studies included PNB and PPB.

3.3.3. MRI characteristics

The magnetic field strength was 1.5 and 3 Tesla in 2 and 6 studies, respectively. Six studies used both 1.5 and 3 Tesla MRI systems. In 2 studies the magnetic field strength was not specified. DWI, DCE and ADC were used in 14, 12 and 4 studies, respectively.

3.3.4. Definition of negative MRI

The definition of nMRI varied little across studies. Fifteen studies used PI-RADS score <3 and one study PI-RADS score <2.

3.3.5. Biopsy methods

Regarding the reference standard, TRUS-guided biopsies were used in 7 studies, trans-perineal biopsies in 6 studies, and mixed TRUS-guided and trans-perineal biopsies in 2 studies.

In one study, the biopsy approach was unclear. The number of cores per biopsy procedure was <18 in 9 studies, >18 in 4 studies, and variable among patients in 2 studies. In one study, the number of biopsy cores taken was unclear.

3.3.6. Definition of clinically significant prostate cancer

The definition of csPCa varied little across studies. 12 studies used Gleason Grade Group (GGG) ≥2 (Gleason score ≥ 3+4). Perlis *et al*. (15) used two definitions: (*i*) Gleason 7 or a cancer core length of 6 mm or greater or (*ii*) any Gleason 7. Druskin *et al*. (16) used GGG ≥2 or GGG =1 in >2 cores or >50% of any core). Panebianco *et al.* (17) used the EAU-ESTRO-SIOG guidelines selection criteria for isPCa, eligible for active surveillance (18). Numao *et al*. (19) used 3 definitions: (*i*) GS ≥4+3 and/or

percent positive core>20% and/or maximum cancer length ≥5mm; (*ii*) GS ≥3+4 and/or percent positive core>20% and/or maximum cancer length ≥5mm; (*iii*) GS ≥ 3+4 and/or percent positive core > 20%.

3.4. Predictive factors

Four studies reported predictive factors for PCa in patients with nMRI at biopsy (clinical predictors of biopsy outcome) and one study reported predictive factors for csPCa in patients with nMRI during follow-up. Twelve studies reported NPV of MRI combined with a marker or a clinical factor.

3.4.1. PSA

Two studies reported NPV of MRI combined with PSA. Thompson *et al*. (20) reported NPV of MRI for csPCa among BN men with a pre-biopsy PSA≥10.0ng/ml or an abnormal DRE of 100%. Among all men with a normal DRE and a PSA<10.0 ng/ml, the NPV was 90% for csPCa. *Otti et al*. (21) reported a NPV of 85.1% in BN patients with nMRI. Patients were stratified in three groups: PSA <5ng/ml, PSA between 5 and 10ng/ml and PSA ≥10 ng/ml. In those patients, NPV of csPCa was 90.2%, 84.8% and 79.2%, respectively. MRI NPV increased when PSA rates decreased.

PSA was reported in 5 studies (17,19,22–24), and was a significant predictive factor of csPCa in 2 studies (17,24). In multivariate analysis, results were contradictory. PSA was reported in 2 studies (17,23) and was a significant predictive factor of csPCa in one study (17) (HR=1.21 (1.1-1.32) p<0.001).

3.4.2. PSA density

Eight studies (21,22,25–30) reported NPV of MRI combined with a PSAD<0.15ng/ml/ml. NPV of MRI coupled with PSAD<0.15ng/ml/ml varied between 84 and 100%, independently of biopsy status. PSAD was reported in 4 studies (17,19,22,24), and was a significant predictive factor of csPCa in all 4 studies, except for definition 3 in reference (19). In multivariate analysis, PSAD was reported in one study (17) and was a significant predictive factor of csPCa (HR = 7.57 (2.73-21) p<0.001). PSAD<0.15 ng/ml/ml was reported in one studies (22) and was a significant predictive factor of no csPCa (OR = 7.7 (2.8-21.3) p<0.001).

3.4.3. PCA3

Perlis *et al*.(15) reported results on patients with PNB and some with previous positive biopsy (PPB). 154 patients with PCA3 score and MRI had repeat biopsy. No patient (0/26) with nMRI and a normal PCA3 score had csPCa on biopsy (NPV=100%).

3.4.4. PHI and PHI density

Druskin *et al*. (16) reported results on Prostate Health Index (PHI) density (PHID) combined with MRI in PNB for the diagnosis of csPCa. 241 cancer naive patients were included, 104 had MRI. MRI NPV for csPCa was 90% (18/20 patients). NPV of MRI coupled with PHID ≥0.44 was 100%.

Gnanapragasam *et al*. (31) reported results of PHI test and MRI in 279 patients (PNB and PPB), including 94 with nMRI. In those patients, and with a PHI score ≥35, the NPV for csPCa was 97% (84–100%) whereas NPV of MRI alone was 75.5% (71/94 patients).

3.4.5. Age

Age was reported in 4 studies (17,19,22,23) and was a significant predictive factor of csPCa in one study (17). In multivariate analysis, results for age were contradictory. Age was reported in 2 studies (17,23) and was a significant predictive factor of csPCa in one study (HR=0.93 (0.89-0.98) p=0.005).

3.4.6. Prostate volume

Prostate volume was reported in 3 studies (19,22,23), and was a significant predictive factor of csPCa in 2 studies (19,22). In multivariate analysis, results were contradictory. Prostate volume was reported in two studies (19,23), and was a significant predictive factor of csPCa, according to all 3 definitions of csPCa (OR =8.1 (2.1–54) $p < 0.01$; OR =5.2 (1.8–19) $p<0.01$; OR=4.8 (1.6–18) $p<0.01$) in reference (19).

3.4.7. Family history

No study reported results on family history in multivariate analysis. In univariate analysis, family history was reported in 2 studies (19,22), and was never a significant predictive factor of csPCa.

3.4.8. Clinically palpable tumor (>T1c)

Clinically palpable tumor was reported in one study (23) and was not a significant predictive factor of csPCa or PCa (OR=1.41 (0.21-9.55) p=0.73).

3.4.9. Previous negative biopsy status

Previous negative biopsy status was reported in 2 studies (17,22). It was a significant predictive factor in both studies . In multivariate analysis, PNB status was reported in two studies (17,22), and was a significant predictive factor of the absence of csPCa (OR = 5.2 (1.6-16.5) p = 0.005) one study (22), but not in the other, HR = 1.01 (0.53-1.93), p=0.97 (17).

3.4.10. Other factors

Ethnicity (23), %free PSA (19), risk calculator (Prostate Cancer Prevention Trial PCPT) (24), biopsy naive status (yes or no) (22), PIRADS v.1 vs PIRADS v.2 (22) were each reported in one study. None of these factors except PCPTRC were significant predictive factors of csPCa. In multivariate analysis, ethnicity was not a predictive factor of PCa in one study (23) (OR=0,98 (0,23-4,19) p=0,98). PCPTRC was a significant predictive factor of csPCa in one study (24) (OR=1.01, $p<0.01$).

3.5. Meta-analysis

Eight studies reported NPV of MRI coupled with PSAD< 0.15ng/ml/ml for csPCa and fulfilled the inclusion criteria for meta-analysis. Six studies included BN patients, and 4 studies included PNB. All studies used GGG ≥ 2 for defining csPCa, and all studies used a score of PIRADS < 3 for defining nMRI. When combined with PSAD<0.15ng/ml/ml, the NPV of MRI increased from 84.4%, 82.7% and 88.2% to 90.4%, 88.7% and 94.1% in all cancer naive, biopsy naive and previous negative biopsy patients respectively (table 4 and figure 3).

4. Discussion

4.1. Principal findings

PSAD was the most relevant predictive factor of csPCa studies in the literature in men with nMRI. When MRI was combined with PSAD<0.15ng/ml/ml, the MRI NPV in cancer naive, biopsy naive and previous negative biopsy groups increased from 84.4%, 82.7% and 88.2% to 90.4%, 88.7% and 94.1%, respectively.

4.2. Reference standard

We included only studies that reported the results of systematic/standard biopsy in patients with nMRI and used the systematic/standard biopsy as a reference standard. The RoB was unclear in most studies because none of the studies reported whether anatomopathologists were blinded to the results of MRI.

Ideally, the gold standard of systematic biopsies should be 5mm template prostate mapping biopsy (TPM-biopsy) as it was performed in PROMIS (1).

TPM-biopsy is the only test able to perfectly characterize disease status by sampling the whole prostate every 5 mm. None of the studies included in this review performed such sampling. This represents a limitation. Beside TPM-biopsy, a follow-up with no cancer occurrence may validate retrospectively MRI NPV. One of the papers assesses outcomes of men with nMRI and clinical follow-up. CsPCa survival probability at 4 years was 95% (17).

Ongoing studies of AS series where patients were selected based on nMRI will help determine the NPV of nMRI (32).

4.3. Impact on clinical practice and research

When combined with PSAD<0.15, the NPV of MRI increases, and this does not depend on biopsy status (all patients, BN patients and PNB patients). Patients with nMRI and PSAD<0.15ng/ml/ml could avoid biopsy. This proposed pathway is in accordance with Padhani *et al*. (33), who proposed that biopsy was not performed in patients with nMRI and PSAD<0.1-0.15ng/ml/ml, but only if surveillance of PSA is an available follow up strategy. The use of PSAD therefore supports the use of pre-biopsy MRI as it allows more men with nMRI to avoid biopsy safely, and the cost effectiveness of the pre-biopsy MRI pathway is dependent on the avoidance of biopsy in the men with nMRI (34,35).

PHI, PHID and PCA3 were combined with MRI in one study each, and increased sharply the NPV of MRI for csPCa. The results of those three studies were limited by the small population, twenty patients in reference (16), and by the inclusion of patients under active surveillance (15,31). Our literature search found only limited data concerning the NPV of MRI combined with PCA3 for the detection of csPCa. Most studies included less than 50 patients, with unclear definition of nMRI and unknown reference standard. Moreover, results were reported for all PCa and not csPCa. The same comments could be made with PHI and PHID.

PSA coupled with MRI showed a small increase in NPV, and appeared inferior to PSAD in that regard. PSA is an androgen-regulated serine protease produced by both prostate epithelial cells and PCa. Serum total PSA levels are increased in PCa, although high PSA levels are predictive of advanced PCa, a large fraction of organ-confined cancers present with much lower total PSA values that overlap those levels found in men without PCa large prostates. It could, however, be used as a surveillance marker (33) for patients with nMRI who didn’t undergo biopsy.

Risk calculators may be useful in informing decisions for biopsy by combining a number of different clinical predictors. Several tools (36) are available as the PCPT cohort (PCPTRC 2.0), which does not include MRI score but does include urinary markers such PCA3, or the ERSPC cohort. Recently a new tool from ERSPC including MRI results has been published, which may help selecting patients for biopsy (37). Nomograms for the diagnostic of PCa and csPCa seem to be useful (38), but none of these has been used in a nMRI population only, and none has been externally validated for csPCa screening.

4.4. How this review compares with other reviews

To our knowledge, this review is the first assessing the use of PSAD in patients with nMRI. Moreover, it is the first to date to have tried to identify predictive factors of csPCa in patients with a nMRI.

4.5. Strengths and limitations

It is limited by the small number of studies reporting predictive factors of csPCa in nMRI patients. Only PSAD was described enough as significant in several studies to perform a meta-analysis. Some studies included PPB (not cancer naive patients), which can make interpretation of the results more challenging to tease out.

Only one study (17) detailed the calculation of PSAD (using prostate volume calculated on MRI). The 15 other studies did not specify if prostate volume was assessed by echography or MRI to calculate the PSAD.

One limitation is that patients received different types of systematic biopsies which we called reference standard. These types included various numbers of systematic biopsies ranging from 12-14 in 8 series, 18 in 2 series and 24 in 6 series. In addition, transperineal approach as an alternative to transrectal was used in 9 out of 15 series for which it was reported. In 2 studies (19,21) all patients did not receive the same reference standard. In 1 study (21), all patients who had MRI did not undergo biopsy (231 patients), in which 84.8% had nMRI, leading to selection bias. That might lead to differences in assessing accuracy of preoperative factors.

As routine use of pre-biopsy MRI is relatively new to most international guidelines, and the management of nMRI is a recent concern in PCa screening, literature is scarce. Prospective studies studying all possible factors in large nMRI populations are needed to address the capital problem of selecting which patients can safely avoid biopsy.

5. Conclusion

PSAD<0.15ng/ml/ml was the most well studied and accurate negative predictive factor for clinically significant prostate cancer diagnosis. We recommend the use of PSAD<0.15ng/ml/ml along with negative MRI results to omit biopsy indication in cancer-naive patients

Figure 1: flow chart

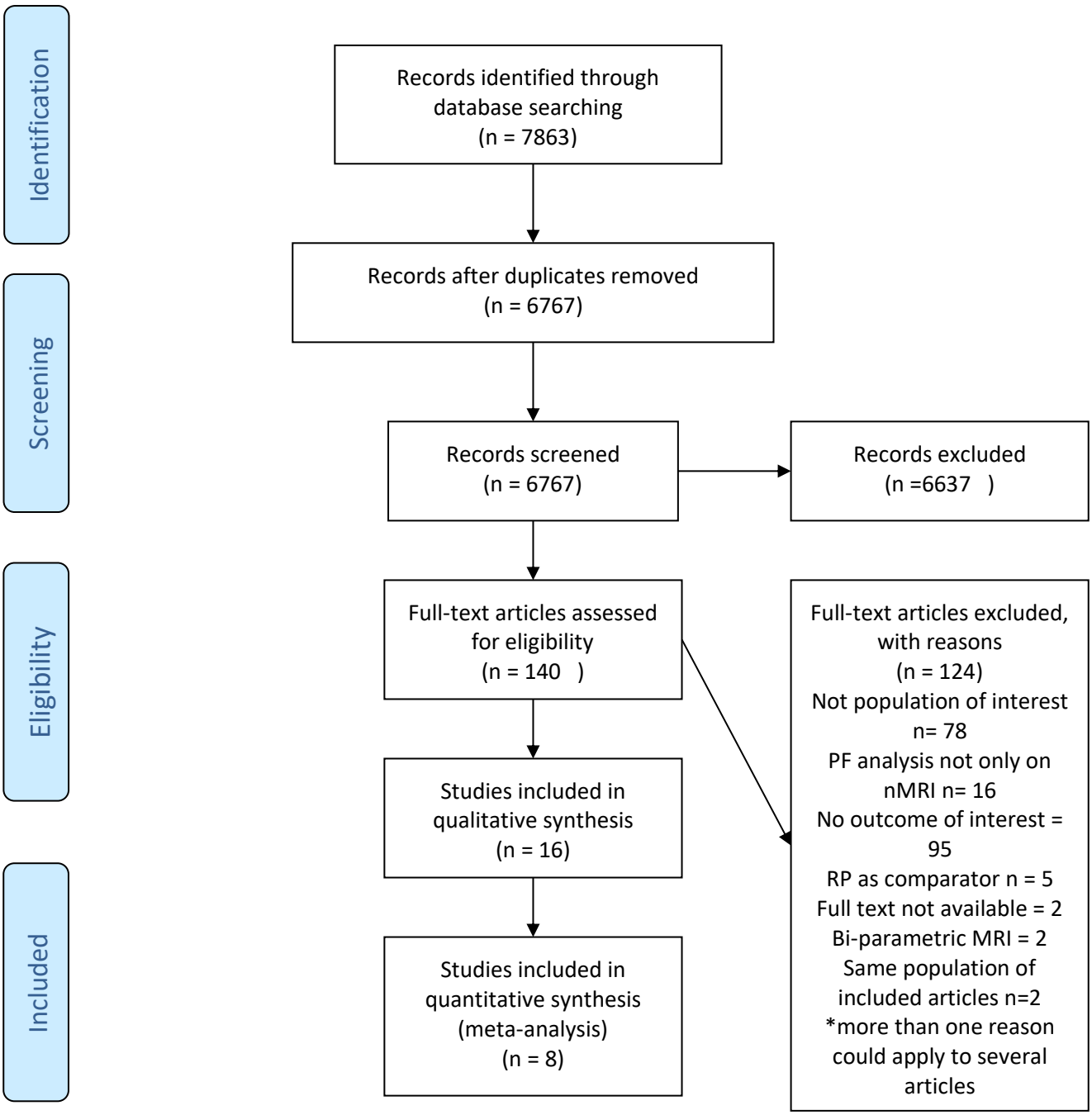

Figure 2: risk of bias QUADAS-2

(A) Assessment of the risk of bias for included studies.

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Wang(24)	☺	☺	?	☺	☺	☺	☺
Numao(19)	?	☺	?	☺	☹	?	☺
Oishi(22)	☺	☺	?	?	☺	☺	☺
An(23)	?	☺	?	☹	☺	☹	?
Panebianco(17)	☺	☺	?	☺	☺	☺	☺
Hansen(25)	☺	☺	?	?	☺	☺	☺
Distler(27)	☺	☺	?	?	☺	☺	☺
Hansen(26)	☺	☺	?	?	☺	☺	☺
Kotb(28)	☺	☺	?	☹	☺	☺	☺
Washino(29)	?	☺	?	☺	☺	☺	☺
Otti(21)	?	☺	?	☹	☺	☺	?
Bryant(30)	?	☺	?	☹	☺	☺	☺
Gnanapragasam(31)	☺	☺	?	☹	☺	☺	☺
Druskin(16)	☺	☺	☹	☹	☺	☺	☹
Perlis(15)	?	☺	?	☹	?	☺	☺
Thompson(20)	?	☺	?	☹	?	☺	☺

(B) Risk of bias summary graph.

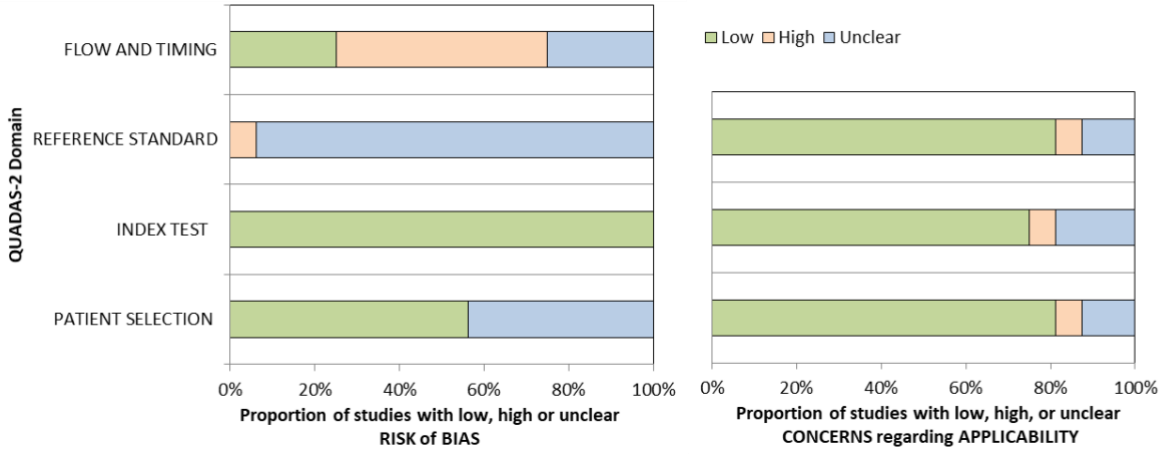

Figure 3: Forest plot showing the NPV for csPCa of MRI alone and combined with PSAD<0.15ng/ml/ml, in cancer naive patients, biopsy naive patients and patients with previous negative biopsies

Figure 3A: NPV of MRI in cancer naive patients

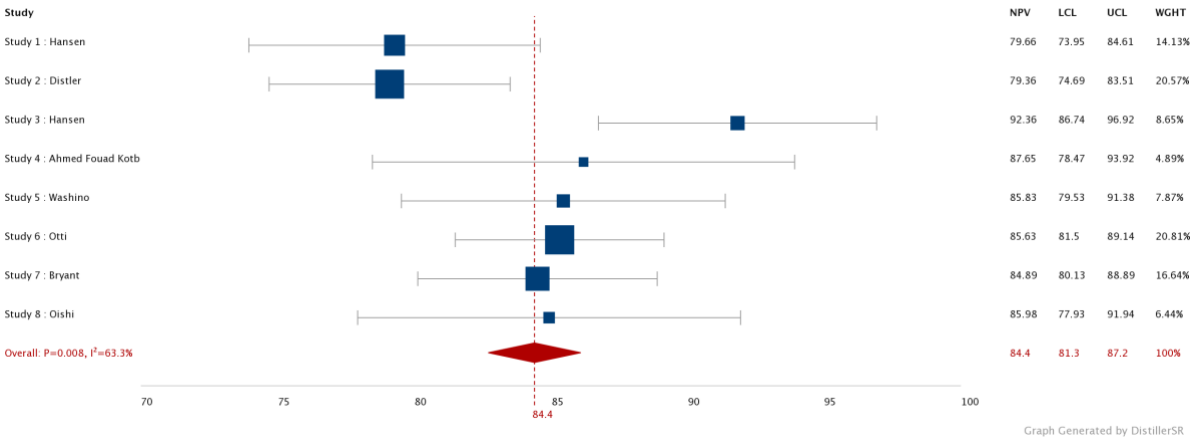

Figure 3B: NPV of MRI in biopsy naive patients

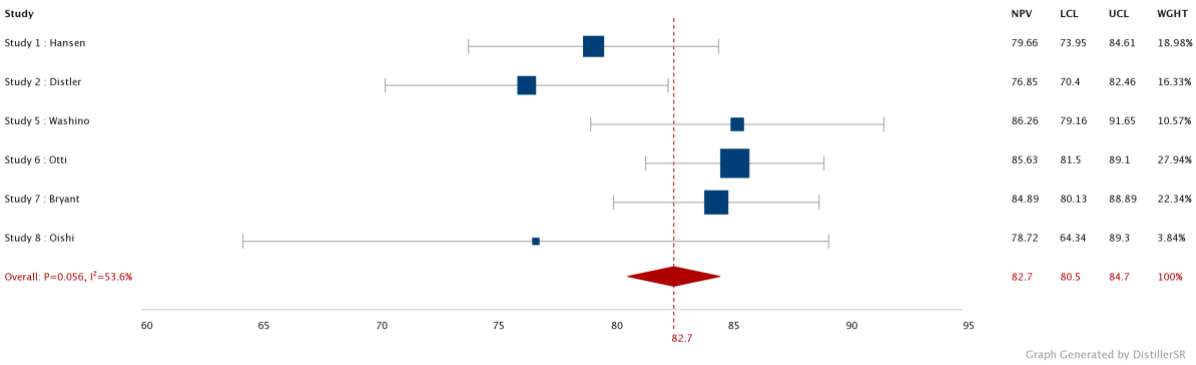

Figure 3C: NPV of MRI in patients with previous negative biopsies

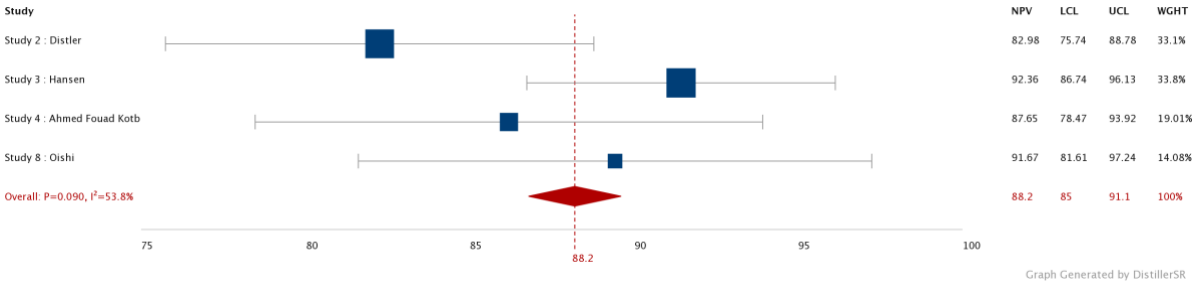

Figure 3D: NPV of MRI combined with PSAD<0.15ng/ml/ml in PCa naive patients

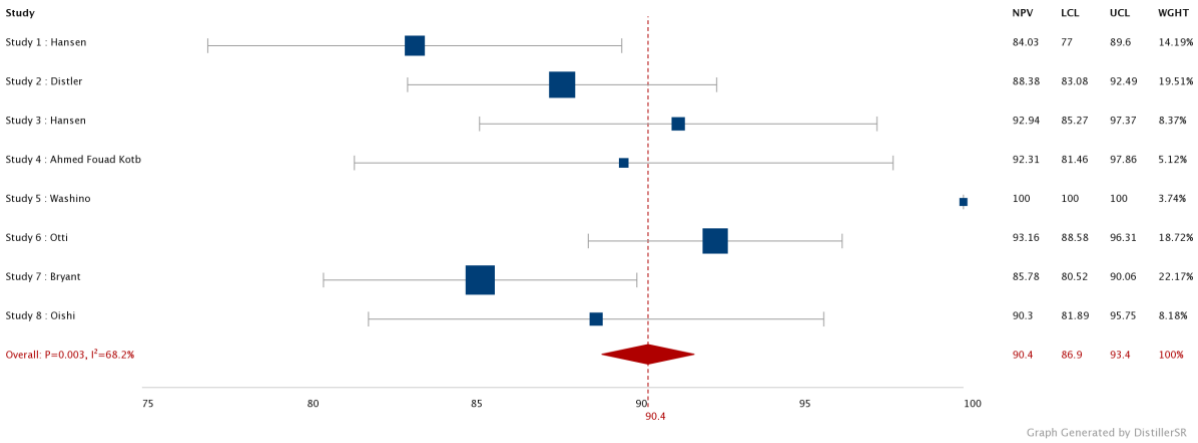

Figure 3E: NPV of MRI combined with PSAD<0.15ng/ml/ml in biopsy naive patients

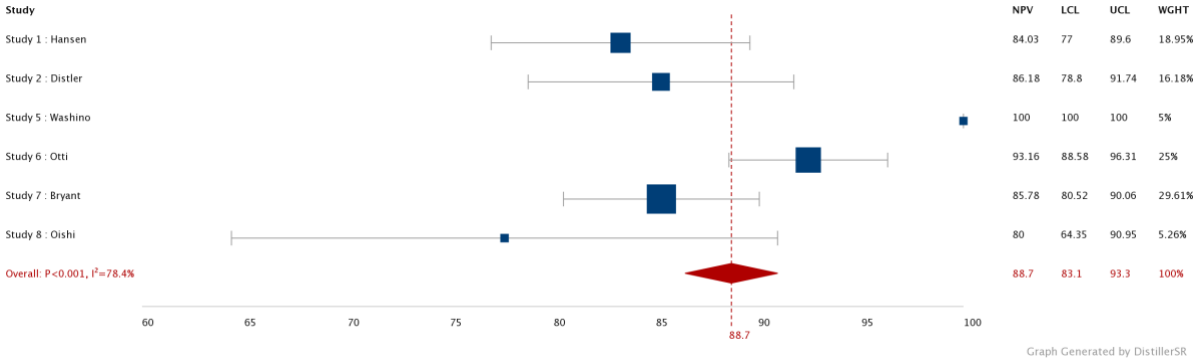

Figure 3F: NPV of MRI combined with PSAD<0.15ng/ml/ml in patients with previous negative biopsies

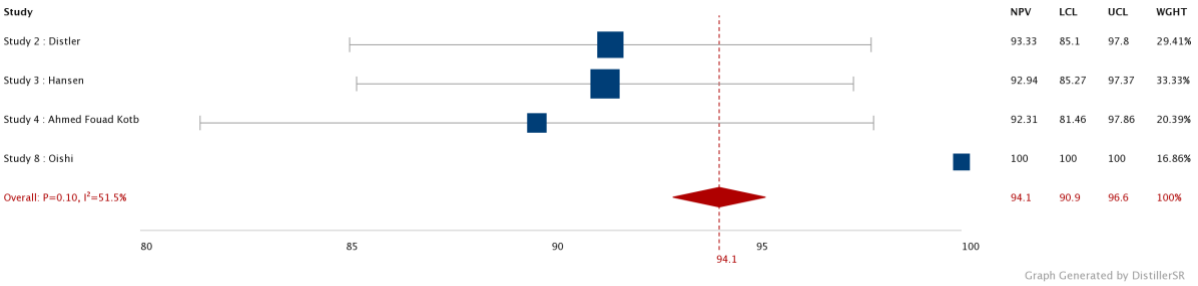

Table 1: Baseline characteristics of included studies

Study	Year	Study design	Center	Period	Magnetic field	MRI sequences	Definition of nMRI	Definition of csPCa	Reference standard	n° of biopsies
Wang(24)	2017	retrospective	Monocentric	2012 - 2015	3T	T2WI ; DWI ; DCE ; ADC	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transrectal	12
Numao(19)	2013	prospective	Monocentric	2006 - 2010	1,5T	T2WI ; DWI ; DCE	PIRADS <3	1. GS ≥4+ 3 and/or percent positive core >20% and/or maximum cancer length≥5mm 2. GS ≥ 4+ 3 and/or percent positive core >20% and/or maximum cancer length ≥ 5 mm 3. GS ≥ 4 + 3 and/or percent positive core > 20%	Transrectal or transperineal	21 (14-29)
Oishi(22)	2018	prospective	Monocentric	2011 - 2017	3T	T2WI ; DWI ; DCE ; ADC	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transrectal	13(12-14)
An(23)	2017	retrospective	Monocentric	2013 - 2017	3T	T2WI ; DWI ; DCE ; ADC	PIRADS 1	GGG ≥2 (Gleason score ≥ 3+4)	Transrectal	12
Panebianco(17)	2018	retrospective	Monocentric	2010 - 2015	3T	T2WI ; DWI ; DCE	PIRADS <3	>G6 or >T2a or >3 positive biopsies or >50% cancer involvement on each positive core.	Transrectal	14(12-18)
Hansen(25)	2018	prospective	Multicentric	2012 - 2016	1,5 & 3T	T2WI ; DWI ; DCE	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transperineal	18-24
Distler(27)	2017	prospective	Monocentric	2012 - 2015	3T	T2WI ; DWI ; DCE	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transperineal	24
Hansen(26)	2017	prospective	Multicentric	2012 - 2015	1,5 & 3T	T2WI ; DWI ; DCE	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transperineal	24
Kotb(28)	2018	retrospective	NR	2015-2016	3T	T2WI ; DWI ; DCE	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transrectal	12
Washino(29)	2017	retrospective	Monocentric	2010 - 2014	1,5 & 3T	T2WI ; DWI	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4) or CCL ≥4mm	Transperineal	14
Otti(21)	2019	retrospective	Monocentric	2013 - 2016	1,5T	T2WI ; DWI ; DCE ; ADC	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transrectal or transperineal	TRUS : 12 ; TP : NR
Bryant(30)	2019	retrospective	Monocentric	2015 - 2017	1,5 & 3T	T2WI ; DWI ; DCE	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transrectal	8-12
Gnanapragasam(31)	2016	prospective	Monocentric	2013-2015	1,5 & 3T	T2WI ; DWI ;	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transperineal	24
Druskin(16)	2017	prospective	Monocentric	NR	NR	NR	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4) or GGG 1 in >2cores or >50% of 1 core	NR	NR
Perlis(15)	2017	retrospective	Monocentric	2011 - 2016	NR	NR	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4) or > 50% core involvement, or >3 positive cores	Transrectal	12-16
Thompson(20)	2016	prospective	Multicentric	2012-2014	1,5 & 3T	T2WI ; DWI ; DCE	PIRADS <3	GGG ≥2 (Gleason score ≥ 3+4)	Transperineal	18

Study	n° patients	n° nMRI	BN	PNB	PPB	Age (median)	PSA (median)	Abnormal DRE (>T1c)	Prostate volume (median)	PSAD (median)	origins	Family history of PCa
Wang(24)*	84	84	39	30	15						Afro-American	
		BN				61.9(+-7.2)	5.9(+-6.3)	18%		0.17(0.15)	2(5%)	18%
		PNB				64(+-7.6	9.4(+-6.9)	10%		0.19(0.21)	1(3%)	20%
Numao(19)	351	194	194	0	0	65 (59–70)	6.3 (4.9–9.1)	19%	32 (24–42)	0.19 (0.14–0.30)	Japanese	5.9%
Oishi(22)	135	135	48	60	27	64(58-69)	5.9(4.1-8.0)	8.1%	55(38-79)	0.1 (0.073-0.15)		24%
An(23)	114	114	20	53	41	61 (57-67)	5,5(3,6-8,7)	4.4%	57(42-80)		Caucasian 93(81.6%); AA 12(10.5%); other 9(7.9%)	
Panebianco(17)	1255	1255	659	596	0							
		BN				66(62-69)	5.9(3.9-7.6)	10%	50(42-68)	0.11(0.08-0.14)		11%
		PNB				68(60-72)	5.6(3.2-7.8)	9%	60(38-73)	0.11(0.08-0.15)		7%
Hansen(25) ×	807	236	236	0	0	65(59-70)	6.5(4.9-8.8)	23%	42(30-58)	0,15(0,10-0,22)		
Distler(27) ×	1040	344	NR	NR	0	65	7,2		45	0,16		
Hansen(26) ×	487	144	0	144	0	66(60-71)	9,0(6,7-13,4)	NR	56(40-80)	0,15(0,10-0,24)		
Kotb(28) ×	228	81	0	81	0	64.6 ± 6.6	8.1 ± 7.8			0.14 ± 0.16		
Washino(29) ×	288	131	131	0	0	69(64-74)	7,5(5,5-11,0)	16%	28,7(23,3-39,4)	0,26(0,17-0,38)		
Otti(21) ×	792	348	348	0	0	66 +- 10	6.75 (4.18)	19.5%	49.50 (36)	0.13 (0.12)		
Bryant(30) ×	1789	278	278	0	0	68 (37-88 ; 63-73)	7.6 (0.4-2668 ; 5.7-11.5)	48%	56.1 (10.4-244 ; 40.1-79)	0.13 (<0.1-42.4 ; 0.09 - 0.23)		17%
Gnanapragasam(31) ×	279	94	0	NR	NR	66 (45-80)			52 (11-230)			
Druskin(16) ×	104	20	NR	NR	0	65.0(59.3-70.8)	7.0(4.9-.10,2)	0%	50 (37.32-70,0)	0.14(0.096-0.21)	AA 11,6%	
Perlis(15) ×	286	58	0	NR	NR	62.5(58-68)	6.35(4,6-8,8)	5,6%	47(35-63)			18,8%
Thompson(20) ×	344	79	79	0	0	62.9	5.2	44.40%	40			26,70%

* mean results and not median; × results on all patients (and not only nMRI)
NR: Non Reported
AA: Afro-American ; BN : biopsy naive ; PNB : previous negative biopsy ; PPB : previous positive biopsy ; DRE : digital rectal examination ; PSAD : PSA density

Study	Rate of csPCa	n° of csPCa	Rate of csPCa in BN	n° of csPCa in PNB	Rate of csPCa in PNB	n° csPCa in PNB	Rate of csPCa in AS/PPB	n° of csPCa in AS/PPB	NPV of MRI for csPCa	NPV of MRI for csPCa in BN	NPV of MRI for csPCa in PNB	Rate of nMRI
Wang(24)	13.1%	11	10.30%	4	16.70%	5	13.3	2	86.90%	89.7%	83.3	18.1%
Numao(19)	20%	38	20%	38					(*i*). 90.7% ; (*ii*). 87.4% ; (*iii*). 88.1%	(*i*). 90.7% ; (*ii*). 87.4% ; (*iii*). 88.1%		55%
Oishi(22)	18%	24	21%	10	8%	5	33%	9	82%	79%	92%	12.90%
An(23)	3.60%	4	0%	0	0%	0	9.80%	4	96.5%	100%	100	NA
Panebianco(17)®	4.80%	60	5.50%	36	4.00%	24			NA	NA	NA	31%
Hansen(25)	20,3%	48	20.30%	48					80% (75-85)	80% (75-85)		29.2%
Distler(27)	20.60%	71							79.4% (75.3-82.9)		83.0 (76.6-87.9)	33.10%
Hansen(26)	7,6%	11			7.60%	11			92.4%		92.40%	29.7%
Kotb(28)	12.40%	10							88%		88%	24.10%
Washino(29)	13.70%	18	13.70%	18					86.30%	86.30%		44.40%
Otti(21)	14.40%	50	14.40%	50					85.60%	85.60%		44%
Bryant(30)	15.10%		15.1%						84.90%	84.90%		35.10%
Gnanapragasam(31)	22.3%	21			22.3%	21			78%		78%	33.7%
Druskin(16)	10%	2							90%			19.20%
Perlis(15)	NR								NR			55.2%
Thompson(20)	8%								92%	92%		23%

® results after median follow-up of 38 months in BN and 60 months in PNB

csPCa : clinically significant prostate cancer ; BN : biopsy naive ; PNB : previous negative biopsy ; AS/PPB : active surveillance/ previous positive biopsy ; NPV : negative predictive value ; nMRI : negative MRI

Table 2: NPV of MRI combined with markers for the diagnostic of csPCa

Study	Definition of nMRI	Definition of csPCa	n° nMRI	n° BN	n° PNB	n° PPB	NPV MRI	NPV MRI in BN	NPV MRI in PNB	Markers	Cutoff value	NPV of MRI + markers
Hansen(25)	PIRADS <3	GGG ≥2	236	236	0	0	80%	80%		PSAD DRE -	**<0,15** <0.10 0.10-0.20 ≥0.20	**84%** 91% 79% 66% 80%
Distler(27)	PIRADS <3	GGG ≥2	344	203	141	0	79.4%	76.8%	83.0%	PSAD	**<0,15** <0.07 0.07-0.15 ≥0.15	**89%** 86.5% 88.9% 66.9%
Hansen(26)	PIRADS <3	GGG ≥2	144	0	144	0	92.4%		92.40%	PSAD	**<0,15** ≥0.15	**93%** 92%
Kotb(28)	PIRADS <3	GGG ≥2	81	0	81	0	88%		88%	PSAD	<0,15	93%
Washino(29)	PIRADS <3	GGG ≥2 and/or CCL ≥4 mm	131	131	0	0	86.30%	86.30%		PSAD	**<0,15** 0.15-0.29 ≥0.3	**100%** 80% 70%
Bryant(30)	PIRADS <3	GGG ≥2	278	278	0	0	84.90%	84.90%		PSAD PSAD + DRE-	**<0,15** <0.10 <0.2 <0,15 <0.10 <0.2	**85.8%** 87% 85.2% 89.6% 92% 88.3%
Oishi(22)	PIRADS <3	GGG ≥2	135	48	60	27	82% (86% if PPB not included)	79%	92%	PSAD	**<0,15** ≥0.15 <0.10 0.10-0.15	**90% (90%)** 60% (71%) 94% (93%) 82% (85%)
Otti(21)	PIRADS <3	GGG ≥2	348	348	0	0	85.60%	85.60%		PSAD PSA	**<0,15** <0.12 0.12-0.15 ≥0.15 <5 5-10 ≥10	**95.2%** 95% 86.7% 76.8% 90.2% 86.6% 79.2%
Druskin(16)	PIRADS <3	GGG≥2 or GGG 1 in >2cores or >50% of 1 core	20	NR	NR	0	90%			PHID	**>0.44**	**100%**
Perlis(15)	PIRADS <3	GGG≥2 or CCL >4mm	58	0	NR	NR	NR			PCA3	**<35**	**100%**
Thompson(20)	PIRADS <3	GGG≥2	79	79	0	0	92%	92%		PSA or DRE + PSA and DRE -	≥10 <10	100% 90%
Gnanapragasam(31)	PIRADS <3	GGG≥2	94	0	NR	NR	78%			PHI	**≥35** ≥25 ≥30 ≥40	**97%** 89% 95% 90%

Table 3: studies including predictive factors of all prostate cancer / csPCa.

Study	n° nMRI	Definition nMRI	Definition csPCa	Univariate analysis										Multivariate analysis								
				Age	PSA	Prostate volume	PSAD	PSAD < 0.15	Previous negative biopsy	> T1c	Ethnicity (AA vs non AA)	Family history of PCa	Other	Age	PSA	Prostate volume	PSAD	PSAD <0.15	Previous negative biopsy	> T1c	Ethnicity	other
Predictive factors of csPCa at biopsy																						
Wang(24)	84 BN 39 PNB 30 PPB 15	PIRADS <3	GGG ≥2		6.1 +4.6 vs 14.4 +10.5 p<0.01		0.14 + 0.12 vs 0.63 + 0.28 p<0.01						PCPTRC 8.4 +6.5% vs 23.1 + 22.7% p<0.01									PCPTRC * OR = 1.01 p< 0.01).
Numao(19)	151 BN 151	PIRADS <3	1. GS ≥4+3 and/or percent positive core greater than 20% and/or maximum cancer length ≥5 mm	OR = 1.1 (0.37–3.5) p=0.81	OR = 1.4 (0.46–14) p= 0.56	OR = 8.1 (2.1–54) p<0.01	OR = 11 (2.1–200) p<0.01					OR = 1.4 (0.07–9.0) p =0.76	% fPSA (cutoff 15%) OR=3.3 (1.1–11) p=0.03			OR =**8.1** (2.1–54) p< 0.01						
			2. GS ≥3+4 and/or percent positive core greater than 20% and/or maximum cancer length ≥5 mm ;	OR =1.3 (0.49–3.5) p = 0.60	OR =1.5 (0.56–4.0) p= 0.44	OR =5.2 (1.8–19) p < 0.01	OR =3.0 (1.0–11) p < 0.04					0.99 (0.05–6.1) p=0.99	% fPSA (cutoff 15%) OR = 2.6 (0.97–7.1) p = 0.06			OR =**5.2** (1.8–19) p< 0.01						
			3. GS ≥ 3+4 and/or percent positive core greater than 20%	OR =1.5 (0.55–4.1) p = 0.44	OR =1.7 (0.63–4.9) p= 0.30	OR =4.8 (1.6–18) p < 0.01	OR =2.8 (0.94–10) p < 0.06					1.1 (0.05–6.5) p = 0.96	% fPSA (cutoff 15%) OR =2.3 (0.85–6.4) p =0.10			OR =**4.8** (1.6–18) p< 0.01						
Predictive factors of no csPCa at biopsy																						
Oishi(22)	135 BN 48 PNB 60 PPB 27	PIRADS <3	GGG ≥2	OR = 0.99 (0.94-1.06) p = 0.97	OR = 1.02 (0.93-1.11) p = 0.7	OR = 1.03 (1.01-1.06) p=0.001	OR = 0.01 (0.0002-0.55) p = 0.02	OR = 5.93 (2.32-15.2) p <0.001	OR = 3.73 (1.3-10.7) p=0.01	OR = 0 (0-1.81) p = 0.2		OR = 0.96 (0.35-2.68) p = 0.9	BN (Y/N) OR = 0.73 (0.30-1.79) p = 0.5 PPB (Y/N) OR = 0.32 (0.12-0.85) p = 0.02 PIRADS v.1 vs v.2 OR = 0.62 (0.24-1.56) p = 0.31					OR = **7.7** (2.8-21.3) p <0.001	OR = **5.2** (1.6-16.5) p = 0.005			
Predictive factors of all PCa at biopsy																						
An(23)	114 BN 20 PNB 53 PPB 41	PIRADS <2	GGG ≥2	OR = 0.99 (0.93-1.05) p = 0.79	OR = 0.90 (0.80-1.01) p = 0.19	OR = 0.99 (0.97-1.00) p=0.06				OR = 2.36 (0.37-14.95) p=0.36	OR = 1.14 (0.29-4.58) p = 0.85			OR = 1.01 (0.95-1.07) p = 0.82	OR = 0.99 (0.81-1.21) p = 0.93	OR = 0.98 (0.96-1.01) p=0.19				OR = 1.41 (0.21-9.55) p = 0.73	OR = 0,98 (0,23-4,19) p=0,98	
Predictive factors of csPCa with follow-up																						
Panebianco(17)	1255 BN 659 PNB 596	PIRADS <3	>G6, >T2a	NR●	NR●		NR●		NR●					HR = **0.93** (0.89-0.98) p = 0.005	HR =**1.21** (1.1-1.32) p<0.001		HR = **7.57** (2.73-21) p<0.001		HR = 1.01 (0.53-1.93) p = 0.97			

Table 4: meta-analysis of studies reporting results of MRI coupled with PSAD<0.15ng/ml/ml

Critères	Nb. studies	Nb. patients	Pooled rates (95%CI)	I^2, %	*P* *
NPV alone					
All cancer naive men	8	1665	84.4 (81.3 to 87.2)	63.3	0.008
In biopsy naive patients	6	1243	82.7 (80.5 to 84.7)	53.6	0.056
In previous negative biopsy patients	4	426	88.2 (85.0 to 91.1)	53.8	0.090
NPV coupled with PSAD < 0.15ng/ml/ml					
All cancer naive men	8	1015	90.4 (86.8 to 93.4)	68.2	0.003
In biopsy naive patients	6	760	88.7 (83.1 to 93.3)	78.4	<0.001
In previous negative biopsy patients	4	255	94.1 (90.9 to 96.6)	51.5	0.10

* *P*-value associated with Chi-squared test for heterogeneity.

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En utilisant les facteurs trouvés dans la revue de la littérature, et en étudiant d'autres, nous avons ensuite étudié le risque de ne pas diagnostiquer un cancer cliniquement significatif au diagnostic et pendant le suivi malgré une IRM non suspecte, si des biopsies n'avaient pas été réalisées. Notre étude « *Negative Prebiopsy Magnetic Resonance Imaging and Risk of Significant Prostate Cancer: Baseline and Long-Term Follow-up Results* » a été publiée dans la revue Journal of Urology en Mars 2021. L'analyse a montré que ce risque était de 9% au moment du diagnostic et de 4% supplémentaire lors du suivi. Ce risque initial diminuait de 9% à 2.4% en in tégrant dans l'analyse au moment du diagnostic la densité de PSA, le toucher rectal ou les antécédents familiaux pour décider ou non d'une biopsie.

Negative pre-biopsy Magnetic Resonance Imaging and risk of significant prostate cancer: baseline and long-term follow-up results.

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Key words: negative MRI, Predictive factors, prostate cancer, PSA density, biopsy.

Abstract

Purpose: Prostate biopsy should be discussed with the patient in case of negative-MRI(nMRI) and low clinical suspicious of prostate cancer(PCa).

Objectives: Primary objective was to describe the risk of clinically significant PCa(csPCa) in a nMRI biopsy-naïve population at baseline and during long-term follow-up. Secondary objective was to evaluate clinical-factors and PSA as predictors of csPCa at baseline.

Materials and Methods: All 503consecutive biopsy-naïve patients referred in 2007-2017 for biopsy with nMRI(PIRADS1-2) who had systematic-12-core-biopsies(SB) at baseline were included. Clinical factors were digital-rectal-examination(DRE), PCa-family-history and PSA. In case of suspicious-DRE or PSA-kinetics during follow-up, MRI and biopsy were performed. CsPCa was defined as either GG1 with cancer-core-length>5mm or ≥3positive-SB in addition to GG≥2(csPCa-1) or any GG≥2(csPCa-2). Non-clinically-significant-PCa was defined as either GG1 with cancer-core-length≤5mm and<3positive-SB(non-csPCa-1) or any GG1(non-csPCa-2). Definition of high-risk-csPCa was GG≥3. Univariate and multivariate-models were fitted to identify predictors of CsPCa-risk.

Results: At baseline,biopsy showed csPCa-1 in 9%(n=45) and csPCa-2 in 6%(n=29) and non-csPCa in 22%(n=111). At median follow-up of 4yrs(IQR:1.6-7.1),31%(95%CI:27-36) of 415untreated patients had a second MRI and 24%(95%CI:20-28) a second biopsy which showed csPCa-1 in 5%(21/415,95%CI:3-7),csPCa-2 in 2%(7/415,95%CI:1-3) and non-csPCa in 8%. Overall incidence was 13%(n=66/503,95%CI:7-21) for csPCa-1, 7%(n=36/503,95%CI:5-9%) for csPCa-2 and 2%(n=12/503,95%CI:1.1-3.7)for high-risk-PCa. Predictors of CsPCa-risk were PSAd≥0.15ng/mL/mL(OR=2.43[1.19-4.21]),clinical-stage≥T2a(OR=3.32[1.69-6.53]) and PCa-family-history(OR=2.38[1.10-6.16]). Performing biopsy in patients with nMRI and PSAd≥0.15ng/ml/ml or abnormal DRE or PCa-family-history would have decreased from 9% to 2.4% the risk of missing csPCa-1 at baseline while avoiding biopsy in 56%.

Conclusion: Risk of csPCa in a negative MRI biopsy-naïve population was 6%-9% at baseline and 7%-13% at long-term follow-up depending on csPCa definitions.

Introduction

Guidelines recommend that prostate biopsy should be discussed with the patient in case of negative MRI(nMRI) and low clinical suspicious of prostate cancer(PCa)(1,2). Hence pre-biopsy MRI is non-suspicious in 20 to 40% of cancer-naive patients with suspicious PCa(PSA>4ng/ml or suspicious digital rectal examination[DRE])(3–5). MRI has been proposed as a “triage-test” for the indication of biopsy in these patients to decrease the number of unnecessary biopsies, overdiagnosis and overtreatment of non-clinically significant PCa(non-csPCa). In the PRECISION study, prostate MRI improved the diagnosis of clinically significant prostate cancer(csPCa) and reduced the diagnosis of non-csPCa compared to traditional systematic transrectal ultrasound (TRUS)-guided-prostate-biopsy(4).
MRI has been shown to have a negative predictive value(NPV) between 85 to 95% for PCa prevalence of 50%. The risk of non-detection of up to 15 % of csPCa is the primary reason that biopsy omission in the setting of nMRI is still debated(3, 6–8). Validation of the NPV of MRI can be performed by correlation of MRI result to histopathological reference standard such as, template prostate biopsies or radical prostatectomy specimens or by longitudinal evaluation of csPCa incidence over time(3). Indeed, a recent study from Panebianco showed that 95% of biopsy-naive patients with negative MRI(nMRI) were free of csPCa at two years(9). The addition of other clinical (prostate volume, age, body mass index [BMI], PCa-family-history and T-stage) or biological factors (PSA kinetics or PSA density[PSAd]) to nMRI could increase the diagnostic accuracy of MRI by reducing the risk of false negatives. Among all these factors, PSAd with a threshold<0.15ng/ml/ml is the most studied for excluding csPCa in patients with nMRI(10).

The main objective of our study was to describe the csPCa cumulative incidence in a nMRI biopsy-naïve population with long-term follow-up. Secondary objectives were to evaluate clinical factors (prostate volume, age, BMI, PCa-family-history and cancer stage) and biological markers (PSA kinetics, PSAD) as predictors of risk of csPCa at baseline.

Material and Methods

Study Design and Population: We conducted a single-centre, retrospective cohort study of all consecutive patients from January 2007 to December 2017 for which pre-biopsy MRI and prostate biopsy were performed in our center. Database protection authorization and patient consent was obtained as requested by ethical committee.

During the study period, 2321 consecutive cancer naïve patients who had prebiopsy MRI were referred for biopsy. We included all cancer-naïve, biopsy-naïve patients referred with suspected PCa (PSA>4ng/ml or and/or suspicious digital rectal examination) who undergone biopsy series after pre-biopsy MRI. Minimum follow up was one year. Patients with previous biopsy, previous prostate cancer diagnosis and patients treated with 5-alpha-reducase inhibitors were excluded (11). Patients with missing MRI or markers data were also excluded (Figure1).

MRI protocol and Reporting: pre-biopsy MRI was performed using a 1.5-Tesla system with a pelvic-phased array coil. Protocol included T2-weighted imaging(T2W) and functional imaging sequences (diffusion weighted imaging, dynamic contrast-enhanced imaging, and apparent diffusion coefficient mapping). Images were interpreted by uroradiologists with>10 years’ experience in prostate MRI reading. MRI was considered negative if the Prostate Imaging–Reporting and Data System (PI-RADS)V1 then V2 score was 1-2(12)

after 2012, or Likert1-2 before.

Biopsy technique and PCa classification: all patients underwent systematic 12-core-systematic-US-guided (TRUS) prostate biopsy, including six lateral and six mid-lobar cores from base, mid and apex of the gland. Targeted biopsies were added if abnormality was found at DRE or TRUS. Each biopsy core was submitted separately, and in the case of malignant cores, overall and separated Gleason and ISUP Gleason Grade Group(GG) classification and maximum cancer-core-length(MCCL) were reported(13). CsPCa was defined as either GG1 associated with criteria of tumor extent (MCCL>5mm or ≥3positive SB) in addition to GG≥2(csPCa-1) or any GG≥2(csPCa-2). Non-csPCa was defined as either GG1 with cancer-core-length≤5mm and<3positive SB(non-csPCa-1) or any GG1(non-csPCa-2). Definition of high risk csPCa was GG≥3.

Clinical data that was collected before first biopsy, included: age, BMI; obesity was defined as BMI>30kg/m2), family history of PCa(defined as at least one first degree relative with PCa) and clinical T-stage at DRE. Biological data included PSA, PSA density and PSA kinetics (PSA velocity and PSA doubling-time) calculated with the MSKCC calculator using at least two measurements over a period of at least 3months(14).

Follow-up monitoring for untreated patients was based on annual PSA and DRE. All patients with non-csPCa1 had were offered active surveillance which consisted of on 6 months PSA and annual DRE. If a curative treatment option was chosen for non-csPCa, follow-up was discontinued at the date of diagnosis. Two-thirds(66%) of our population had follow-up until the last two years of the follow-up period. The main endpoint was occurrence of a csPCa-1. MRI and biopsy during follow-up were performed in cases of rising PSA or suspicious DRE. Time to CsPCa-2 occurrence was calculated from the date of first biopsy series to the diagnosis of csPCa-2.

Statistical analysis: Categorical variables were expressed as numbers and percentages. Quantitative variables were expressed as medians, with interquartile ranges(IQR). Normality of distributions was assessed using histograms and the Shapiro-Wilk test. In csPCa1-naive patients, cumulative incidence of csPCa1 was estimated using the Kalbfleisch and Prentice method, by taking into account death as a competing event. In the overall population, potential predictive factors of csPCa1 diagnosis at first biopsy series were evaluated by using logistic regression models, odds-ratios, and their 95% confidence intervals(95%CI). Optimal threshold values of PSA density and previously published thresholds (with their 95%CIs) were evaluated by calculating sensitivity, specificity, positive predictive value and negative predictive value. Decision curve analysis (DCAs) assessed the net benefit associated with the adoption of each model: PSAd≥0.10ng/ml, PSAd≥0.14ng/ml, PSAd≥0.15ng/ml and association of PSAd≥0.15ng/ml, PCa-family-history and DRE. All statistical tests were done at the two-tailed α level of 0.05 using SAS software, release 9.4(SAS Institute, Cary, NC).

Results

Study population: For the whole cohort of 2321 patients with pre-biopsyMRI, results showed in 56% any Pca(n=1290), in 50% csPCa1(n=1156) and in 40%csPCa-2(n=927). MRI was positive in 73%(n=1694) and negative in 27%(n=627). Out of 627 negative MRI patients, 124(20%) were excluded(Figure 1).

Baseline results: A total of 503 patients with nMRI underwent analyses. Baseline clinical, biological and pathological data are shown in Table 1. Biopsy at baseline was positive for CsPCa-1 in 9%(n=45; 95%CI,6-11%), for csPCa-2 in 6%(n=29; 95%CI,4-8%), for high-grade cancer in 1.6%(n=8; 95%CI:0.5-2.7) and for non-csPCa in 22%(n=111; 95%CI,19-26%). Patients' clinical characteristics and outcome for high risk PCa at baseline are shown in Supplementary data 1. All patients with CsPCa-1(n=45) and 43/111 non-csPCa received curative treatment (prostatectomy, radiotherapy or focal-therapy). The remaining 68/111 patients with non-csPCa were on AS. The only patient at metastatic stage received hormonal treatment. During follow-up, 29 patients(5.8%) with no diagnosis of csPCa died of other causes than PCa. One patient with diagnosis of csPCa at first biopsy series died from metastatic PCa.

Long-term follow-up results: After a median follow-up of four years(IQR, 1.6-7.1), 31%(n=130/415, 95%CI:27-36) of the 415 untreated patients (patients on AS and patients without cancer at baseline biopsy) underwent at least a second MRI. Table2 shows the MRI, biopsy, curative treatment and histology follow-up data. At a mean follow-up of 30 months, 24%(n=98/415,95%CI:20-28) underwent at least a second biopsy series, which was positive for csPCa-1 in 5%(n=21, 95%CI: 3-7) and for csPCa-2 in 2%(n=7, 95%CI:1-3) and non-csPCa in 8%(n=33, 95%CI:5-11). Overall incidence was 13%(n=66/503,95%CI:7-21) for csPCa-1, 7%(n=36/503,95%CI:5-9%) for csPCa-2 and 2%(n=12/503,95%CI:1.1-3.7) for high-risk PCa. During follow-up, when patients had a csPCa-1, MRI was suspicious in 94% of cases(n=16/17,95%CI:83-100). Overall non-csPCa was diagnosed in 123/503 patients(24%). Cumulative incidence of CsPCa diagnosis during follow-up after biopsy at baseline was 1.7% at 2 years(95%CI,0.7-3.5),4.6% at 5years(95%CI,2.6-7.4) and 11.3% at 10years(95%CI,6.0-18.3)(Figure3).

Risk factors analyses: at univariable analyses, PSAd(OR=1.06[1.03–1.09],p=0.032), T-stage>T1c(OR=3.32[1.69–6.53],p<0.001) and PCa-family-history(OR=2.38[1.10–6.16],p=0.028) were significantly associated with csPCa-1 diagnosis at first biopsy series in men with nMRI. At multivariate analyses, these factors remained significantly associated with the diagnosis of csPCa-1 at baseline: PCa-family-history OR=2.31(95%CI:1.12-5.26,p=0.043),T-stage OR=2.43 (95%CI:1.12-5.26,p=0.025) and PSAd OR=1.06(95%CI:1.03-1.10,p=0.001)(Table3). The area-under-the-receiver-operating-characteristic-curve for diagnosis of csPCa at various PSA densities was 0.67(95%CI:0.58-0.76) (Supplementary data 2). Analyses of single and combined risk factors are shown in supplementary data 3. Use of a PSAd threshold of≥0.15ng/mL/mL in nMRI patients would have decreased the risk of missing csPCa from 9% to 4.6%, while avoiding biopsy in 65%. Used in combination, PSAd≥0.15ng/ml/ml, abnormal DRE and family history with MRI results would reduce the risk of missing a csPCa-1 to 2.4% and of avoiding biopsy in 56% of patients. Furthermore, the model including PSAd≥0.15ng/ml/ml+positive DRE+PCa positive family history showed the highest net benefit for predicting diagnosis of csPCa in biopsy naïve nMRI patients at DCAs(Figure 2).

Discussion

In this retrospective study of 503 patients, nMRI was associated with a 6 or 9% rate of csPCa, at biopsy at baseline depending on csPCa definition. Combining nMRI with PSAd and others clinical factors appears to have utility as a triage test to help identify patients with csPCa for which biopsy could be safely omitted. This strategy of triage to biopsy combining MRI and clinical factors to risk stratify minimizes harms and maximizes detection of significant cancer

The results presented here mirror those found in the literature, with an incidence of csPCa in nMRI around 5-15%. Norris et al. recently showed that in case of nMRI, application of a PSAD threshold of 0.15ng/ml/ml reduced the proportion of men with undetected cancer(15). Definition of csPCa is still debated but we trust that GG1 high extent PCa should be still considered as CsPCa as it was the case during the study period. Our CsPCa-2 incidence that was used in csPCa definition of PRECISION study was 40%, which is close to the 38% incidence in PRECISION study. This strengthens the reproducibility of our data and allows comparisons with other series.

At long-term follow-up, a 5%risk of finding csPCa after baseline biopsy is also reassuring for patients and corroborates results previously described. Panebianco found that 95% of nMRI-negative biopsy patients had no PCa at 48months(9). In the study of Venderink et al. more than half of patients had negative MRI, CsPCa(GG≥2) diagnosis-free survival was 99.6% after 3years. These good results are partly due to the the fact that half of the series had previous negative biopsy(16).

One of the main barriers to widespread screening for prostate cancer is the lack of specificity of traditional diagnostic tools(PSA and DRE) and the risk of over-treatment for clinically insignificant cancers. Whilst MRI does offer hope of improving screening NPV for csPCa, several studies, including the MRI-first study(7), have shown that avoiding biopsy for patients with negative MRI carries the risk of missing 5 to 15% of csPCa, thus potentially missing the window of disease curability(9,17-19). Furthermore, aggressive histopathological subtype as cribriform or ductal cancer may play an important role in MRI visibility(20). In PRECISION study, pre-biopsy MRI not only increased the detection of csPCa in positive tests(+12%), but also decreased the number of non-csPCa diagnoses(-13%), because nMRI patients were not biopsied(4). We showed that 90% of patients with a csPCa diagnosis during follow-up had a suspicious follow-up MRI. The onset of a lesion at MRI during follow-up was strongly predictive of a csPCa.

Our results regarding predictors of csPCa in this population are also consistent with previous studies. We showed that the density PSA is a strong predictor to help exclude csPCa in cases of nMRI. A PSAd threshold of<0.15ng/mL/mL appears to be a good selective marker that increases the NPV of MRI, and thus facilitates omission of prostate biopsies in appropriate cases(10). Padhani *et al.* have recently also proposed that patients with nMRI and PSAD<0.1-0.15ng/mL/mL could avoid prostate biopsy, but only if longitudinal PSA(and probably PSAd) monitoring was implemented(20).

The superiority of PSA density as a potential predictor could be explained by the fact

that PSA is a hormonal marker produced by both normal prostate epithelial cells and PCa. A proportion of localized PCa have moderate PSA levels that are similar to those of high-volume prostates(without PCa), as blood PSA levels are related to prostate volume. Therefore, it would seem plausible that PSAd could be a more accurate marker for the diagnosis of csPCa than PSA, as gland volume is accounted for.

Currently, there are few data available on predictive factors for csPCa in patients with nMRI. In our study, age was not associated with presence of csPCa, as also demonstrated in several previous studies(21-24). Similarly, obesity was not associated with csPCa in our study of nMRI patients; however, in studies without pre-biopsy MRI, obesity was associated with presence PCa at first biopsy, and more specifically, with high-grade disease(25). Interestingly, the evidence surrounding PSA as predictive factor for csPCa in nMRI patients is debated(9,10). It appears that PSA kinetics could also be useful for predicting the risk of significant cancer in the context of prostate cancer screening or active surveillance. However, in our study, we found no correlation between PSA kinetics and csPCa diagnosis at baseline, although this may be due to PSA 'noise' from non-cancer sources(26).
Family history has been reported in various studies as a potential predictive factor of PCa, but has never been shown to be a significant predictive factor of csPCa(10,22,23). In our study, we found correlation between family history and csPCa, however our definition was extended to include all patients with at least one first degree relative with PCa. Lastly, clinically palpable tumor was reported in two nMRI studies, but was not found to be a significant predictive factor PCa(9,10,23,24).

Our study has several limitations. Whilst limited by being both single-centre, and retrospective, the strength provided by the long follow-up in our study would only be possible in centers that embraced pre-biopsy MRI before incorporation into national guidelines. A minimal follow-up of 1 year is short and biases the results. The retrospective nature of the study resulted in a loss of data, which also reduces the power of the results. Next, radiologist expertise is important for diagnosis of negative MRI for csPCa(27). In our study, radiologists had over 10years of MRI interpretation which favors better results, only single MRI readings were performed. We used a 1.5-Tesla MRI with high b-values. A 3-Tesla machine may have been associated with better results. If the high-level of radiological expertise in our study may hinder extrapolation of our results to the general population, especially outside expert centers, the widespread use of MRI for the diagnosis of prostate cancer and the recent uptake of big-data technology, may improve training of radiologists, and as such, the generalizability of our results to future practice(28). Imaging exams other than MRI could also be tested for the detection of significant prostate cancers before biopsies(29). The lack of other biomarkers data such as 4K-score or genetic scores due to absence of our routine use and due to the retrospective analysis is a limitation of the work. We are aware that definition of CsPCa has evolved with time.

Conclusion

Risk of csPCa in a negative MRI biopsy-naïve population was 6%-9% at baseline and 7%-13% at long-term follow-up depending on csPCa definitions. Performing biopsy in patients with nMRI and PSAd≥0.15ng/ml/ml or abnormal DRE or PCa-family-history would have decreased from 9% to 2.4% the risk of missing csPCa-1 at baseline while avoiding biopsy in 56%.

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Table 1 Baseline clinical, biological and biopsy results

Variable	**n***	
Median age, yr (IQR)	503	62.94 (58 - 68)
Median BMI, kg/cm^2 (IQR)	406	26.27 (23 - 28)
PCa family history, n (%)	447	60 (13.4)
Median PSA, ng/mL(IQR)	503	6.85 (4.7 - 8)
Median prostate volume, mL(IQR)	489	59.89 (40 - 70)
cT stage, n (%) : T1c T2a T2b T2c T3/T4	503	 428 (85) 65 (13) 6 (1.2) 6 (1.2) 0 (0)
Median PSA density, ng/mL/mL (IQR)	489	0.13 (0.08 - 0.16)
PSA density≥ 0.15 ng/mL/mL, n (%)	489	164 (33.5)
PSA doubling time, month(IQR)	320	38.81 (12.4 - 51)
Median PSA velocity, ng/mL/yr (IQR)	320	1.77 (0.4 - 1.9)
ISUP Grade Group at 1st biopsy series, n (%): GG 1 GG 2 GG3-5	156	 127 (81) 21 (14) 8 (5)
Diagnosis of PCa at baseline biopsies, n (%) csPCa-1 csPCa-2 non-csPCa (all definitions) benign (no PCa)		156 (31) 45 (9) 29 (6) 111 (22) 347 (69)
Metastatic stage at diagnosis, n (%)	503	1 (0.2%)

* Data were not available for all patients (missing data or only one pre-biopsy PSA results)
BMI = body mass index, PSA = prostate specific antigen, ISUP = International Society of Urological Pathology, csPCa = clinically significant prostate cancer. non-csPCa = non-clinically significant prostate cancer

Table 2 MRI and biopsy results during follow-up for 415 untreated cases with no PCa (n=347) at baseline or with non-csPCa on active surveillance (n=68)

Variable	**n**	
Median follow-up, month (IQR)	415	47.28 (19 - 85)
MRI during follow-up, n (%): For cases with no PCa at baseline, n (%) For cases with non-csPCa at baseline and untreated, n (%)	415 347 68	130 (31) 84 (24) 46 (68)
Cases referred for second biopsy series, n (%) **Diagnosis at second biopsy series**, n (%) csPCa-1, n (%) csPCa-2, n (%) non-csPCa, n (%) Benign, n (%)	415	98 (24) 43 (10) 21 (5) 7 (2) 33 (8) 55 (13)
Overall incidence of PCa during FU*, n (%) csPCa-1, n (%) csPCa-2, n (%) non-csPCa n (%) Benign, n (%)	503	189 (38) 66 (13) 36 (7) 123 (24) 314 (62)
Cumulative incidence of ≥GG3 PCa, n (%)	503	12 (2)
Metastatic stage, n (%)	503	1 (0,2)

* Data were not available for all patients (missing data or only one pre-biopsy PSA results)
BMI = body mass index, PSA = prostate specific antigen, ISUP = International Society of Urological Pathology, csPCa = clinically significant prostate cancer. non-csPCa = non-clinically significant prostate cancer

Table 3 Univariate and multivariate analyses of predictive factors for clinically significant prostate cancer

		Univariate analyses		Multivariate analyses	
Variable	N*	OR (CI-95%)	p	OR (CI-95%)	p
Age (year)	459	0.99 (0.95 - 1.04)	0.71		
BMI (kg/cm^2)	369	0.95 (0.87 - 1.04)	0.27		
BMI ≥30		0.79 (0.32 - 1.96)	0.61		
PCa family history	406	2.38 (1.10 - 6.16)	0.028	2.31 (1.03 - 5.21)	0.043
PSA (ng/mL)	457	1.03 (0.97 - 1.09)	0.4		
Prostate volume (mL)	459	0.99 (0.97 - 1.01)	0.072		
cT stage (≥cT2a)	457	3.32 (1.69 - 6.53)	< 0.001	2.43 (1.12 - 5.26)	0.025
PSA doubling time (month)	297	1.02 (0.98 - 1.06)**	0.29		
PSA velocity (ng/ml/yr)	297	1.00 (0.9 - 1.06)	0.87		
PSA density (ng/mL/mL)	445	1.06 (1.03 - 1.09)***	< 0.001	1.06 (1.03 - 1.10)***	<0.001
≥ 0.15 ng/ml/ml		2.43 (1.19 - 4.21)	0.012		
≥ 0.14 ng/ml/ml		3.14 (1.64 - 6.00)	< 0.001		
≥ 0.10 ng/ml/ml		3.2 (1.39 - 7.34)	0.006		
* Data were not available for all patients (missing data or only one pre-biopsy PSA results) **OR calculated for 10 units increase. *** OR calculated for 0.01 units increase.					

Supplementary data 1 Patient clinical characteristics and outcome for the 8 high risk csPCa cases at baseline

Patients	PSAd (ng/ml/ml)	Stage at DRE	PCa Family history	Grade Group at biopsy	Metastatic stage	Last status
1	0.05	**T2b**	**Yes**	5 (4+5)	Yes (Bones)	Deceased at 5 years
2	0.12	**T2b**	**Yes**	5 (3+5)	No	RP - 10 years remission
3	**0.48**	T1c	No	3 (4+3)	No	RP - 4 years remission
4	0.12	T1c	No	3 (4+3)	No	RP - 2 years remission
5	**0.23**	T2a	No	5 (3+5)	No	RP + RT - 1 year remission
6	0.10	T1c	No	5 (3+5)	No	RP - 6 years remission
7	0.09	T1c	Unknown	3 (4+3)	No	RP - 4 years remission
8	0.11	T1c	No	3 (4+3)	No	RP - 6 years remission

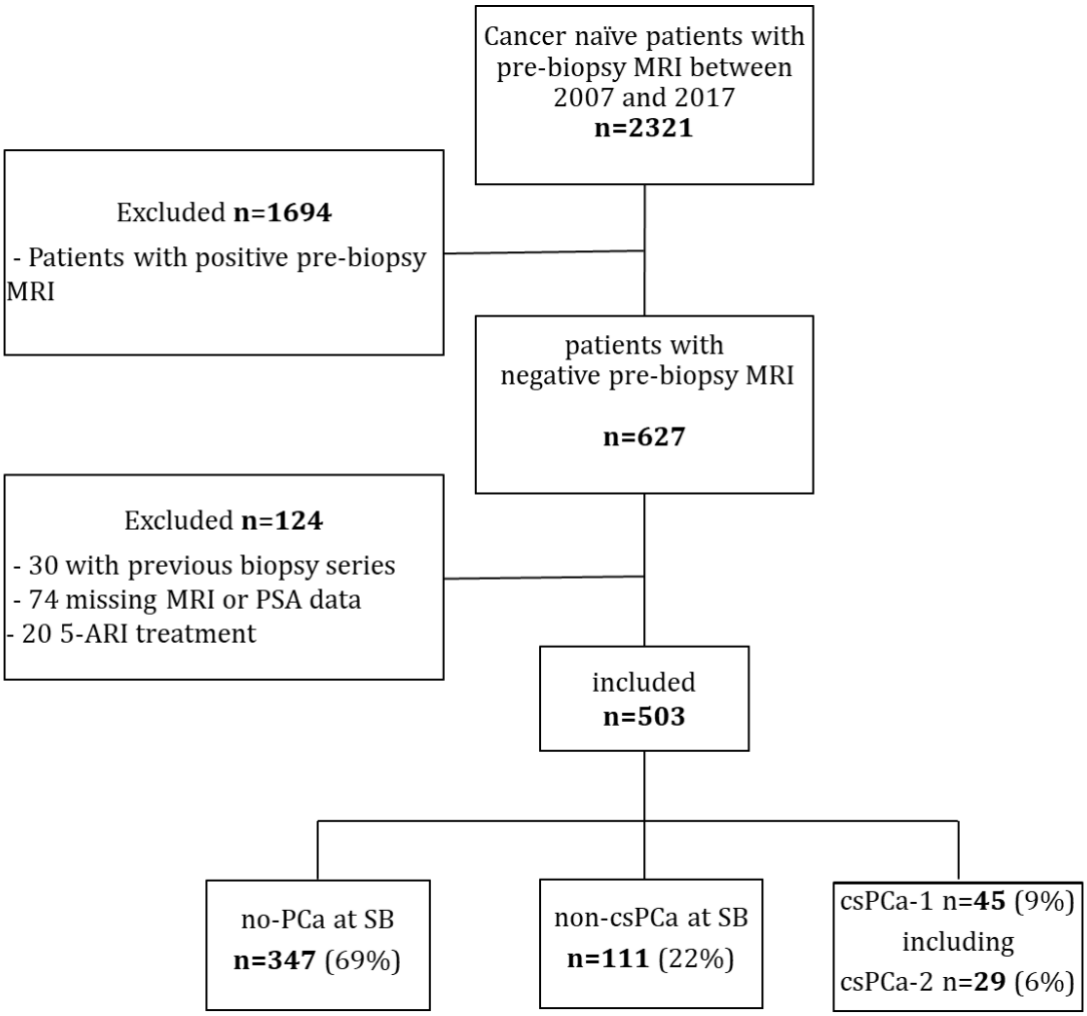
Characteristics in bold are the positive predictive factors available at baseline which might be used for biopsy indication
PSAd= PSA density, DRE = digital rectal examination, RP = radical prostatectomy, RT = radiotherapy

Supplementary data 3 Analyses of single and combined risk factors to predict risk of missing csPCa at baseline biopsy in case of not performing biopsy for the 503 patients with negative MRI

Predictive factors	Missed csPCa-1 (%)	Missed csPCa-2 (%)	Number of biopsy avoided (%)
nsMRI	45 (9.0)	29 (6)	503 (100)
nsMRI + ≥ 0.15 ng/ml/ml	23 (4.6)	14 (2.8)	325 (65)
nsMRI + ≥ 0.14 ng/ml/ml	16 (3.2)	11 (2.2)	306 (61)
nsMRI + ≥ 0.10 ng/ml/ml	9 (1.8)	6 (2.1)	178 (35)
nsMRI + ≥ 0.15 ng/ml/ml + DRE + PCa FH	12 (2.4)	8 (1.6)	281 (56)

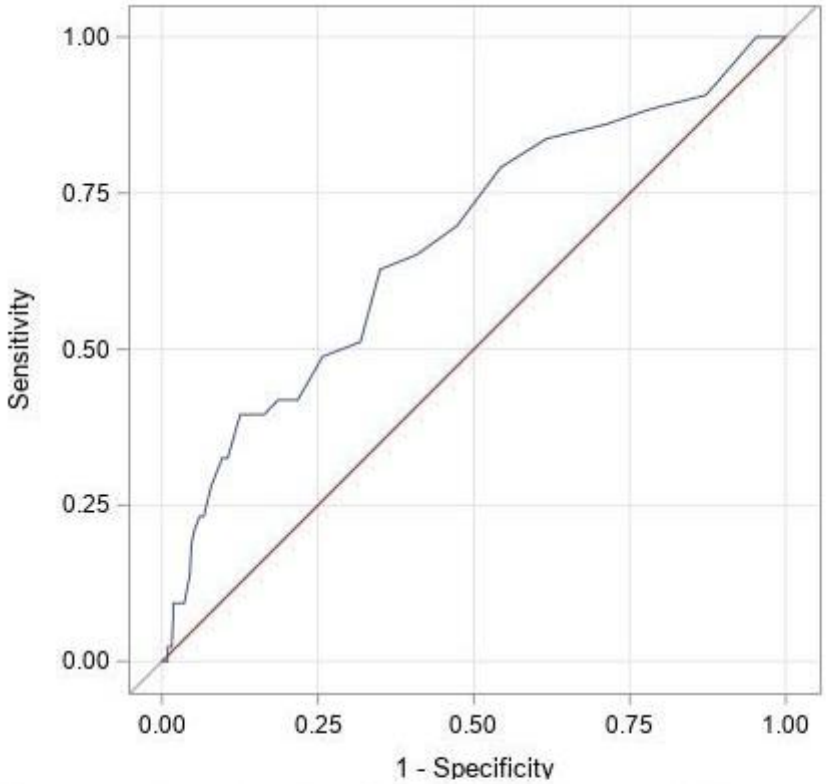
DRE = abnormal digital rectal examination, FH = family history

Figure 1 Patient inclusion and exclusion flowchart.



PCa = Prostate cancer; non-csPCa = non-clinically significant prostate cancer
csPCa = clinically significant prostate cancer; csPCa-1=GG1 with cancer core length >5mm or ≥3 positive SB in addition to GG≥2; csPCa-2 = any GG≥2

Supplementary data 2 Receiver operating characteristic (ROC) curve of PSA density for diagnosis of csPCa at baseline



PSA density (ng/ml/ml)	Sensitivity	Specificity	PPV	NPV
≥ *0.15 ng/ml/ml*	51.2	68.2	13.4	93.5
≥ *0.14 ng/ml/ml*	63.8	65.0	14.8	94.8
≥ *0.10 ng/ml/ml*	83.7	38.3	11.6	96.1

PPV = positive predictive value, NPV = negative predictive value, PSA = prostate specific antigen

Figure 2: Decision-curve analyses (DCA) demonstrating the net benefit associated with the use of different models predicting diagnosis of csPCa-1 in biopsy naïve negative MRI patients

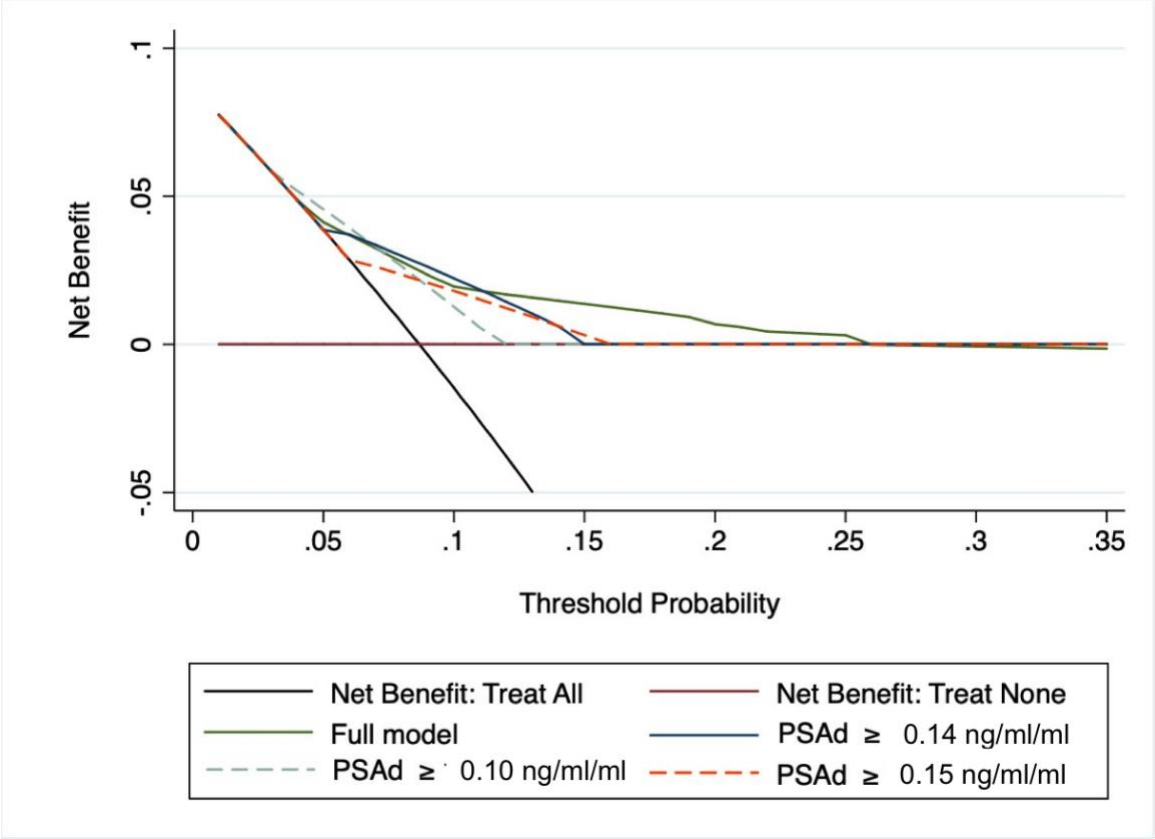
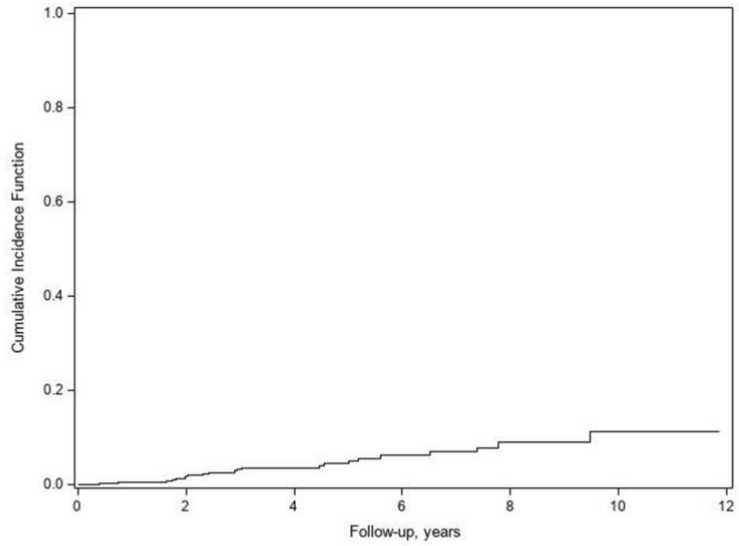


Figure 3 Cumulative incidence curve of csPCa-1 diagnosis during follow-up for 415 untreated patients after biopsy at baselin



Year of follow up	0	2	4	6	8	10
Patients	415	284	180	108	59	21
csPCa-1	0	7	12	17	20	21

csPCa = clinically significant prostate cancer

Discussion de la première partie

La première étude a permis de mettre en évidence que peu d'auteurs avaient étudiés des facteurs prédictifs de cancers de la prostate cliniquement significatifs en cas d'IRM non suspecte et que la densité du PSA était le facteur le plus étudié et le plus discriminant. La réalisation de biopsies en cas d'IRM non suspecte et de densité de PSA ≥ 0.15ng/ml/ml permet de réduire le taux de faux négatif (1-VPN) de l'IRM de 15.6% à 9.6% et d'améliorer la VPN de l'IRM. Cette méthodologie d'étude nous a limité aux facteurs prédictifs publiés et ne nous a pas permis d'étudier la combinaison de plusieurs facteurs prédictifs.

C'est pour cette raison que nous avons souhaité valider et améliorer ces résultats à partir d'une étude de cohorte rétrospective. Cette étude, incluant 503 patients avec une IRM non suspecte à l'inclusion et des biopsies systématisées a permis d'évaluer le taux de cancer cliniquement significatif au moment de l'inclusion et pendant un suivi médian de 4 ans puis d'étudier des facteurs prédictifs de cancer cliniquement significatifs. Nous avons conclu, en fonction de 2 définitions de cancer significatif que ce risque était entre 6 et 9% (VPN de l'IRM de 91%) au moment du diagnostic et de 4% supplémentaire lors du suivi. Ce risque initial diminuait de 9% à 2.4% en cas d'intégration de la densité de PSA du toucher rectal ou des antécédents familiaux à la décision de réaliser ou non une biopsie.

Ces résultats étaient en accord avec ceux de Norris et al. qui avait décrit les cancers significatifs non vus à l'IRM dans l'étude PROMIS (34). Entre 7% et 13% des patients avaient un cancer cliniquement significatif malgré une IRM non suspecte. Biopsier les patients avec une densité de PSA ≥ 0.15ng/ml/ml dans cette étude diminuait de 13% à 9% le taux de cancers cliniquement significatifs non diagnostiqués. Les caractéristiques de ces cancers manqués étaient des cancers de plus faible grade et taille que les cancers vus à l'IRM (cancers de score de Gleason 3+4 maximum) (34).

Ne pas réaliser de biopsies en cas d'IRM non suspecte est une décision qui doit être expliquée aux patients et acceptée. Cette décision impose un suivi des patients et la réalisation de nouvelles IRM en cas de cinétique suspecte du PSA. Le suivi de ces patients n'est pas encore consensuel et une attention particulière doit être portée chez ces patients. De nouvelles études permettront de clarifier le suivi de ces patients.

Ces résultats ont permis de proposer aux patients de notre service ayant une IRM non-suspecte, une densité du PSA < 0.15ng/ml/ml, un toucher rectal et des antécédents familiaux non suspect de ne pas réaliser de biopsies. Un suivi semestriel du PSA est en revanche réalisé.

En conclusion de la première partie, nos 2 études ont permis de prouver que l'IRM pouvait servir de test de triage entre le dépistage et les biopsies. Cela va permettre de diminuer le nombre de biopsies nécessaires après un test de dépistage suspect et ainsi de réduire le sur-diagnostic des cancers cliniquement non significatifs.

L’IRM non-suspecte et la surveillance active

Seconde partie – Article 3

Pour évaluer le statut de l’IRM suspect ou non-suspect à l’inclusion comme critère de sélection en plus des critères de cancers cliniquement non significatifs de faibles volume et grade, nous avons mené une étude rétrospective multicentrique, comparant 1035 patients ayant une IRM non suspecte et 1084 une IRM suspecte à l’inclusion grâce à la base de données multicentrique internationale GAP3-Movember. Notre étude “*mei*. La conclusion est que Suivi

Prostate cancer patients under active surveillance with a suspicious MRI are at increased risk of needing treatment: Results of The Movember Foundation's Global Action Plan Prostate Cancer Active Surveillance (GAP3) Consortium.

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[a] *The Movember Foundation's Global Action Plan Prostate Cancer Active Surveillance (GAP3) consortium members presented in Appendix A.*

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Abstract

Background: Inclusion criteria for active surveillance (AS) are low or intermediate risk prostate cancer. The predictive value of the presence of a suspicious lesion at MRI at the time of inclusion is insufficiently known.

Objective: To evaluate the percentage of patients needing active treatment stratified by presence or absence of suspicious lesion at baseline MRI.

Design, Setting, and Participants: Retrospective analysis of data from the multicentric AS GAP3 Consortium database. Inclusion criteria were men with grade groups (GG) 1 or GG2 prostate cancer combined with PSA<20 ng/ml. We selected a subgroup of patients who had MRI at baseline and for whom MRI results and targeted biopsies were used for AS eligibility. Suspicious MRI was defined as a MRI lesion PI-RADS/Likert $\geq$3 and for which targeted biopsies did not exclude the patient for AS.

Outcome Measurements and Statistical Analysis: Primary outcome was treatment free-survival (FS). Secondary outcomes were histological GG progression FS and continuation of AS (discontinuation FS).

Results and Limitation: The study cohort included 2119 patients (1035 non-suspicious MRI and 1084 suspicious MRI men) with a median follow up of 23 months (12-43). For the whole cohort, 3-years treatment-FS was 71% (95%CI: 69-74). For non-suspicious MRI and suspicious MRI groups, 3-years treatment-FS were 80% (95%CI: 77-83) and 63% (95%CI: 59-66). Active treatment (HR=2.0, p<0.001), grade progression (HR=1.9, p<0.001) and discontinuation of AS (HR=1.7, p<0.001) were significantly higher in suspicious MRI group than non-suspicious MRI.

Conclusion: The risk of switching to treatment, histological progression and AS discontinuation are higher in cases of suspicious MRI at inclusion.

Patient summary: In men with low or intermediate risk prostate cancer who choose active surveillance, those with suspicious MRI at time of inclusion in AS are more likely to show switch to treatment than men with a non-suspicious MRI.

Introduction:
Active surveillance (AS) decreases the harms of screening and over-detection of men with a low or intermediate risk of prostate cancer (PCa) progression. The main goal of AS is to avoid or delay the use of treatments without compromising patients' long-term survival (1).
Selection criteria were traditionally based on PSA<10ng/ml, T-stage≤T2a, and ISUP grade group (GG) 1 at standard transrectal biopsy systematic biopsy (SB) defining cancers at low risk (2). Standard transrectal biopsy is associated with misclassification due to underestimation of the tumour volume or GG at entry (1). Adding MRI as a selection tool decreases the risk of missing clinically significant disease before active surveillance is started. It was reported that 10% of men eligible for AS based on SB TRUS-guided biopsy are reclassified to a clinically significant PCa (CSPCa) by MRI and TB (3). In the ASIST study, use of MRI at entry or during the first year of AS resulted in significantly fewer rates of AS discontinuation (19% vs 35%) and progression at biopsy to GG≥ 2 cancer (9.9% vs 23%) after 2 years of follow-up (4). MRI and MRI-targeted biopsy for a suspicious MRI (PIRADS score 3-5), are now recommended in the EAU 2020 guidelines, in addition to standard biopsy for men on AS (5).
In the Movember multicentric international GAP3 database (6), we identified a subgroup of patients who had MRI at baseline, and for whom MRI results and targeted biopsies were used for AS eligibility. Importantly, these were patients who still met AS-eligibility criteria after their initial targeted biopsies. The aim of our study was to evaluate the percentage of patients needing active treatment stratified by presence or absence of suspicious lesion at baseline MRI. The primary outcome was treatment free-survival (FS). Secondary outcomes were histological GG progression FS and all men continuing active surveillance (discontinuation FS).

Patients and Methods

Study population
Between 2014 and 2016, the GAP3 database was created by combining patient data from established AS cohorts worldwide. Requirements for participation included an active registry of AS patients over the last 2 years or more, including at least 50 patients annually and ethical approval for sharing digital patient data in a centralized uniform, and consensus-based AS database (v3.3). To date, 25 cohorts from the USA, Canada, Asia, Australia, the United Kingdom, and Europe fulfilled the requirements for participation and joined the initiative, resulting in data for a total of 21,647 men on AS.
We retrospectively included from the GAP3 database all patients from 13 cohorts in 8 countries on AS who had MRI performed at “baseline” with its results documented **(Figure 1)**. The use of MRI and inclusion in the GAP3 database differed between cohorts. In this study, baseline MRI definition was an MRI performed in the 3 months before diagnosis or during the first year after inclusion. Some investigators performed MRI upfront at the time of the first diagnosis, and therefore cases reclassified to CSPCa not considered for AS were not included in the GAP3 database. Other investigators included in the GAP3 database patients selected for AS based on PSA, T-stage and ISUP GG based on systematic biopsies (SB), and performed an MRI during the first year of follow up. Some of these patients were reclassified to CSPCa at re-biopsy based on MRI-TB results and were therefore

excluded. Since baseline MRI results when performed within the 12 months after AS inclusion can lead to reclassification up to 6 months after MRI, the period range for reclassification was up to 18 months after diagnosis.

Exclusion criteria:
Baseline MRI performed earlier than 3 months before diagnosis or more than a year after diagnosis. PSA>20ng/ml, GG 3,4 or 5 at inclusion or patients who were reclassified within 18 months after diagnosis if baseline MRI was performed after inclusion. Also cohorts with less than 25 patients with an MRI at inclusion were excluded.

Definition of suspicious and non-suspicious MRI
Baseline MRI was considered as suspicious when the item *"Suspicious lesions found on MRI"* was filled with "Yes" or "Equivocal", and as non-suspicious when the column was filled with "No". A sub-analysis of patients (n=737) with available Likert scores for clinically significant disease (1, "highly unlikely"; 2, "unlikely"; 3, "indeterminate" or "equivocal"; 4, "likely"; and 5, "highly likely") or PI-RADS score (ref) was performed by stratifying patients into three groups with subsequently assessment scores 1-2 (low risk), score 3 (equivocal), and scores 4-5 (likely) for the likelihood of PCa.

Collected Data
Available data, as described previously (6), included: age, PSA at inclusion, PSA density, T-stage at DRE, number of biopsy cores with PCa, maximum % PCa in any core, Gleason grade group.
For MRI data, available data were suspicious lesion found on MRI, number and location of the lesion on MRI, Likert or PiRADS score (reported in 737 cases). The concordance between MRI and TB results was not available. We collected the AS discontinuation status and date, the cause of discontinuation, and the death status. With respect to reasons for discontinuation, the following information was available for most cohorts: 'Convert to watchful waiting', 'Clinical progression', 'Pathological progression', 'Clinical and Pathological progression', 'PSA progression (PSA-DT < 3 years)', 'Other PSA kinetics', 'Patient choice/Anxiety', 'Doctors Anxiety', 'Radiological progression', 'Died', 'Lost to FU', 'Other/Unknown' or 'Still on active surveillance'.

Study design
The primary outcome was active treatment FS, which was defined as undergoing radical prostatectomy, radiation therapy, brachytherapy, focal therapy, or androgen deprivation treatment. Censoring time was defined as the date of the last recorded clinical appointment or stopping active surveillance due to other reasons. Secondary outcomes were GG progression which was defined as upgrading at follow-up biopsy (GG>1 for GG1 at inclusion and GG>2 for GG2 at inclusion) or high grade progression defined as upgrading at follow-up biopsy >GG2 based on per-protocol or for cause biopsy and all causes AS discontinuation defined as progression, conversion to active treatment without evidence of progression, transition to watchful waiting, anxiety, non-PCa death and other/unknown (Supplementary Figure 1). A sub analysis of Gleason GG 1 patients was also performed.

Statistical analysis:
We used survival analysis to compare the risks of switching to active treatment, cancer grade progression, and AS discontinuation for patients in different MRI groups. The event in survival analysis is defined as switching to active treatment, cancer grade progression, or AS discontinuation. Firstly, we performed the Kaplan-Meier analysis and estimated the probabilities of survival for patients in suspicious and non-suspicious MRI groups. Subsequently, we fitted stratified Cox proportional hazard models (7), with stratified baseline survivals for different cohorts, to estimate pooled hazard ratios for the covariates of interest. Covariates include patients' baseline MRI and PSA density (calculated as PSA level divided by prostate volume). Analyses were performed in R version 3.6.1.

Results:

Cohort characteristics:
In total, 2119 patients were included from 13 cohorts (Table1). Our study cohort contained 1035 men with a non-suspicious MRI and 1084 with a suspicious MRI, 1875 men (88%) had GG1 at baseline, whilst the remaining 244 (12%) had GG2 cancer. The median age at diagnosis was 64 years (IQR: 59-69), median PSA was 5.3ng/ml (3.8-7.3) and 65% of men had a non-palpable tumour. Patients and tumour characteristics were comparable between non-suspicious MRI and suspicious MRI except for tumour visibility at MRI. Patients' characteristics are summarized in Table 2. Median follow up for the cohort was 23 months (IQR: 12-43).

Clinical events:
Whole cohort
For the whole cohort, treatment FS, biopsy upgrading FS and AS discontinuation FS were 71% (95% CI: 69-74), 84% (95%CI: 82-86) and 67% (95%CI: 65-71) at 3-years respectively.
An overview of clinical outcomes is summarized in Table 3. Types of active treatment, number with histological progression at biopsy and cumulative reasons for AS discontinuation during follow-up in both MRI groups for the whole series are shown in Table 4 and supplementary Figure1. No cancer specific deaths were reported. Results per centre are described in Supplementary tables1 & 2. At 3 and 5-years treatment FS was 80% (95% CI: 77-83), 70% (95%CI: 66-74) for non-suspicious MRI and 63% (95%CI: 59-66), 49% (95%CI: 44-54) for suspicious MRI patients respectively (Figure 2A). Switch to treatment was significantly higher in suspicious MRI men than in non-suspicious MRI men (HR=2.00,p<0.001). In total, 392 men had histological progression at biopsy during follow-up including 101 (5%) patients who had a high-grade progression >GG2 (Table 4). The 3-yr and 5-yr histological progression FS rate were 89% (95%CI: 86-91), 81% (95%CI: 77-85) in non-suspicious MRI and 79% (95%CI: 76-82), 70% (95%CI: 65-75) in suspicious MRI men, respectively (Table 3). Histological progression at biopsy was significantly higher in suspicious MRI than in non-suspicious MRI men (HR=1.88, p<0.001). High grade histological progression FS rate were at 3 years 97% (95%CI: 95-98) in suspicious MRI men and 93% (95%CI: 91-95) in suspicious MRI men. Histological progression at biopsy to a higher grade (GG>2) was significantly higher in suspicious MRI than in non-suspicious MRI men (HR=2.85, p<0.001). At 3 and 5-

years, all causes AS discontinuation FS were 76%(95%CI:73-79), 63%(95%CI:59-67) for non-suspicious MRI and 58%(95%CI:54-62), 42%(95%CI:38-47) for suspicious MRI patients respectively (Figure 2B). Causes of discontinuation in the two groups are summarized in Table 4. AS discontinuation was significantly higher in -suspicious MRI than in non-suspicious MRI men (HR=1.77, p<0.001).

Sub-cohort of GG1 only
The 3-yr and 5-yr treatment FS were 82% (95%CI: 79-85), 72% (95%CI: 68-77) for those with non-suspicious MRI and 66% (95%CI: 62-70), 52% (95%CI: 47-57) for those with suspicious MRI respectively. Switch to treatment was significantly higher in the suspicious MRI group (HR=1.93,p<0.001). The 3-yr and 5-yr histological progression FS rate were 89% (95%CI:86-91), 80% (95%CI:76-84) for those with non-suspicious MRI and 78% (95%CI:74-81), 69% (95%CI:64-74) for those with suspicious MRI respectively. Histological progression at biopsy was significantly higher in the suspicious MRI group (HR=1.97,p<0.001) (Figure2C). The 3-yr and 5-yr AS discontinuation FS rate were 78% (95%CI:75-81), 66% (95%CI:61-70) for patients with non-suspicious MRI and 62% (95%CI:58-66), 47% (95%CI:42-52) for patients with suspicious MRI at inclusion respectively. AS discontinuation was significantly higher in the suspicious MRI group (HR=1.67, p<0.001).

Sub-analysis of Likert/PIRADS data
At 3-years and 5 years treatment FS were at 3-years and 5 years 83% (95%CI:78-89), 74% (66-82) for those with Likert/PIRADS score 1-2, 70% (95%CI:63-77), 59%(95%CI:50-69) for those with score 3 and 55%(95%CI:50-61), 42%(35-49), for those with score 4-5 respectively. Switch to treatment was significantly lower in the score 1-2 vs score 3 vs score 4-5 (HR=2.12,p<0.001), (HR=4.18,p<0.001). The three years histological progression FS rate were 83%(95%CI: 78-89), 71% (95%CI: 75-88) and 65% (95%CI:59-71) for those with Likert/PIRADS score 1-2, score 3 and score 4-5 respectively. There was no significant difference for histological progression between patients with a score 1-2 at MRI vs score 3, but there was significantly more histological progression for score 4-5 (HR=2.05, p<0.001). The 3-yr AS discontinuation FS rate were 82% (95%CI:76-87), 67% (95%CI:41-75) and 52% (95%CI:47-59) for patients with an MRI score 1-2, score 3 and score 4-5 at inclusion. AS discontinuation was significantly lower in the score 1-2 vs score 3 vs score 4-5 (HR=1.92,p<0.001), (HR=3.75,p<0.001).

Discussion:

We need AS outcomes from international multicentric cohorts. One of the main outcomes is the length of time we can defer treatment and have the patient on AS. In the whole GAP3 MRI cohort, 3 years and 5 years treatment FS were 71% and 60% respectively. These outcomes can be shared with patients at the time of treatment decision.

AS eligibility criteria are of importance for this risk of switch to treatment. MRI has proven to increase staging and grading and as such resulted in a decrease of 10% of reclassification rate within the first year on AS (3). Some patients with a suspicious MRI are still eligible for AS. Our work was aimed to compare the risk of switching from active surveillance to active treatment depending on MRI risk category at baseline. We showed that the risk to switch to treatment, the risk of histological

progression and the risk of AS discontinuation are lower if the MRI at the time of inclusion is non-suspicious.

These results confirm that was already reported in monocentric studies (8-10). Stavrinides et al. showed that event FS (defined as prostate cancer treatment, transition to watchful waiting, or death) and treatment FS were lower in patients with MRI-visible (Likert 4-5) disease (9). In the Princess Margaret Cancer Centre cohort, it was reported that 51% of men with suspicious baseline MRI received definitive treatment within 5 years, compared to 27% and 21% of men with equivocal and negative MRI, respectively (11). This is in agreement with our results, where the estimated treatment FS rates at 3-years are 70% for non-suspicious MRI and 49% for suspicious MRI group. In the Lille and Cambridge cohorts who received MRI at inclusion (12,13), histological progression FS at a median follow-up of 36 and 39 months were close to the results of 89% and 81% for non-suspicious MRI and 79% and 70% for suspicious MRI at 3 years and 5 years in the GAP3 cohort. Mamawala et al. showed that the 2- and 4-year upgrade FS rates were significantly lower for the negative MRI group (93% and 83%) than for the positive MRI group (74% and 59%) (8). These rates are close to the 27.5% progression in the whole Movember cohort at 5 years (14).

Our results show that men with a suspicious MRI are more likely to receive definitive treatment, more likely to have an upgrading on follow-up biopsy and more likely to discontinue AS, compared to men with a negative MRI.
The potential impact of these findings is that a suspicious MRI does not necessarily exclude a patient from AS since a substantial number of men with a suspicious MRI did not progress on AS but clearly suggests that those with a suspicious MRI may have to be followed more closely than a patient with a non-suspicious MRI. It questions also the accuracy of targeted biopsy to sample an MRI lesion and eventually reclassify PCa before inclusion.

These rates may reflect in part tumours with rapid growth and in part tumours that were missed by the diagnostic tests used for selection criteria. Hence, if MRI accuracy is high to eliminate significant tumours, its NPV goes from 75% to 95%, explaining that there are still some significant tumours that are missed at entry. Progression happens over time when an initial non-significant PCa progresses or when a new significant lesion grows. Inoue et al. modelled that the probability of true grade progression ranges from 1.2% to 2.4% per year of AS (15). Theoretically, 5 to 10% of CS-PCa are missed at entry and progression ranges from 1.2% to 2.4% per year of AS which means that reclassification/progression should range from 8.6% to 17.2% at 3 years and from 11% to 22% at 5 years. Our results showing a 70% (66-74) 5-yr treatment FS for non-suspicious MRI patients are concordant with this model. It is important to note that only 2119 patients out of 21,643 patients (10%) of the world largest AS cohort had a baseline MRI. MRI was not routinely used when GAP3 started but has since become an increasingly utilized diagnostic tool.

There are some limitations that need to be considered in this study. The GAP3 database is purely a retrospective database resulting in a limited control over data collection and lack of availability of some data of interest. Another limitation was the impossibility from the database to cross the MRI and TB data. In consequence, the definition of positivity and negativity of the MRI were purely at imaging and not

confirmed by TB data in the database. However, all investigators said that in case of suspicious MRI, TB were performed and results taken into account to exclude patients if at high risk. Also, outcomes for primary and secondary endpoints differ between cohorts. Criteria for inclusion differ and this was reported for our GAP3 consortium (1). Likelihood of a suspicious MRI prompting treatment is also likely to differ between cohorts, as does the likelihood of men being offered, or choosing AS for GG 2 disease. The time of follow-up was limited and so, mid to long-term differences in oncologic outcomes could not be evaluated. Heterogeneity of AS protocol and the heterogeneity of included patients as described in previous papers may include a selection bias but the prevalence of outcomes reported in our study are, therefore, likely more representative for the average prostate cancer population (16).

Conclusion:
The risk of switching to treatment, histological progression and AS discontinuation are higher in cases of suspicious MRI at inclusion. This information should be shared with the patients at inclusion.

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Table 1: Patients and AS characteristics for the 13 GAP 3 selected cohorts (n=2119)

Cohorts	Number of patients	Median age (IQR)	Median follow up (IQR)	Suspicious MRI n= (%)	Switch to active treatment n= (%)	Biopsy progression n= (%)	AS discontinuation all causes n = (%)
Atlanta	53	62 (56-69)	13 (10-25)	46 (87)	13 (25)	1 (2)	18 (34)
Bordeaux	166	65 (60-68)	27 (11-47)	93 (56)	49 (30)	6 (4)	50 (30)
Helsinki	42	66 (61-72)	36 (23-43)	27 (64)	16 (38)	1 (2)	18 (43)
Hopkins	216	66 (62-69)	19 (13-30)	175 (81)	57 (26)	12 (6)	80 (37)
Lille	227	65 (60-69)	29 (17-51)	127 (56)	58 (26)	12 (5)	66 (29)
London-UCL	303	62 (57-67)	26 (3-51)	138 (46)	90 (30)	10 (3)	108 (36)
Melbourne	73	65 (58-69)	14 (0-27)	65 (89)	17 (23)	0 (0)	24 (33)
MUSIC	305	64 (59-69)	12 (6-16)	158 (52)	46 (15)	8 (3)	54 (18)
PRIAS	225	64 (59-69)	21 (13-31)	72 (32)	32 (14)	8 (4)	45 (20)
Singapore	48	66 (61-70)	17 (13-34)	5 (10)	28 (58)	3 (6)	29 (60)
Sydney	104	59 (53-66)	48 (38-64)	44 (42)	35 (34)	10 (10)	40 (38)
UCSF	194	62 (57-67)	47 (26-75)	67 (35)	45 (23)	21 (11)	51 (26)
Valencia	163	65 (60-70)	38 (14-55)	67 (41)	67 (41)	8 (5)	75 (46)

Table 2: AS patients' characteristics at entry (n=2119)

Baseline Characteristics	Non suspicious MRI	Suspicious MRI	Overall Patients
Number of patients	1035	1084	2119
Age at diagnosis, year, median (IQR)	63 (58-68)	65 (59-69)	64 (59-69)
Follow-up months, median (IQR)	27 (13-51)	18 (11-38)	23 (12-43)
PSA, ng/mL, median (IQR)	5.3 (3.7-7.3)	5.4 (3.8-7.2)	5.3 (3.8-7.3)
PSA density, ng/mL^2, median (IQR)	0.11 (0.07-0.16)	0.11 (0.07-0.16)	0.11 (0.07-0.16)
Number of biopsy cores with prostate cancer, median (IQR)	1 (1-2)	2 (1-3)	1 (1-2)
Maximum percentage of cancer in any core, %, median (IQR)	10 (5-20)	14 (5-29.3)	10 (5-24.89)
T-Stage at DRE, number (%):			
T1	730 (71)	650 (60)	1380 (65)
T2	97 (9)	107 (10)	204 (10)
TX	208 (20)	327 (30)	535 (25)
Grade Group 2, n (%)	110 (11)	134 (12)	244 (12)

Table 3: Cumulative incidence of switch to active treatment, histological progression at biopsy and AS discontinuation (all causes) during follow-up % (95%CI) for the whole series of 2119 patients

		Number at risk	All-time HR (p-value)	3 years % (95%CI)	HR (p-value)	5 years % (95%CI)	HR (p-value)
Switch to active treatment for all GG 1 and 2	All	2119		29 (26-31)		40 (37-43)	
	Non-susp MRI	1035		20 (17-23)		30 (26-34)	
	Susp MRI	1084	2.00 (<0.001)	37 (34-41)	1.72 (<0.001)	51 (46-56)	1.86 (<0.001)
	Likert1-2	185		17 (11-23)		26 (18-34)	
	Equivocal	208	2.12 (<0.001)	31 (24-37)	1.56 (<0.05)	42 (32-50)	1.80 (<0.01)
	Likert 4-5	344	4.18 (<0.001)	45 (39-51)	2.79 (<0.001)	58 (50-64)	3.75 (<0.001)
Switch to active treatment for GG 1	All	1875		26 (23-28)		37 (34-40)	
	Non-susp MRI	925		18 (15-21)		28 (24-32)	
	Susp MRI	950	1.90 (<0.001)	34 (30-38)	1.64 (<0.001)	47 (42-52)	1.76 (<0.001)
AS discontinuation for all GG 1 and 2	All	2119		32 (30-35)		46 (43-49)	
	Non-susp MRI	1035		23 (20-26)		37 (32-41)	
	Susp MRI	1084	1.77 (<0.001)	41 (37-45)	1.67 (<0.001)	56 (51-61)	1.67 (<0.001)
	Likert1-2	185		18 (12-24)		34 (25-43)	
	Equivocal	208	1.92 (<0.001)	33 (25-39)	1.56 (<0.05)	45 (36-54)	1.58 (<0.05)
	Likert 4-5	344	3.75 (<0.001)	48 (41-53)	2.87 (<0.001)	60 (53-67)	3.29 (<0.001)
AS discontinuation for GG 1	All	1875		30 (27-32)		43 (40-47)	
	Non-susp MRI	925		22 (19-25)		34 (30-39)	
	Susp MRI	950	1.67 (<0.001)	38 (34-42)	1.58 (<0.001)	53 (48-58)	1.57 (<0.001)
Biopsy upgrading for all GG 1 and 2	All	2119		16 (14-18)		24 (21-27)	
	Non-susp MRI	1035		11 (9-14)		19 (15-23)	
	Susp MRI	1084	1.88 (<0.001)	21 (18-24)	1.69 (<0.001)	30 (25-35)	1.69 (<0.001)
	Likert1-2	185		17 (11-22)		27 (18-35)	
	Equivocal	208		19 (12-25)		27 (18-36)	
	Likert 4-5	344	2.05 (<0.001)	35 (29-41)	1.34 (<0.05)	48 (39-55)	1.72 (<0.01)
Biopsy upgrading for GG 1	All	1875		17 (14-19)		25 (22-28)	
	Non-susp MRI	925		11 (9-14)		20 (16-24)	
	Susp MRI	950	1.97 (<0.001)	22 (19-26)	1.80 (<0.001)	31 (26-36)	1.72 (<0.001)

Non-susp MRI : Non-suspicious MRI ; Susp MRI : Suspicious MRI ; HR : Hazard ratio vs. 'Non-susp MRI' or 'Likert 1-2

Table 4: Types of active treatment, number of histological progression at biopsy and reasons for AS discontinuation during follow-up in both MRI groups for the whole series of 2119 patients

		Non-suspicious MRI	**Suspicious MRI**	**All**
Types of treatment*	Radical prostatectomy	126	208	334
	Radiation-therapy	30	54	84
	Brachytherapy	20	41	61
	Focal therapy	37	42	79
	ADT/others	18	26	44
Histological progression at biopsy	GG1 to > GG1	125	165	290
	GG1 and GG2 to > GG2	37	63	100
Reasons for discontinuation n=1624	Pathological progression	96	136	232
	Clinical progression	21	47	68
	Clinical and Pathological progression	12	27	39
	Radiological progression	14	10	24
	Radiological and pathological progression	5	2	7
	PSA progression (PSA-DT < 3 years	5	16	21
	Other PSA kinetics (PSA V > 0.5 ng/ml)	4	5	9
	Patient choice/Anxiety	23	39	62
	Physician Anxiety	7	0	7
	Death from other cause	9	8	17
	Lost to FU	17	13	30
	Convert to WW	8	4	12
	Other/Unknown	506	590	1096

*Some patients had multiple treatments

Figure 1: Flowchart

Sur71%

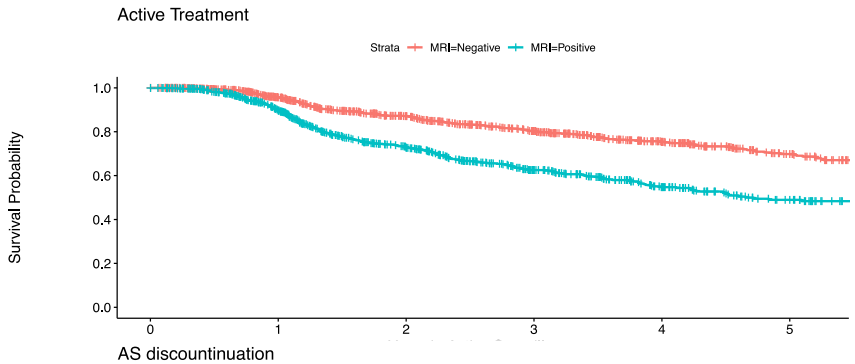
Figure 2:

A: Active treatment free-survival curves for GG1+GG2 patients according to MRI groups.

B: AS discontinuation free survival curves for GG1+GG2 patients according to MRI groups

C: Histological GG progression free-survival curves for GG1+GG2 patients according to MRI groups

A



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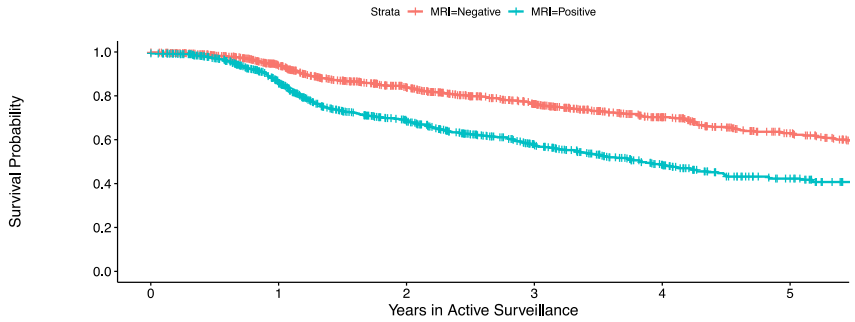
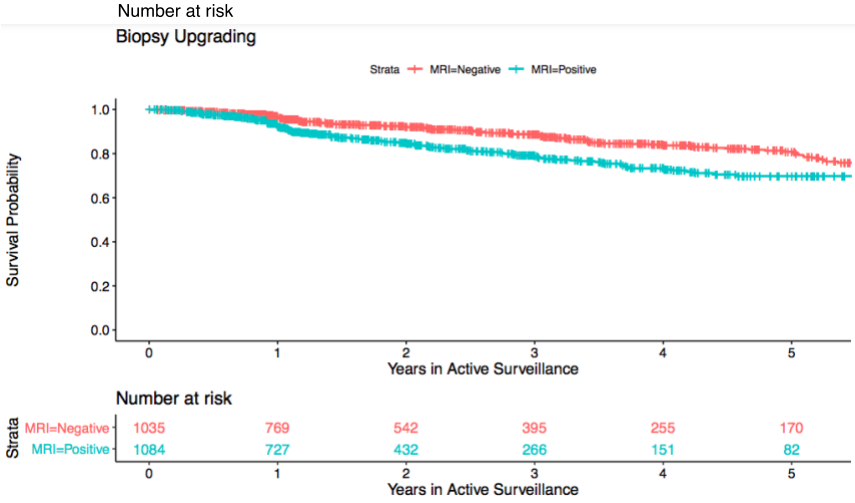


C



Seconde partie – Article 4

Nous avons aussi évalué les examens de suivi des patients en surveillance active avec IRM non suspecte et plus particulièrement la réalisation de biopsies de confirmation à un an. En effet le risque de progression en l'absence de cancer significatif à l'inclusion est très faible lors des premières années de suivi et les biopsies de confirmation ne montrent pas de progression dans 85% des cas. Notre . Une étude prospective incluant 2 cohortes successives de 78 et 71 patients avec et sans biopsies de confirmation à un an a comparé les taux de diagnostic de progression tumorale à 2 ans et le rôle de la cinétique du PSA pour cette prédire cette progression. Notre étude "*Low-risk prostate cancer selected for active surveillance with negative MRI at entry: can repeat biopsies at 1 year be avoided? A pilot study* " a été publiée dans la revue World Journal of Urology en Juillet 2018. La conclusion était qu'il n'y avait pas de différence de diagnostic de progression entre les 2 groupes et que la cinétique du PSA suspecte à elle seule pouvait faire indiquer une biopsie. Cette étude nous a permis de proposer de ne plus réaliser ces biopsies de confirmation en cas d'IRM non suspecte au diagnostic.

Low-risk prostate cancer selected for active surveillance with negative MRI at entry: can repeat biopsies at one year be avoided? A pilot study.

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Purpose: In patients considered for active surveillance (AS), the use of MRI and targeted biopsies (TB) at entry challenges the approach of routine "per protocol" repeat systematic biopsies (SB) at one year. This pilot study aimed to assess whether an approach of performing repeat biopsies only if PSA kinetics are abnormal would be safe and sufficient to detect progression.

Methods: Prospective single-centre study of 149 patients on AS with low risk PCa, a negative MRI at entry and followed for a minimum of 12months between 01/2007 and 12/2015. Group1 (n=78) patients had per-protocol 12-mo repeat SB; group2 (n=71) patients did not. Surveillance tests for tumour progression were for both groups: for cause SB and MRI-TB biopsies if PSA velocity (PSA-V)>0.75ng/ml/year, or PSA doubling time (PSADT)<3years. The main objectives are to compare the 2-year rates of tumour progression and AS discontinuation between groups. The secondary objectives are to estimate the diagnostic power of PSA-V and PSA-DT, to predict the risk of tumour progression.

Results: Overall 21 out of 149 patients (14.1%) showed tumour progression, 17.1% for group1 and 12.3% for group2 and 31(21.2%) discontinued AS at 2 years. There was no difference between the 2 groups (p=0.56). The area under the PSA-V and PSADT curves to predict tumour progression was 0.92 and 0.83, respectively.

Conclusions: We did not find any significant difference for progression and AS discontinuation rate between the 2 groups. The PSA kinetic seems accurate as a marker of tumour progression. These results support the conduct of a multi-centre prospective trial to confirm these findings.

Introduction

Active surveillance(AS) reduces the harms of screening and overtreatment of men with a low risk of prostate cancer (PCa) progression(1). This therapeutic strategy concerns about 20% of newly diagnosed PCa. It aims to avoid or delay the use of curative treatments without compromising the long-term survival of patients(2).
Many prospective cohorts have validated AS, but there is no consensus on inclusion or follow-up criteria(3). Selection criteria based on "blind" 12 systematic biopsies(SB) of the posterior part of the prostate are associated with a reclassification rate of 20 to 30% at one year using per protocol" repeat SB. However when selection criteria are based on MRI, targeted biopsies(TB) and the addition of SB, 10% of patients eligible for AS are reclassified at entry, before inclusion(4) and the reclassification/progression rate is only 16% at 2 years(5).
The use of MRI and TB at entry questions the need for routine "per protocol" repeat SB at one year for only 16% of patients. “Per protocol" repeat SB are associated with complications including severe urinary tract infection, sepsis and poor acceptability by patients(6).
To replace "per protocol" repeat SB, it has been suggested to use PSA kinetics(PSAk) followed by “for cause only" repeat SB if the PSA kinetics were abnormal(7,8).
A high PSA velocity (PSA-V) or short PSA doubling time (PSA-DT) are related with an unfavourable outcome and should lead to performing an additional prostate biopsy or to deferred radical treatment during follow-up of men on AS(9). Cooperberg recently validated the role of PSAk as an independent predictor of reclassification/progression in a AS multicentre cohort with PSA data collected at protocol-mandated intervals, relatively long follow-up, and centralized analysis(10).
The question is to assess the value of "per protocol" repeat SB at one year to diagnose progression. One way to answer is to rely only on for cause biopsies, skipping "per protocol" repeat biopsies. However this strategy may be associated with a risk of missing progression for some patients. Our study was designed as a pilot study to look at progression rate in 2 groups (with and without "per protocol" repeat SB) and to assess whether the rate of progression is at least non-inferior in the group without repeat biopsy. If the rate is non-inferior within at least 2 years of follow-up, results may be used as a rational for a cohort or randomised prospective study.
In our series of patients on AS, with baseline MRI and TB assessment and during surveillance we studied 2 consecutive groups with and without "per protocol" repeat 12-mo SB. The main objectives were to compare the 2-year rates of tumour reclassification/progression and AS discontinuation between groups. Secondary objectives were to estimate the diagnostic power of PSA-V and PSA-DT, to predict the risk of tumour reclassification/progression.

Methods

Study design and population. Prospective cohort study enrolling men on AS at Lille University Hospital (data base protection authorization obtained and patients' consents collected). All consecutive patients between January 2007 and December 2015, who accepted AS were enrolled.

Inclusion criteria were life expectancy>10 years, low risk PCa as determined by trans-rectal ultrasound 12 SB with number of positive biopsies ≤3, Gleason score 6(3 + 3) and maximum cancer core length (MCCL) ≤ 5mm, clinical stage ≤cT2, no abnormality or false positive abnormality for significant cancer at MRI. Mp-MRI were performed as previously described (4) using a 1.5 Tesla system with a pelvic phased array coil and interpreted by a uroradiologist with 15 years of experience in MRI prostate reading. No abnormality on MRI was defined as Likert score $\leq$ 2 and abnormal MRI suspicious for malignancy as Likert score $\geq$3. In case of abnormality at MRI suspicious for cancer, negative targeted biopsies were defined as a false positive abnormality. Exclusion criteria were history of 5a-reductase inhibitor(5-ARI) use, absence of pre-biopsy MRI and less than one year of follow-up. Out of 233 patients enrolled on AS, 149 met the inclusion criteria.

Data collection. Clinical data including age and Charlson score, PSA at baseline and every 6-mo, PSA density, PSA kinetics(PSA-V and PSADT) calculated with the MSKCC calculator(https://www.mskcc.org/nomograms/prostate/psa_doubling_time) using at least three measurements over a period of at least 6 mo, pathological data including(number of positive SB, MCCL at baseline and for follow-up biopsies, Gleason score) and prostate volume at ultrasound.

AS follow-up protocol. Visit every year with DRE, PSA every 6-mo, per-protocol MRI and repeat SB at 12 mo for patients enrolled in years 2007-2012(group 1, n=78). Our AS cohort has matured with follow-up. Interim observation in 2012 showing low rate of progression at 12-mo (85%) with calculated PSA-V NPV>95% to predict progression, per-protocol 12-mo repeat SB were removed from AS protocol for patients included in years 2012-2015(group 2, n= 71). During the year 2012, 17/28 patients had 12-mo repeat SB and 11/28 did not. For both groups, other tests including for cause MRI and SB or TB biopsies were indicated for all patients in case of 2 consecutive PSA rises (PSAV > 0.75ng/ml). A for cause MRI plus biopsy were scheduled in addition to a third PSA dosage 3 months later. If PSA kinetics was still suspicious MRI and biopsies indication were confirmed and performed.

Definition of cancer reclassification or progression. Presence of any amount of Gleason grade 4, >3 positive biopsies, MCCL >5mm at 12 SB or MRI-TB with pathological progression criteria in case of newly abnormality at MRI, any pelvic or extra-pelvic metastasis or death due to prostate cancer were defined as reclassification (during first year of follow-up) or progression (after first year of follow-up). Starting from the year 2013 the AS criteria for reclassification or progression were modified and presence of Gleason score 7(3+4) in only 1 core and (MCCL) ≤ 3mm was accepted as non progression. The causes of AS interruption were tumour reclassification/progression, patient choice (anxiety) or physician choice leading to treatment despite the absence of progression), patient decision not to be followed (watchful waiting) and lost to follow up.

Radical prostatectomy assessment. For patients who underwent radical prostatectomy(RP) after AS pathological stage was recorded.

Statistical Analysis

Qualitative variables are expressed as count and percentage. Continuous variables are reported as median and interquartile range (IQR). Normality of continuous variable are checked graphically and by using Shapiro-Wilk test. Main patient's characteristics were compared between groups using Chi-square test for qualitative variables and Mann-Whitney-U test for continuous variables. The 2-year rates of tumour reclassification/progression and active surveillance discontinuation were estimated using Kaplan Meier curves and compared between groups using the log-rank test. To estimate the diagnostic power of PSA-V and PSA-DT, sensitivities, specificities, PPV, NPV, and areas under the curve (AUC) were calculated using patient reclassification/progression as a gold standard. PSA-V threshold values of >0.75ng/ml/year and >0.5ng/ml/year were used (NCCN). PSA-DT threshold value of <3 years was used)(9). All statistical tests were performed at the 2-tailed α level of 0.05. Data were analysed using SAS version 9.4[SAS Institute Inc., Cary, NC 27513, USA].

RESULTS

Population

The clinical, pathological, biological and imaging data for both groups of patients at inclusion are shown in Table I. Patients of group 2 had a shorter follow up (42.5 months (IQR, 26 to 70) vs.32 (IQR, 23 to 49) (p=0.034). No other significant difference was found between the 2 groups.

Follow-up

Of the 78 patients of group 1, 36(46.2%) had negative repeat SB, 33(42.3%) had positive repeat SB but /progression criteria and 9(11.5%) had positive repeat SB with reclassification/progression criteria. Four patients had positive "for cause" biopsies during the first 2 years. For these 13patients, reclassification/progression at 2 years was due to a Gleason grade progression in 4, a size>5mm or >3 positive biopsies in 4 and a progression of size and grade in 5 (Table 2). Overall, 19 patients (24.4%) discontinued AS at 2 years, 13(17.1%) for cancer reclassification/progression, 2(2.8%) for patient choice of treatment (1 negative SB, 1 positive RB without reclassification/progression), 3(3.8%) for physician choice (all 3 had positive SB without reclassification/progression), and 1(1.5%) lost to follow-up. After 2 years, 12 additional patients discontinued AS (7 for tumour progression, 2 for patient choice, 1 for physician choice and 2 lost to FU).

Of the 71 patients of group 2, 10(14.1%) had for cause biopsies during the first 2 years of AS. Two had positive repeat SB but without reclassification/progression criteria and 8 had positive repeat SB with reclassification/progression criteria. For these 8 (12.3%) patients, reclassification/progression at 2 years was due to a Gleason grade progression in 4, a size>5mm or >3 positive biopsies in 3 and a progression of size and grade in 1 (Table 2). Two (3%) patients discontinued for patient choice (with no for cause biopsy indication during their FU) and 2(3%) were lost to follow-up. After 2 years, 2 additional patients discontinued AS 2(3.0%) for cancer reclassification/progression.

Overall, of the 149 patients on AS, 21(14.1%) men were reclassified or progressed at 2 years: 17.1%(95% CI, 10.3% - 27.7%) in group 1 and 12.3%(95% CI, 6.3% - 23.1%) in group 2. There was no statistically significant difference between the 2 groups at 2 years (p=0.56)(Figure 1a) or for the entire follow up period (p=0.19).

At 2 years of FU, 31 patients (21.2% 95%CI,15.4%-28.8%) stopped AS. Nineteen patients in group 1 (24.4%; 95%CI,16.3%-35.5%) and 12 patients in group 2 (17.7%; 95%CI, 10.5%-29.2%) (Figure 1b).

Of these 31 patients, 21 were referred for RP, 2 for radiation therapy, 2 for brachytherapy, 2 for HIFU hemi-ablation, 2 for watchful waiting and 2 were lost to follow-up. No patient had diagnosis of metastasis or death from any cause.

Radical prostatectomy assessment

For 15 patients in group 1, PT specimens showed in 4 (27%) a Gleason score of 6(3+3), in 5 (33%) a Gleason score of 7(3+4) and in 4 (27%) a Gleason score>7(3+5 or 4+4). The pathological stages were pT2 in 10(67%), pT3a in 3(20%). Data are missing for 2 patients (13%). For 6 patients in group 2, PT specimens showed in all of them a Gleason score of 7(3+4). The pathological stages were pT2(83%) in 5 and 1 pT3a(17%) in 1. All patients for both groups were N0 and M0 at imaging. One patient had a detectable PSA during follow-up and had salvage radiation therapy (anteriorly located cancer with stage pT3a, R1).

PSA-V and PSADT and prediction of reclassification/progression

PSA-V. PSA-V>0.75ng/ml/year in the first 2 years, was associated with tumour reclassification during repeat SB in 12/18(66.7%) of cases. Sensitivity of PSA-V for the detection of tumour reclassification was 92% and specificity was 91%. The PPV and NPV of this test were 67% and 98% respectively. The AUC of the PSA-V diagnostic test>0.75ng/ml/year was 0.92 (95% CI, 0.83 - 1.00]). The accuracy of PSA-V for tumour progression is shown in Figure 2a. For PSA-V threshold value of >0.5ng/ml/year sensitivity, specificity, PPV, NPV were respectively: 92%, 86%, 57% and 98%

In case of PSA-V >1 ng/ml/year sensitivity, specificity, PPV, NPV were respectively: 92.3%, 93.8%, 75% and 98.4%.

PS-DT. PSADT<3 years in the first 2 years, was associated with tumour reclassification in 81.8% of cases (9/11). The sensitivity of PSADT for detection of tumour progression was 69% and its specificity was 97%. The VPP and VPN for this test were 82% and 94%, respectively. The AUC for PSADT diagnostic test <3 years was 0.83 (95%CI, 0.70-0.96). The accuracy of PSADT for tumour progression is shown in Figure 2b.

DISCUSSION

In this retrospective pilot study, we evaluated the approach of "per protocol" RB at one year for patients on AS with negative MRI at entry. We did not find any significant difference for reclassification/progression rate between 2 groups in which the only difference for follow up was the use of "per protocol" RB at one year. We also did not demonstrate difference for AS interruption rate at 2 years. This is the first study to report reclassification/progression rates without per-protocol RB at 12-mo. Our results are encouraging since they open the door to obviate unnecessary biopsies, without hampering the possibility of diagnosis to tumour progression to a clinically significant stage.

Our reclassification/progression rate of 14.1% is similar to the 16 % rate at a median follow up of 39 months observed in the Cambridge cohort who receive MRI at inclusion (5). These rates reflect in part tumours with rapid growth and in part tumours that were missed by the diagnostic tests used for selection criteria. Hence, if MRI accuracy is high to eliminate significant tumours, its NPV was ranges from 63% to 98% (11,12), explaining that there are still some significant tumours that are missed at entry. In some of our cases, MRI during surveillance showed a new abnormality suspicious for cancer (confirmed by TB), and which was actually retrospectively seen when reviewing previous MRI. In this case, tumour progression was associated with suspicious PSA-V.

These results, which need to be confirmed in larger series, could have clinical important implications. Indeed, "per protocol" RB are often not accepted by some patients who prefer not to enter AS or to leave AS by choice. In fact, 2 patients of group 1 chose to undergo RP treatment in another institution, following RB, which showed no progression. These choices can be explained, by patient reluctance about the frequent per-protocol based multiple repeated prostate biopsies indications (13). No longer performing "per protocol" RB would therefore improve the comfort of 86% of patients of our series and reduce the risk of choosing radical treatments in patients who may not benefit from radical treatment.

Controversy exists for the use of PSAk to predict PCa progression for patients on AS. PSADT and PSA-V were initially used in AS follow-up protocols but some papers described their inaccuracy. As Van den Bergh et al, described in 2008, the evidence concerning the prognostic value of the PSA-V and PSA DT is sparse, especially in active surveillance (9). Cooperberg recently validated the role of PSAk as an independent predictor of reclassification/progression in a AS multicentre cohort with PSA data collected at protocol-mandated intervals, relatively long follow-up, and centralized analysis (10).

These encouraging data suggest that PSAk or similar assessments of kinetics should be considered in future multivariable models of AS outcomes and must be validated in other surveillance cohorts. Hence, important limitation of their study is the reliability of the end point. Their reclassification itself was imperfect end point, as it may reflect undersampling due to the lack of MRI and targeted biopsies at entry and during follow-up. MRI were only performed at the clinicians' discretion and, as enrolment started in 2008, the majority of men did not undergo this imaging test.

In our series, we found an excellent NPV of PSA-V and PSADT. The PSADT, originally described to predict the biological recurrence of radiotherapy-treated PCa (14), has been found in several studies to be predictive of progression on AS (15,16). Similarly, PSA-V has been tested by many authors as predictors of

progression in AS (17,18) but few studies have shown significant results. Iremashvili et al. showed an association between PSA-V and progression only after 4 sets of biopsies (19). Patel et al. showed that the number of times the PSA-V increased above 0.4ng / ml / year predicted PCa progression in AS (20). Differences in populations chosen for the studies and biopsy protocols may explain differences in findings. We did not use PSA density at inclusion or during follow-up and cannot retrospectively assess its role as a risk factor for progression.
Our study has several limitations: The retrospective mono-centric nature of the study leads to a probable selection bias. The medium duration of follow-up can lead to interpretation bias. Time to assess progression without per-protocol based biopsy is unknown. Longer follow-up in group 2 may show additional progressions which could have been detected by per-protocol 12-mo SB. The lack of power of our study because of the small size in each group could lead to interpretation bias. Finally our centre is a regional tertiary reference centre. That can cause a recruitment bias. Imaging and pathology analyses were performed by radiologists and pathologists dedicated to urology. This may lead to a difficulty in the generalization of our results.

Conclusion.

We did not find significantly less reclassification/progression and AS discontinuation rate in the group without "per protocol" RB at one year. These results support the safety and the need to conduct of a multi-centre prospective trial to confirm these findings.

Table I: Clinical, pathological, biological and imaging data for both groups of patients at inclusion

	Group 1(n=78) With per protocol 12-mo SB	Group 2 (n=71) Without per protocol 12-mo SB
Age	63 (59 - 67)	65 (60 - 70)
Charlson score	2 (2 - 4)	2 (2 - 4)
Total PSA (ng/ml)	6.62 (5.45 - 8.30)	6.21 (4.55 - 8.60)
PSA density (ng/ml/cm^3)	0.13 (0.09 - 0.18)	0.10 (0.07 - 0.16)
Number of positive SB	1 (1 - 1)	1 (1 - 2)
MCCL (mm)	1 (1 - 2)	1 (1 - 2)
Follow-up (mo)	42.5 (26 - 70)	32 (23 - 49)

Values are median (interquartile range). SB: 12 cores systematic biopsies. MCCL: Maximal cancer core length

Table 2: Progression causes at 2 years and pathological results after radical prostatectomy

Patient	Group	Time to progression (mo)	Number of positive SB n,(size)	Number of positive MRI-TB n,(size)	Gleason sum at biopsy	Cause of progression	Treatment	pT-Stage (RP)
P63	1	24	1(8mm)	0	3+4	Grade + Size	BT	
P65	1	11	2 (?)	0	4+3	Grade	RT	
P75	1	24	4 (1-1-1-4mm)	2 (1-3mm)	3+3	Size	RP	T2c
P90	1	9	4 (1-1-3-7mm)	0	3+3	Size	RP	T2c
P94	1	10	0	1 (3mm)	4+3	Grade	RP	T2c
P100	1	12	1 (1mm)	2 (1-9mm)	3+5	Grade + Size	RP	T2c
P102	1	12	0	2 (4-5mm)	4+3	Grade	RP	T3a
P108	1	12	4 (1-1-3-4mm)	0	3+3	Size	RP	T2c
P113	1	14	0	3 (2-2-4mm)	3+4	Grade + Size	RP	T3a
P115	1	13	0	2 (2-8mm)	4+3	Grade + Size	RP	T3a
P130	1	11	1 (1mm)	2 (1-2mm)	4+3	Grade	HIFU	
P135	1	24	3 (1-1-2mm)	1(1mm)	3+3	Size	RP	T2c
P140	1	19	3 (1-2-2mm)	2 (5-7mm)	3+4	Grade + Size	RP	T2c
P2	2	23	4 (2-2-2-2)	0	3+3	Size	HIFU	
P6	2	14	1 (5mm)	0	3+4	Grade	RP	T2c
P7	2	24	2 (2-3mm)	0	3+5	Grade	RP	T3a
P11	2	19	2 (1-5mm)	1 (1mm)	4+3	Grade	RT	
P57	2	18	2 (1-1mm)	2 (2-4mm)	3+3	Size	RP	T2c
P107	2	4	0	2 (1-5mm)	3+4	Grade	RP	T2c
P110	2	5	4 (1-2-2-4mm)	2 (2-6mm)	3+3	Size	BT	
P133	2	8	0	1 (6mm)	3+4	Grade + Size	RP	T2c

BT: Brachytherapy; RT: Radiotherapy; RP: Radical prostatectomy; HIFU: High Intensity Focus Ultrasound.

Figure 1a: Tumour progression free-survival curves according to groups

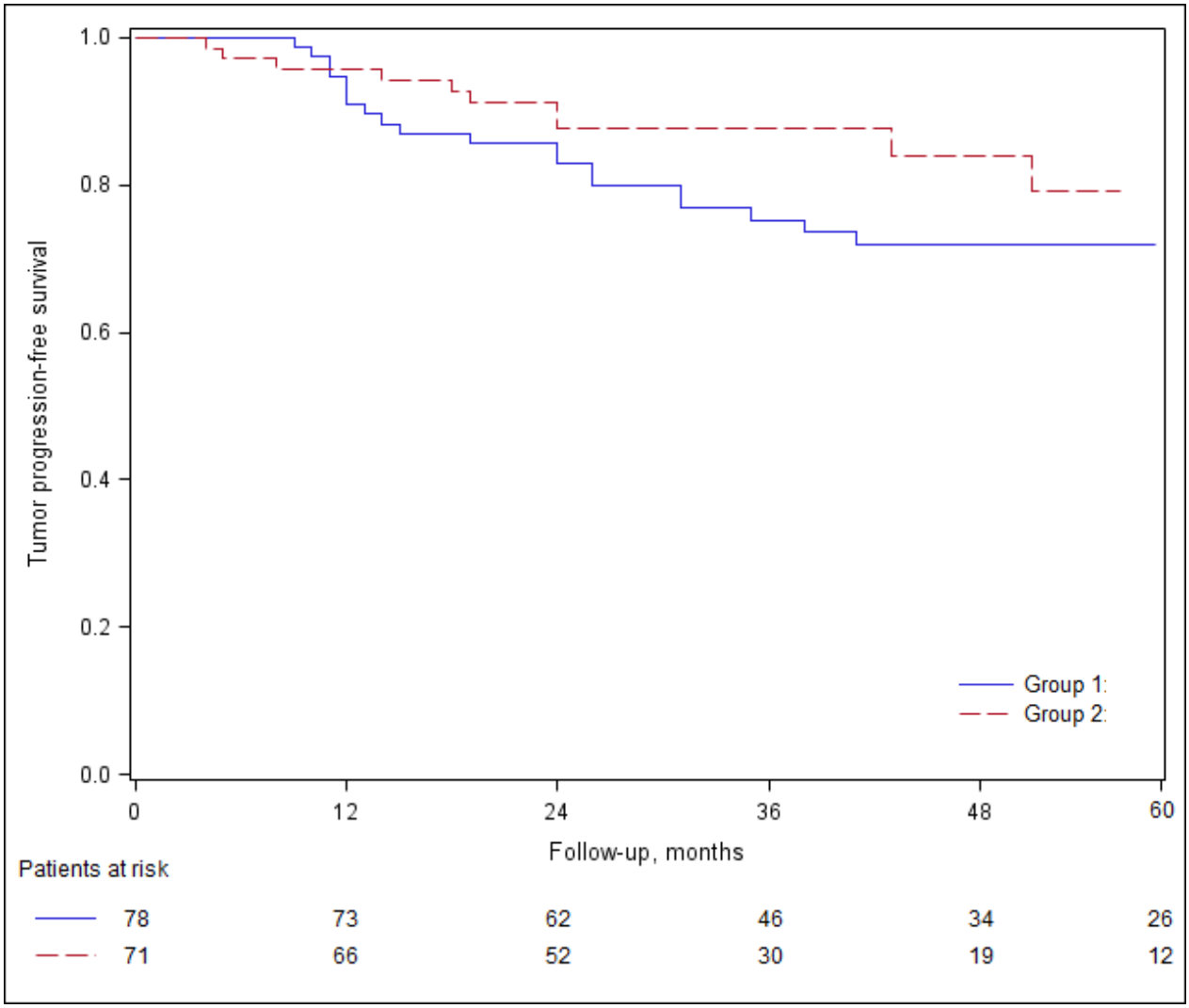


Figure 1b : AS discontinuation free survival curves according to groups.

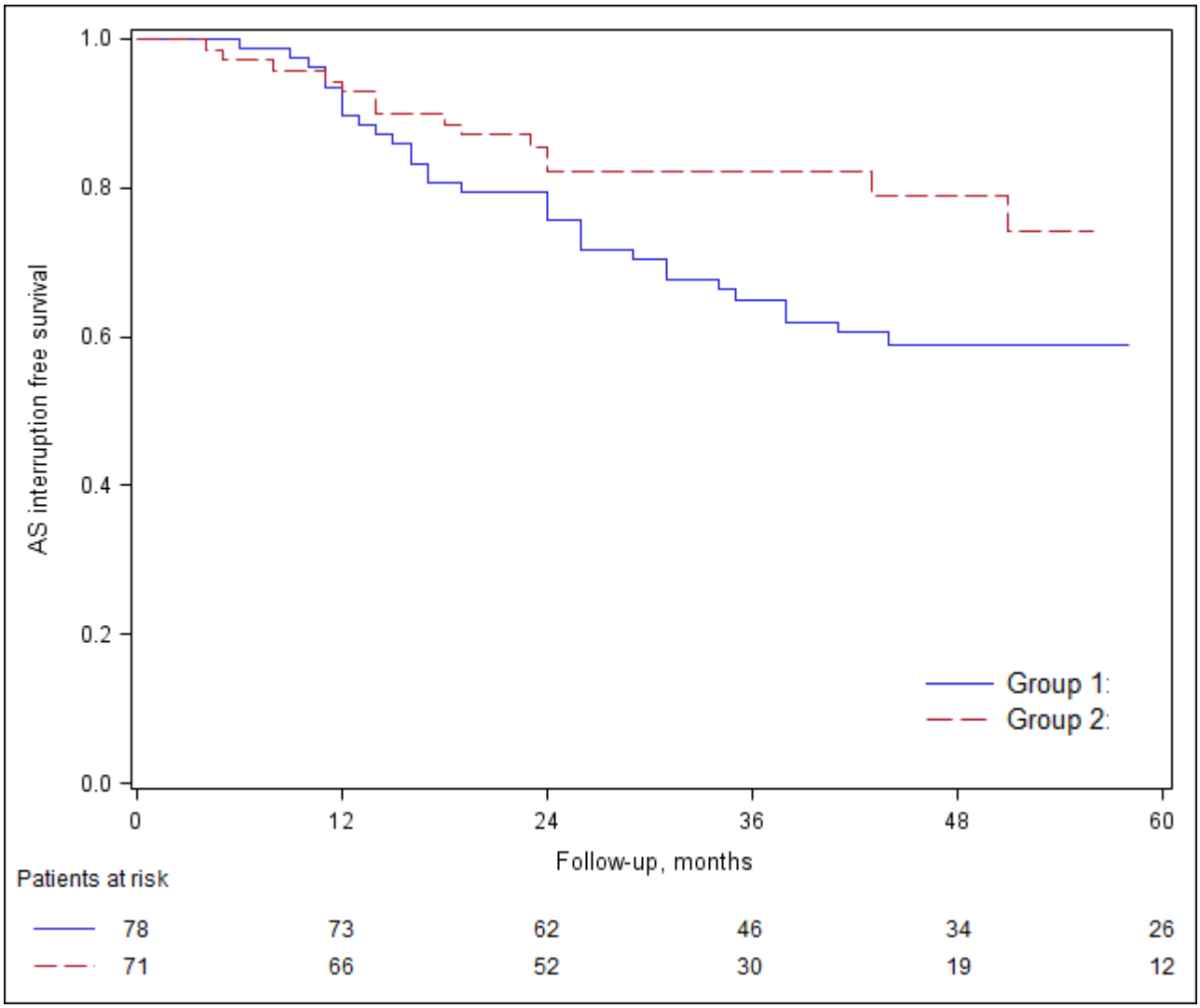


Figure 2a: PSA velocity and progression at repeat biopsies at 1 year

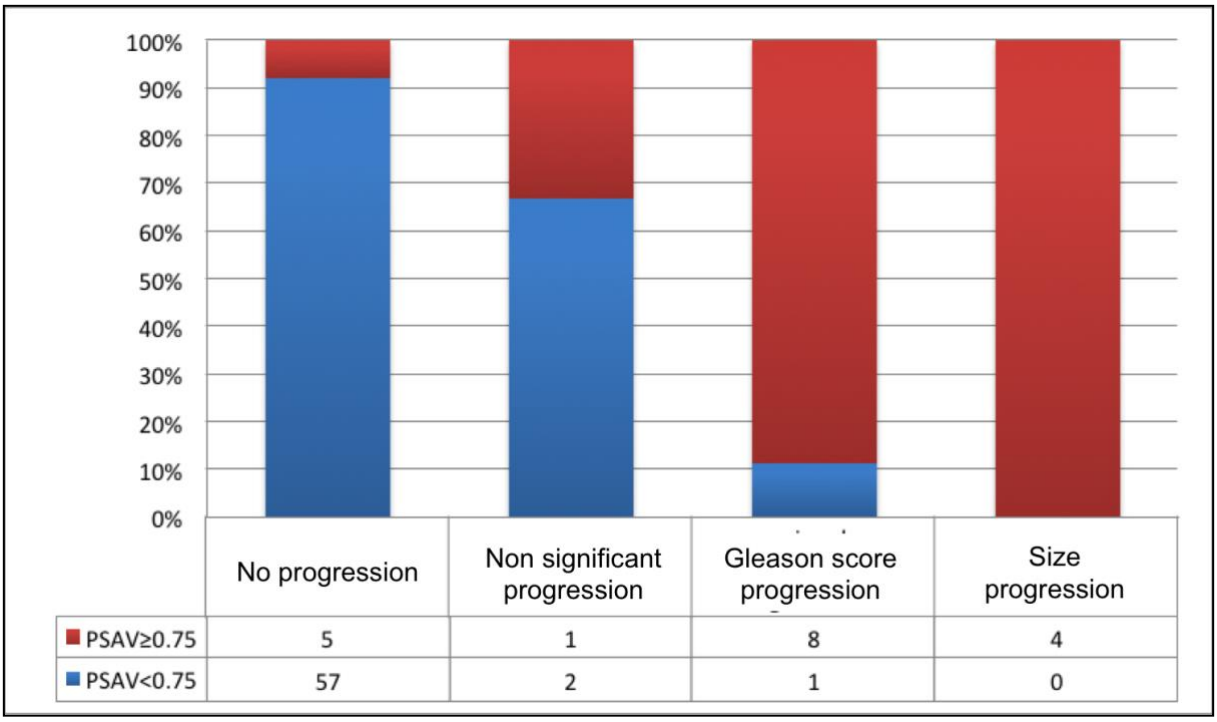
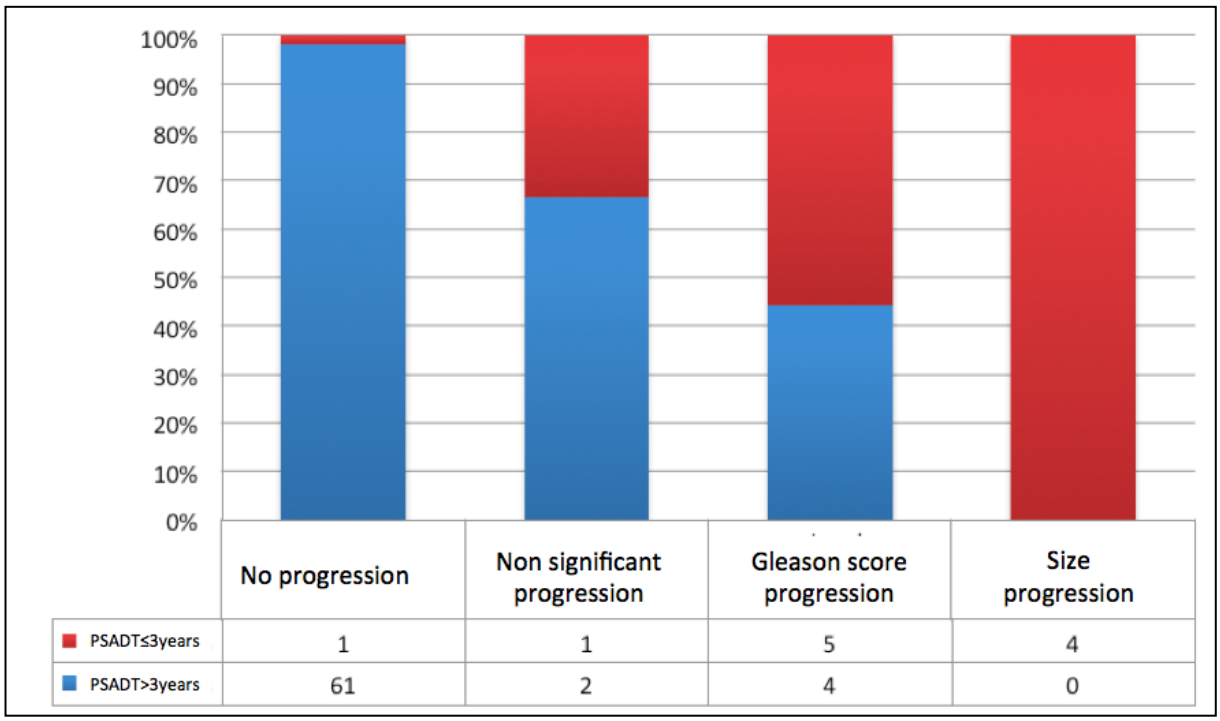


Figure 2b: PSA doubling time and progression at repeat biopsies at 1 year.



	No progression	Non significant progression	Gleason score progression	Size progression
PSADT≤3years	1	1	5	4
PSADT>3years	61	2	4	0

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Discussion de la seconde partie

L'étude 3 a permis de montrer que les risques de traitement curatif, de progression tumorale et de sortie de surveillance active étaient diminués en cas d'IRM non suspecte à l'inclusion. Ces résultats confirment ceux publiés par Stavrinides et al. dans une étude monocentrique concernant 672 patients en surveillance active. Celle-ci montrait que les patients avec une tumeur non visible à l'IRM présentaient moins de risque à un traitement curatif (35). De même, l'étude de Mamawala et al. publiée en 2020 démontrait chez les patients du Johns Hopkins Hospital que le risque de progression tumorale était à 2 ans et 4 ans de 93% et 83% en cas tumeur non visible à l'IRM et de 74% et 59% en cas tumeur visible à l'IRM (HR=1.96, p<0.001) (36). Les impacts potentiels de ces résultats sont multiples. Une IRM positive ne devrait pas exclure d'emblée les patients de surveillance active car 50% d'entre eux sont toujours en surveillance active après 5 ans mais ceux -ci devront être informés du risque augmenté de progression et devront être surveillés de plus près. De plus comme Stavrinides et al. l'a proposé, le caractère visible ou non de la tumeur devrait faire partie des critères d'inclusion au même titre que le score de Gleason (35). En effet dans leur cohorte, les taux de traitements curatifs étaient similaires dans la cohorte de cancers de score de Gleason 3+3 visibles et dans la cohorte de cancers de score de Gleason 3+4 non-visibles. Ceci n'a pas pu être confirmé dans notre étude du fait du nombre trop peu important de patients avec un score de Gleason 3+4 à l'inclusion.

L'étude 4, a montré qu'il n'y avait pas de différence de diagnostic de progression entre le groupe ayant eu des biopsies de confirmation per-protocole à un an et celui n'ayant eu que des biopsies « pour cause ». De plus nos résultats ont proposés que la cinétique du PSA suspecte seule pouvait faire indiquer une biopsie dans le suivi de la surveillance active. L'efficacité de la cinétique du PSA comme test de suivi en surveillance active a été confirmée par Cooperberg et al. En effet, dans une étude incluant 851 patients en surveillance active, les auteurs ont démontré que la cinétique du PSA prédisait de façon statistiquement significative une reclassification de la tumeur aux biopsies (37). La limite de leur étude était l'absence d'IRM dans leur protocole de surveillance active, comme nous l'avions écrit dans une réponse dans European Urology (Annexe 1) (38). Cela a entrainé un risque de sous-estimation des progressions du fait de l'absence de biopsies ciblées.

De même Gallagher et al. a montré dans une étude qui avait inclu 211 patients en surveillance active avec un suivi médian de 4.2 ans, que la vélocité du PSA était significativement associée à une progression histologique chez les patients ayant une IRM non suspecte à l'inclusion (AUC=0.85, p<0.001) (39). Cette aire sous la courbe était proche de celle de notre étude pour la vélocité du PSA (AUC=0.92).
Cette étude nous a permis de proposer de ne plus réaliser ces biopsies de confirmation en cas d'IRM non suspecte au diagnostic.

Impact sur l'actualisation des recommandations des sociétés savantes des 4 publications

Nos études présentées dans le cadre de la thèse ont permis de montrer que l'IRM de la prostate et plus particulièrement l'IRM non suspecte était un examen clé pour la diminution du sur-diagnostic et du sur-traitement du cancer de la prostate non significatifs, de faibles grade et stade.
L'impact de ces études peut être évalué par leur prise en compte dans les mises à jour des recommandations en oncologie des sociétés savantes.

Les résultats de notre étude 1 "*Predictive Factors of Missed Clinically Significant Prostate Cancers in Men with Negative Magnetic Resonance Imaging : A Systematic Review and Meta-Analysis*" associés aux résultats d'autres études ont été cités dans les recommandations de l'EAU 2021 (40):

Paragraphe "*Role of Risk-Stratification*":

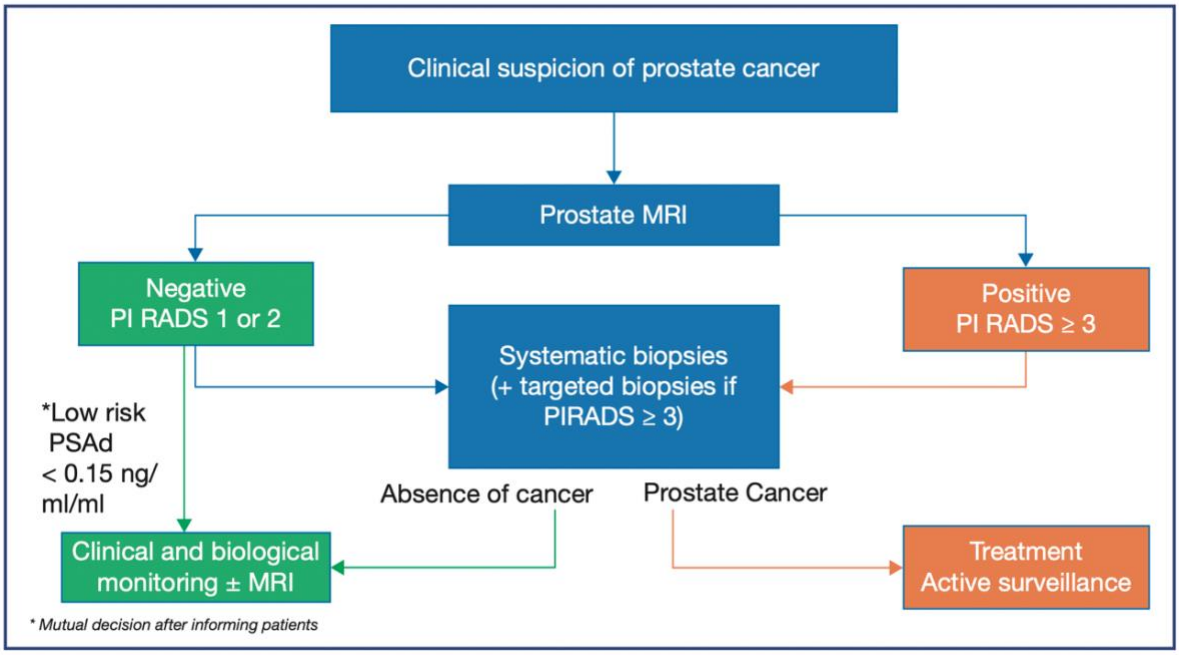
*« Prostate-specific antigen density (PSAD) may help refine the risk of csPCa in patients undergoing MRI as PSAD and the PI-RADS score are significant independent predictors of csPCa at biopsy**. In a meta-analysis of 8 studies, pooled MRI NPV for ISUP grade > 2 cancer was 84.4% (95% CI: 81.3–87.2) in the whole cohort, 82.7% (95% CI: 80.5–84.7) in biopsy-naïve men and 88.2% (95% CI: 85–91.1) in men with prior negative biopsies. In the subgroup of patients with PSAD < 0.15 ng/ml, NPV increased to respectively 90.4% (95% CI: 86.8–93.4), 88.7% (95% CI: 83.1–93.3) and 94.1% (95% CI: 90.9–96.6).** In contrast, the risk of csPCa is as high as 27–40% in patients with negative MRI and PSAD > 0.15–0.20 ng/mL/cc»*

D'autres marqueurs associés à l'IRM ont été étudiés dans des cohortes mono centriques et ne présentent pas de preuves suffisantes pour pouvoir les recommander actuellement. Associer les résultats de l'IRM au score PCA3 ou à des calculateurs de risques tel que le score de l'ERSPC (European Randomised Screening for Prostate Cancer) ou du PBCG (Prostate Biopsy Collaborative Group)

a été étudié mais « *leurs calibrations, nécessaires pour chaque nouvelle population semble difficile* » (40).

Dans l'actualisation de 2020 recommandations française et européennes d'urologie, il est recommandé en cas d'IRM non suspecte de réaliser des biopsies systématisées en cas de suspicion élevée de cancer de prostate. En cas de faible suspicion (densité du PSA < 0.15ng/ml), la possibilité de surseoir aux biopsies doit être discutée avec le patient. Une stratégie diagnostique en fonction du résultat de l'IRM pré-biopsique a été proposée par l'actualisation de ces recommandations 2020 et correspond aux résultats de nos études antérieures (Figure 1) (33).

Figure 1 : stratégie diagnostique en fonction du résultat de l'IRM pré-biopsique selon le CCAFU (33)



Les résultats de notre étude 2 « *Negative Prebiopsy Magnetic Resonance Imaging and Risk of Significant Prostate Cancer: Baseline and Long-Term Follow-up Results* » ont été publiées après la mise à jour des recommandations. Ils ont permis de valider la VPN élevée de l'IRM pour le diagnostic de cancer de la prostate non significatif, de faibles grade et stade. Cette étude complète celle de l'étude PRECISION à laquelle nous étions associés (25).

Les résultats de notre étude 3 "*Prostate cancer patients under active surveillance with a suspicious MRI are at increased risk of needing treatment: Results of The Movember Foundation's Global Action Plan Prostate Cancer Active Surveillance (GAP3) Consortium*." ont été acceptés pour être présenté aux congrès de l'AUA et de l'EAU 2021 dans des sessions thématiques sur la surveillance active. Notre conclusion sera qu'une IRM non suspecte à l'inclusion est un facteur prédictif de maintien sans traitement curatif et peut permettre un allègement des protocoles de suivi qui peuvent présenter un obstacle pour certains patients à la poursuite de la surveillance active.

Les résultats de notre étude 4 "*Low-risk prostate cancer selected for active surveillance with negative MRI at entry: can repeat biopsies at 1 year be avoided? A pilot study***"** ont été cités dans les recommandations de l'EAU 2021 (40):

Paragraphe "*Monitoring during Active surveillance":*

« Protocol-based re-biopsy, without MRI or PSA changes, however, detected pathological progression in only 7% of men. In another serial MRI study on AS, PSA velocity was significantly associated with subsequent requirement for radical therapy in patients with no visible lesions (negative MRI); 0.98 (0.56–1.11) ng/mL/year in progressed disease vs. 0.12 (0.16 to 0.51) ng/mL/year in non-progressed disease (p < 0.01). Prostate-specific antigen doubling time was significant in patients with visible lesions (positive MRI); 3.2 (1.9–5.2) years in histopathological progressed disease vs. 5.7 (2.5–11.1) years in histopathological non- progressed disease (p < 0.01). In patients with no visible lesions on their first MRI, a cut-off of 0.5 ng/mL/year in PSA-velocity had a sensitivity of 89% (8/9 progressions identified) and a specificity of 75% for progression to radical therapy. ***Another study showed similar results on PSA-velocity and PSA doubling times in MRI- negative men on AS. For the PSA-velocity threshold value of > 0.5 ng/mL/year, sensitivity and specificity were 92% and 86%, respectively. Prostate-specific antigen doubling time < 3 years in the first 2 years were associated with tumour reclassification in 82% of cases (9/11) with a sensitivity and specificity for detection of tumour progression of 69% and 97%, respectively».***

L'impact de ces différents résultats a permis une modification de notre protocole de surveillance active au CHU de Lille. Nos critères d'inclusion sont un cancer non visible à l'IRM, de score de Gleason de 6, de longueur maximale de 5mm et 3 biopsies atteintes au maximum. Le PSA et l'âge sont non pris en compte. Les examens de suivi sont un dosage semestriel du PSA et un examen clinique annuel. En cas d'une augmentation du PSA sur 2 dosages successifs avec une vélocité >0.5ng/ml/an, une IRM est réalisée. En cas d'apparition d'une lésion à l'IRM, des biopsies systématisées et ciblées sont alors réalisées.

L'analyse du suivi de notre cohorte de surveillance active au CHU de Lille montre que chez 246 patients avec un suivi médian de 5.3 ans, le maintien en surveillance active est de 80% à 3 ans et de 74% à 5 ans. Dans cette cohorte, tous les patients qui ont progressé étaient dans leur fenêtre de curabilité, sauf 1 patient (0.4%) qui a présenté des métastases ganglionnaires. Aucun décès spécifique n'a eu lieu.

Perspectives

Nous avons mis en évidence que malgré une VPN élevée de l'IRM, environ 10% à 20% des cancers de la prostate cliniquement significatifs sont non-visibles à l'IRM ce qui suscite des inquiétudes compréhensibles chez certains cliniciens et les patients. Dans la pratique urologique actuelle, l'IRM est maintenant utilisée comme test de tri pour stratifier les hommes qui nécessitent une biopsie et ceux qui n'en nécessitent pas. C'est pour cela que récemment, des études ont cherché à comprendre les raisons pouvant expliquer que certaines tumeurs significatives soient non visibles à l'IRM et décrire leurs caractéristiques histologiques et génétiques (41,42).

Miyai et al. a montré sur une cohorte de 59 patients ayant eu une prostatectomie que les tumeurs non visibles avaient une densité de cellules tumorales plus faible et une proportion de stroma plus faible que les tumeurs visibles (43). Norris et al. a proposé comme explication que la visibilité des tumeurs à l'IRM, pourraient être multifactorielle. Au niveau cellulaire, les cancers non visibles ont des volumes, des scores de Gleason, des densités vasculaires et cellulaires plus faibles que les tumeurs visibles. Au niveau génétique, des données préliminaires sont cohérentes avec cette hypothèse. En effet il a été mis en évidence des marqueurs de l'agressivité du cancer augmentés dans les tumeurs visibles à l'IRM. En effet, la perte de *PTEN*, la surexpression de *CENPF* et des scores génomiques tels que Oncotype et Decipher plus élevés ont été rapportés dans les tumeurs visibles à l'IRM. Sur le plan vasculaire, la néo-vascularisation qui est un élément crucial au développement tumoral et à son agressivité est entre autres induite par *VEGF*. Il a été montré une expression plus faible dans les tumeurs non visibles. Au-delà de la densité cellulaire et vasculaire, il semble que le sous-type histo-pathologique joue également un rôle important dans la visibilité IRM des tumeurs. Le cancer de la prostate intra-ductal (ou intra-canalaire) est un sous-type rare, agressif et à faible sécrétion de PSA qui semble avoir tendance à l'invisibilité à l'IRM. De même, les tumeurs cribriformes sont un autre sous type histo-pathologique agressif qui semble également ne pas être difficilement visible à l'IRM (44). Les hypothèses des différences entre les tumeurs visibles et non visibles à l'IRM sont résumées dans le tableau 2.

Tableau 2 : Hypothèses des différences cliniques, histologiques et génétiques entre les tumeurs de prostate visibles et non visible à l'IRM (d'après Norris et al. (44))

	Caractéristiques	Tumeurs visibles	Tumeurs non visibles
Caractéristiques histologiques	Score de Gleason	**Plus élevé**	Moins élevé
	Volume tumoral	**Plus élevé**	Moins élevé
	Densité cellulaire	**Plus élevée**	Moins élevée
	Densité vasculaire	**Plus élevée**	Moins élevée
	Ratio stroma/épithélium	Moins élevée	**Plus élevée**
	Présence de cancer intraductaux	Moins élevée	**Plus élevée**
	Présence de cancer cribiriforme	Moins élevée	**Plus élevée**
Caractéristiques génétiques	Gènes de réparation de l'ADN	**Plus grande dérégulation**	Plus faible dérégulation
	Voies biologiques	**Augmentation du cycle cellulaire**	Réduction du cycle cellulaire
	Angiogenèse	**Plus de promoteurs de l'angiogenèse**	Moins de promoteurs de l'angiogenèse
	Hypoxie	**Plus de promoteurs de l'hypoxie**	Moins de promoteurs de l'hypoxie

Les futures études sur ce sujet, porteront sur la poursuite de la compréhension de la visibilité des tumeurs en fonction de leur agressivité afin de pouvoir associer à l'imagerie des biomarqueurs qui permettront une meilleure caractérisation du risque de progression, un meilleur suivi des patients et un traitement individualisé. Pour cela il est nécessaire de travailler sur des cohortes de patients ayant eu une IRM pré biopsique, des données histologiques aux biopsies (et non sur pièce de prostatectomie car désormais un taux important de patient est sous surveillance active ou reçoit un traitement focal) et un suivi à long terme. Pour mener ce type d'étude, nous avons réalisé des micropuces tissulaires à partir de biopsies d'une cohorte de patients ayant eu une IRM pré-biopsique en collaboration avec le laboratoire du Dr Whitaker à l'University College of London au cours de mon Master 2. La difficulté de réalisation de ces micropuces est liée à la taille des échantillons (une biopsie faisant <1mm sur 15mm) et l'hétérogénéité des tissus (la tumeur faisant très fréquemment que quelques millimètres). Nous avons donc développé une méthode de construction de ces micropuces que nous avons validées à l'aide de biomarqueurs validés dans le cancer de la prostate.

L’article « Immunohistochemical biomarker validation in highly selective needle biopsy microarrays derived from mpMRI-characterized prostates » Olivier et al. a été publié en 2018 dans la revue Prostate (Annexe 2).

Cet article a été cité dans l’article *Molecular Biomarkers in Localized Prostate Cancer : ASCO Guideline* (45) . Ces recommandations de l’ASCO mettent en évidence que la littérature est rare sur ce sujet de la corrélation entre résultats de l’IRM prostatique et les résultats histochimiques ou génétiques.
Dans ces recommandations, à la question : *« Quelles sont les forces et les faiblesses comparatives de la génomique par rapport à l'IRM pour identifier le cancer de la prostate cliniquement significatif ? »* La réponse est : *« Chez les hommes atteints d'un cancer de la prostate nouvellement diagnostiqué éligible à une surveillance active, l'IRM et la génomique visent à identifier les cancers cliniquement significatifs. Le groupe d'experts approuve leur utilisation uniquement dans les situations où le résultat, lorsqu'il est pris en compte avec des facteurs cliniques courants, est susceptible d'affecter la prise en charge. Cela peut inclure, par exemple, la prise en charge initiale des hommes potentiellement éligibles à la surveillance active, où chacune de ces approches peut fournir des informations cliniquement pertinentes et exploitables. Ces tests peuvent fournir des informations indépendantes des paramètres cliniques de routine et indépendants les uns des autres. »*

Ces micro-puces permettront d’étudier l’expression de protéines tissulaires sur les biopsies et de corréler leurs expressions aux données de l’IRM et aux données de suivi afin d’associer les données cliniques, d’imagerie, protéomique et génétiques pour prédire au mieux l’évolution. Des cancers en surveillance active afin d’élargir ses indications en toute sécurité.

Conclusion

Ce travail de thèse a démontré la bonne performance de l'IRM, en association à la densité du PSA et les antécédents familiaux pour prédire l'absence de cancer de faibles grade et stade en cas de biopsie. Les résultats ont été repris dans les recommandations des sociétés savantes d'urologie française et européenne en 2020-2021. Ces résultats participent à l'objectif de réduire le sur-diagnostic et le sur-traitement du cancer de la prostate et par ce fait de mieux défendre le dépistage du cancer de la prostate et ainsi permettre une amélioration de la survie spécifique. En plus des facteurs cliniques, du PSA et de l'IRM, d'autres marqueurs tissulaires sont en cours d'évaluation pour aider à prédire le risque de progression du cancer au cours de la surveillance.

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Annexes

Annexe 1 : Réponse à l'article de Cooperberg et al. publiée dans *European Urology* en 2018

Re: Refined Analysis of Prostate-specific Antigen Kinetics to Predict Prostate Cancer Active Surveillance Outcomes
Cooperberg MR, Brooks JD, Faino AV, et al

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Experts' summary:
The authors analyzed the extent to which prostate-specific antigen (PSA) kinetics might facilitate improved decision- making for men on active surveillance (AS) for low-risk pros- tate cancer. Among all 851 participants, 291 (34%) were reclassified because of an increase in biopsy Gleason grade or tumor volume to 34% of total biopsy cores with cancer. Some 80% of biopsies were per protocol at 12 mo, 24 mo, and every other yr. PSA was measured every 3 or 6 mo. The PSAk marker was independently associated with reclassification.

Experts' comments:
We must applaud this positive study that brings back PSA kinetics into the follow-up protocol for patients on AS. PSA changes frequently drive treatment decision-making in con- temporary practice.

PSA kinetics for prediction of the risk of progression was not easy to establish in this study because of various methodological issues. The authors had to calculate a novel biomarker (PSAk) using a linear mixed-effect model (LMEM), taking into account the logarithm of PSA modeled as a linear function of time and the individual-specific rate of change over time. This methodology does not lend itself to simple calculation at the point of care and requires a robust background of PSA data. This need to use LMEM methodol- ogy may be linked to concerns about the reliability of the reclassification rate (endpoint). Hence, according to authors

it may be affected by undersampling owing to the lack of magnetic resonance imaging (MRI) and targeted biopsies at entry and during follow-up for most patients.

Some current AS protocols include MRI and targeted biopsies in the selection criteria. This allows exclusion from AS of 10% of cases of unrecognized clinically significant cancers in areas undersampled (mostly anteriorly located) via systematic biopsies [1]. This would clarify the endpoint of misclassification at entry or during follow-up and decrease reclassification or progression rate to 16% [2].

The next steps will be to prospectively validate PSA kinetics (why not PSA velocity?) in AS protocols, along with MRI, as a surrogate test to predict progression. Will this allow us (as we do intuitively for patients who have had a negative first biopsy series) to obviate per-protocol repeat biopsies at 12 mo or every other year (Olivier et al, manuscript submitted for publication)? Will more patients eventually decide to remain on AS?

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ORIGINAL ARTICLE

WILEY The Prostate

Immunohistochemical biomarker validation in highly selective needle biopsy microarrays derived from mpMRI-characterized prostates

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Introduction: Diagnosing prostate cancer routinely involves tissue biopsy and increasingly image guided biopsy using multiparametric MRI (mpMRI). Excess tissue after diagnosis can be used for research to improve the diagnostic pathway and the vertical assembly of prostate needle biopsy cores into tissue microarrays (TMAs) allows the parallel immunohistochemical (IHC) validation of cancer biomarkers in routine diagnostic specimens. However, tissue within a biopsy core is often heterogeneous and cancer is not uniformly present, resulting in needle biopsy TMAs that suffer from highly variable cancer detection rates that complicate parallel biomarker validation.

Materials and Methods: The prostate cores with the highest tumor burden (in terms of Gleason score and/or maximum cancer core length) were obtained from 249 patients in the PICTURE trial who underwent transperineal template prostate mapping (TPM) biopsy at 5 mm intervals preceded by mpMRI. From each core, 2 mm segments containing tumor or benign tissue (as assessed on H&E pathology) were selected, excised and embedded vertically into a new TMA block. TMA sections were then IHC-stained for the routinely used prostate cancer biomarkers PSA, PSMA, AMACR, p63, and MSMB and assessed using the h-score method. H-scores in patient matched malignant and benign tissue were correlated with the Gleason grade of the original core and the MRI Likert score for the sampled prostate area.

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Results: A total of 2240 TMA cores were stained and IHC h-scores were assigned to 1790. There was a statistically significant difference in h-scores between patient matched malignant and adjacent benign tissue that is independent of Likert score. There was no association between the h-scores and Gleason grade or Likert score within each of the benign or malignant groups.

Conclusion: The construction of highly selective TMAs from prostate needle biopsy cores is possible. IHC data obtained through this method are highly reliable and can be correlated with imaging. IHC expression patterns for PSA, PSMA, AMACR, p63, and MSMB are distinct in malignant and adjacent benign tissue but did not correlate with mpMRI Likert score.

KEYWORDS
immunohistochemistry, MRI, prostate cancer, tissue microarrays

1 | INTRODUCTION

Prostate cancer is one of the most common non-cutaneous cancer in males, with approximately 417 000 new cases diagnosed in 2012 in Europe alone.[1] PSA testing has resulted in an increase in prostate cancer incidence and to a diagnostic migration toward smaller, low-grade disease with low metastatic potential, and limited impact on mortality.[2–4] In the Western world, it is common practice to diagnose prostate cancer through transrectal, ultrasound-guided systematic needle biopsies (TRUS) in PSA-detected men. Clinico-pathological parameters obtained through this approach including serum PSA, Gleason grade, and maximum cancer core length on biopsy are often used to stratify risk and guide patient management. In the last decade mpMRI has emerged as an important technique for characterising and targeting the biopsy of suspected prostate cancer, as it reduces the number of unnecessary biopsies and efficiently detects clinically significant targets without over-diagnosing insignificant disease.[5–8]

Incorporating tissue biomarkers in the patient stratification process could further refine the emerging imaging-based patient pathways, but selecting molecules for such purposes requires their parallel testing in very small amounts of diagnostic tissue. Tissue microarrays (TMAs) constructed from prostate needle biopsies are a promising tool for high-throughput biomarker development and validation.[9,10] Numerous strategies have been proposed for maximising needle biopsy TMA performance, but a simple and productive approach is the vertical re-orientation of biopsy cores for the construction of high-density arrays.[11,12]

However, a recurring challenge in needle biopsy TMAs is the significant variability in their cancer content. This is generally due to (i) the considerable heterogeneity of prostate cancer; (ii) random tissue sampling approaches that often result in disease misrepresentation (which is not necessarily the case with TMAs derived from prostatectomy specimens); and (iii) tissue loss during sampling, fixation, embedding, or staining. These difficulties are further complicated by the scarcity of biopsy material, which is very precious and cannot be easily substituted if TMA quality is poor.

It follows that, for biomarker validation purposes, the ideal diagnostic needle biopsy TMA should (i) incorporate a large number of specimens; (ii) contain tissue from well-characterized prostate areas with clinically significant disease; (iii) have a high cancer detection rate for maximum performance; and (iv) produce results that can be correlated with imaging data. Here, we present the construction of a biopsy TMA from prostates thoroughly characterized using mpMRI and 5 mm transperineal mapping (TPM) biopsies. We divided the biopsy cores in segments such that only either benign or malignant tissue was included in a specific array position. To test our tissue-selective TMAs, we performed IHC for routinely used prostate biomarkers and correlated IHC h-scores with imaging parameters and original pathology.

2 | MATERIALS AND METHODS

2.1 | Patient cohort

All prostate tissue was acquired during the PICTURE trial, a paired-cohort confirmatory study designed to assess the accuracy of mpMRI in detecting clinically significant cancer.[13] For this purpose, 249 men with a previous TRUS biopsy requiring a repeat evaluation underwent

a 3T mpMRI followed by TPM biopsies of the entire prostate at 5 mm intervals. The likelihood of significant cancer by mpMRI was reported using the Likert scale, as previously defined.[14] In all MRI scans, the base, middle and apex of the prostate were divided in four quadrants resulting in Likert scores assigned to a total of 12 prostate areas for each patient. Ethical approval previously given for the study allowed the use of needle biopsy specimens for TMA construction as described below.

For each patient, the pathology report was reviewed and the biopsy cores with the highest Gleason score and/or the longest maximum cancer core length (MCCL) were identified and selected for retrieval from the UCL/UCLH Biobank for Studying Health & Disease. All cores were previously fixed and routinely processed to formalin fixed paraffin embedded (FFPE) blocks. Haematoxylin and eosin (H&E)-stained 4 µm sections were evaluated by an expert uropathologist (AF and CJ) and tumor foci were identified and graded according to the Gleason grading system. A total of 448 tissue blocks were retrieved. Clinical data (disease stage, age, PSA at study entry) and Likert scores for all prostate regions were collected for all patients. A summary of the pathology of all selected cores and the distribution of their corresponding Likert scores is presented in Table 1.

TABLE 1 Pathological and radiological characteristics associated with the TMA biopsy cores

TMA core characteristic	Number of cores
Pathology	
Benign	26
PIN	16
3 + 3	64
3 + 4	118
3 + 5	1
4 + 3	20
4 + 4	3
5 + 4	1
Likert score for sampled quadrant	
2	73
3	59
4	39
5	77

Pathological and radiological characteristics of the cohort: TPM biopsy reports were scrutinized and cores with the highest Gleason grade and/or maximum cancer core length identified. True benign biopsies or biopsies containing only prostatic intraepithelial neoplasia (PIN) from patients without any cancer were also included in the TMA. MRI images of the base, middle, and apex of each prostate were divided in quadrants and each quadrant assessed using a 5-point Likert scale for the likelihood of underlying clinically significant cancer (where scores of 4 or 5 denote a higher likelihood). For each TMA core, the corresponding Likert score for the sampled prostate quadrant was available.

2.2 | Microarray construction

An overview of the TMA construction process is shown in Figure 1. Diagnostic 4 µm H&E-stained sections were obtained and scanned using a Hamamatsu scanner.The digital H&E images were inspected and 2 mm-long benign or tumor regions were identified and then marked on the original FFPE block for cutting. Each cutting plan was mutually agreed by at least two investigators. FFPE blocks were incubated at 60°C for 20 min and all marked tumor or benign biopsy core tissue was dissected and excised with a microtome blade. In this way, wax chips containing 2 mm-long core segments containing exclusively benign or tumor tissue according to the H&E cutting plan were produced. Each chip was re-marked for orientation purposes and placed onto a new individual plastic cassette before being re-embedded vertically. Once all benign or malignant core segments were vertically re-embedded, an Estigen MTA-1 Manual Tissue Arrayer was used to extract 1.5 × 6 mm wax cores containing the vertical 2 mm core segments from each donor block and place them in the recipient wax block. Benign and tumor core segments were randomly positioned 0.7 mm apart in a 6 × 10 format. Liver tissue and blank positions were also used for orientation purposes. Each newly constructed TMA block was placed on a glass slide for 40 min at 60°C in an incubator and then cooled on a cold plate for tempering. Seven TMA blocks were produced and cut into 4 µm sections, with one slide every 50 retained for H&E staining and quality control.

2.3 | Immunohistochemistry (IHC)

FFPE TMA sections were deparaffinized and rehydrated with successive 5-min washes in xylene and alcohol (100, 90, and 70%). For all immunohistochemical stains, a Leica BOND-MAX Autostainer (Leica Biosystems, UK) was used. Heat-induced epitope retrieval was performed using either a pH 6.0 citrate-based or a pH 9.0 ethylene-diamine-tetra-acetic acid-based, ready-to-use solution (ER1 and ER2, respectively, Leica Biosystems). All sections were incubated with the following primary antibodies under appropriately optimized conditions: PSA (rabbit polyclonal antibody, Dako A/S, Denmark; 1:9000 dilution, no retrieval), PSMA (mouse monoclonal antibody, clone 1D6, Leica Biosystems; 1:50 dilution, ER1 for 20 min), p63 (mouse monoclonal antibody, clone 7JUL, Leica Biosystems; 1:50 dilution, ER2 for 20 min), AMACR (rabbit monoclonal antibody, clone 13H4, Dako A/S, Denmark; 1:100 dilution, ER2 for 20 min), and MSMB (mouse monoclonal antibody, clone YPSP-1, Abcam, UK; 1:2500 dilution, enzymatic pre-digestion with Leica Biosystems Enzyme 1 for 15 min). Diaminobenzidine (DAB) was used as the chromogen and counterstaining was performed with haematoxylin for 1 min. Following dehydration, the slides were cover-slipped using DPX (Leica Biosystems).

2.4 | IHC scoring

Digital images of the IHC slides were obtained using a Hamamatsu scanner. Each individual TMA core was assessed for the presence of cancer and h-scored by at least two independent investigators (HW,

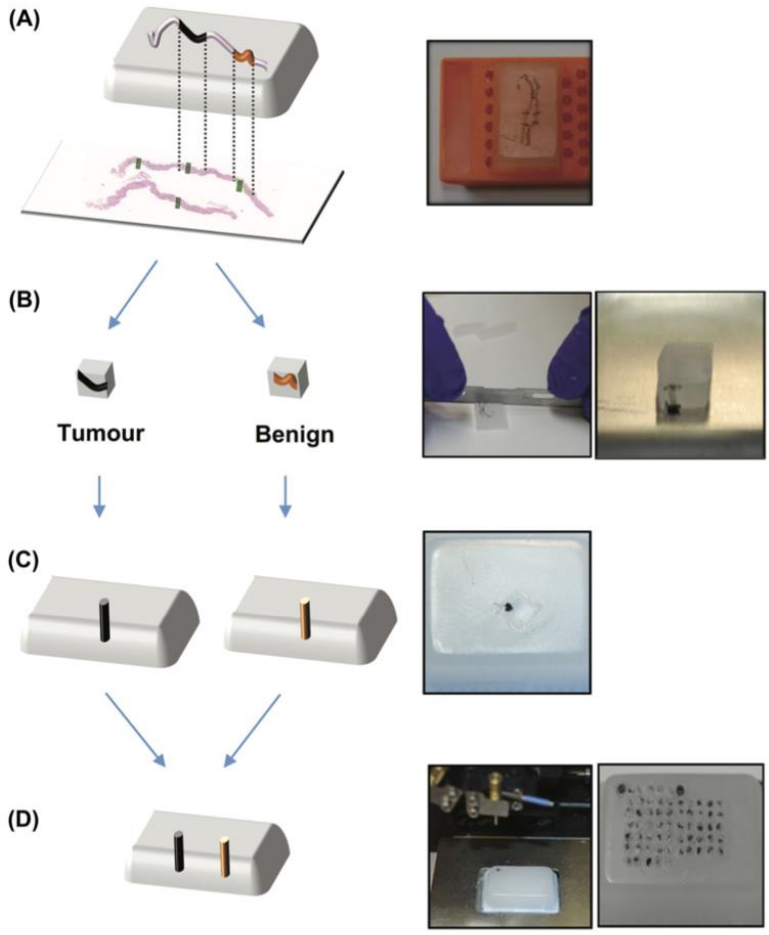

FIGURE 1 TMA construction: Benign and malignant areas of 2 mm were identified within a biopsy core on H&E and selected for inclusion in the TMA (A). Each segment of core was divided according to the H&E cutting plan with a microtome blade, in order to obtain wax chips that contain either 2 mm of malignant or benign tissue core (B). The wax chips were marked on their edges for orientation, re-positioned vertically, and then embedded in a new paraffin donor block (C). All vertically re-embedded core segments are introduced into the final TMA block before tempering at 37°C for smoothing (D). In total, seven TMA slides were constructed and sectioned in their entirety, yielding 200-300 slides per TMA. The first slide of every 50 was stained with H&E for quality control while the rest were stored for immunohistochemistry after dipping in wax. [Color figure can be viewed at wileyonlinelibrary.com]

VS, ZA) without prior knowledge of clinical data. A proportion representing the estimated percentage of positively stained epithelial cells (0-100%) and an intensity score (0 = none; 1 = weak; 2 = intermediate; 3 = strong) were assigned to each core. A final h-score ranging from 0 to 300 was calculated by multiplying the proportion score with the intensity score (h-score = % no staining × 0 + % weak staining × 1 + % moderate staining × 2 + % strong staining × 3). The designation of a core section as "benign" or "malignant" was reassigned in cases of discrepancy with the H&E appearance in the original tissue block. Only tumor tissue was scored in cores containing both tumor and benign tissue. Missing cores or purely stromal areas were excluded from the analysis.

2.5 | Data analysis

All statistical analyses and visualization were performed in the R programming environment (http://www.R-project.org/, version 3.4.1). Continuous data distributions (h-scores) were tested for normality using quantile-quantile plots and the Shapiro-Wilk test. Due to substantial non-normality of the h-score distributions, the paired Wilcoxon signed rank procedure was used to test for significant differences in h-scores between malignant and paired, adjacent benign tissue. Kruskal-Wallis analysis of variation was used for comparisons between multiple groups. All tests were two-sided and a statistical significance level of 0.01 was considered significant.

3 | RESULTS

3.1 | Performance measures

Although other methods of producing efficient biopsy TMAs have been described, data on their performance in terms of cancer detection rates are not comprehensive.[8–11] For this TMA seven blocks were constructed containing 448 core segments in total. Slides were IHC stained against five biomarkers (PSA, PSMA, AMACR, p63, and MSMB), yielding a theoretical maximum of 2240 (5 × 448) stained core sections. A summary of these results is given in Table 2. Of these, 338 (15%) were either lost during the staining process or were not assessable on scoring due to poor tissue quality. In addition, 112 (5%) core sections contained stroma only. When missing, un-assessable or purely stromal tissue was excluded from the analysis, 1790 (83%) of cores remained. Of these 371 (82.8%) core sections were h-scored for PSA, 345 (77%) for PSMA, 343 (76.6%) for p63, 367 (81.9%) for AMACR, and 364 (81.3%) for MSMB. During h-scoring, each core section was re-evaluated to confirm that it contained tumor or benign tissue as designated in the original H&E cutting plan before vertical re-embedding. For each TMA slide three separate levels, each 50 slides apart, were assessed for pathology on H&E appearances and demonstrated consistent tumor or benign content at two or more levels for 81.9% of cores (Figure 2A and Table 3). In total, IHC and H&E appearances at a single level agreed in 1670 out of 1790 cases, with concordance in 349 cores stained for PSA, 324 for PSMA, 325 for p63, 342 for AMACR, and 330 for MSMB. Concordance rates (ie, number of h-scored core sections with IHC-H&E concordance/total number of h-scored core sections) were 94, 94, 95, 93, and 90% for each stain, respectively.

3.2 | IHC correlations with pathology and Gleason grade

Gleason grade is routinely used in indicate the aggressiveness of prostate cancer and markers preferentially diagnosing clinically significant disease (often characterized by the presence of ≥Gleason 4 pathology) are increasingly sought. For data analysis only patient matched pairs of malignant tissue and paired, adjacent benign tissue (from the same tissue block) were considered. The number of h-scored scored malignant-benign pairs was 105, 92, 101, 103, and 99 for PSA, PSMA, p63, AMACR, and MSMB, respectively, There statistically significant difference between the h-scores for malignant and paired benign tissue for PSA ($P < 0.001$), PSMA ($P < 0.00001$), p63 ($P < 0.00001$), AMACR ($P < 0.00001$), and MSMB ($P < 0.00001$) (Figures 2B and 2C). Overall, AMACR and PSMA h-scores were higher in tumor tissue compared to matched benign, whereas the opposite was true for p63, MSMB, and PSA. These differences were also seen when visualising h-scores for all cores (including unmatched) (Supplementary Figure S2), although no statistical tests were performed as each group contained a mixture of paired and unpaired values.

As Gleason grade ≥4 is often associated with more aggressive disease each of the benign and malignant groups were analyzed separately to investigate h-score differences between different Gleason grades at diagnosis (Figure 3). Non-parametric analysis of variation failed to demonstrate any significant h-score difference between both the benign and tumor tissues originating from cores with different Gleason grades indicating that protein expression of these markers was associated with tumorigenesis but not aggressiveness of disease.

3.3 | IHC correlations with mpMRI

mpMRI has been shown to efficiently diagnose clinically significant prostate tumors and is rapidly becoming a mainstay of prostate cancer diagnosis. Despite this, very few routinely used biomarkers have been studied in conjunction with mpMRI data. In this study paired h-scores were compared for different mpMRI Likert scores (Figure 4). When each benign and malignant group was divided to two Likert subgroups ("lower" Likert ≤ 3 vs "higher" Likert ≥ 4), there was a significant

TABLE 2 TMA IHC outcomes

	PSA	PSMA	p63	AMACR	MSMB	Total
Missing or unassessable	42	87	95	68	46	338
Stroma only	35	16	10	13	38	112
Concordant	349	324	325	342	330	1670
Benign	167	155	158	170	160	810
Tumor	182	169	167	172	170	860
Re-assigned	22	21	18	25	34	120
Benign→tumor	9	7	4	10	10	40
Tumor→benign	13	14	14	15	24	80
Total	448	448	448	448	448	2240

TMA quality assessment: All IHC was performed on the BondMax Autostainer for PSA, PSMA, p63, AMACR, and MSMB. Digital images of the IHC slides were obtained using a Hamamatsu scanner. Each individual TMA core was assessed for the presence of cancer and h-scored by at least two independent investigators (HW, VS, ZA) without prior knowledge of clinical data. The designation of a core section as "benign" or "malignant" was reassigned in cases of discrepancy with the H&E appearance in the original tissue block.

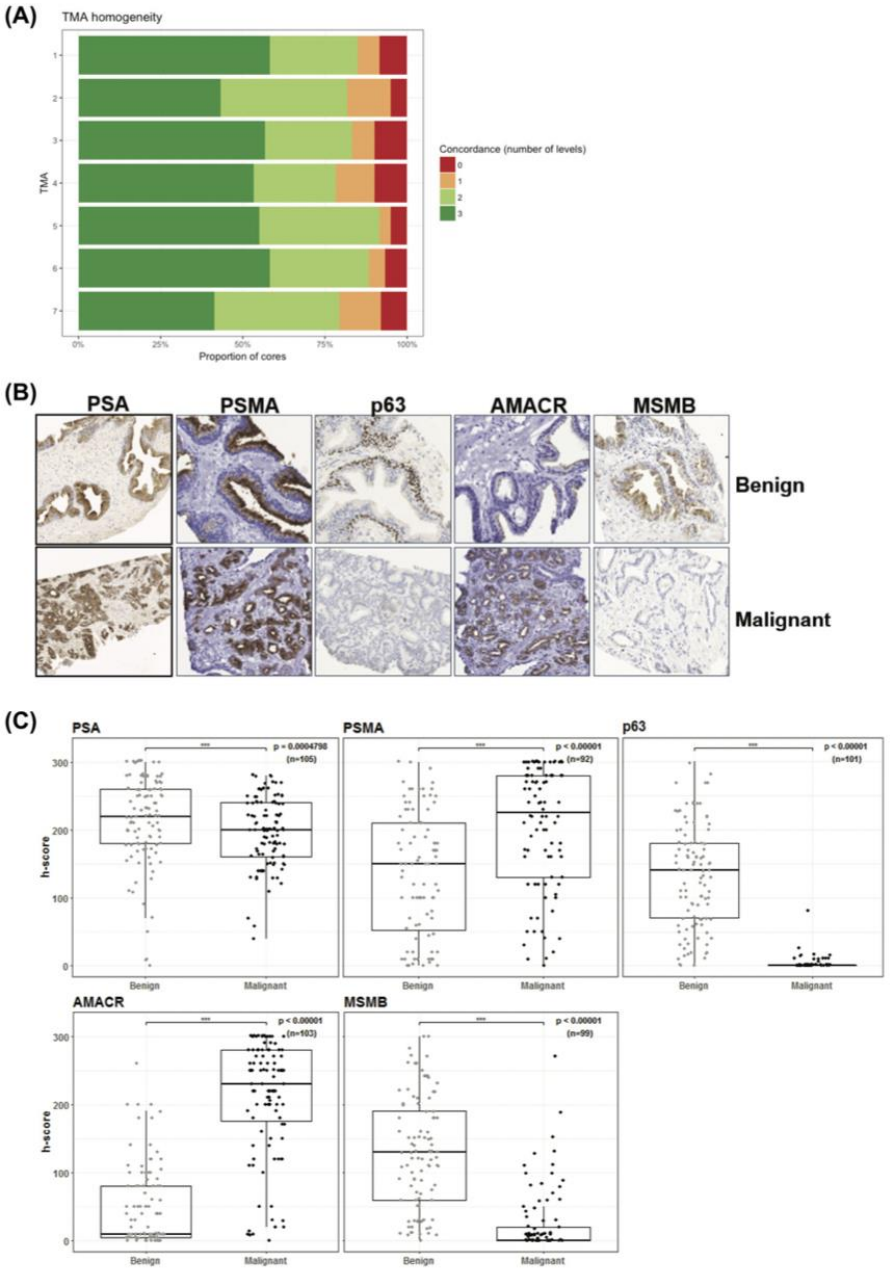

FIGURE 2 IHC for common prostate cancer biomarkers on paired samples: The seven TMA blocks were cut in their entirety and slides were wax dipped to prevent from oxidation. Slides at three levels (typically slide 50, 100, and 150) from each of the seven TMA slides were H&E stained and each core assessed for tumor or benign content. Concordance was measured as slides that had the same pathology at all three levels (dark green), two levels (pale green), one level (orange), and no levels (red). All IHC was performed on the BondMax Autostainer with staining shown in brown and nuclei are shown in blue. Representative images are shown for PSA, PSMA, p63, AMACR, and MSMB (20× magnification) (B). IHC for paired samples was analyzed using h-score method that takes into account staining intensity and the number of positively stained cells. Data for all samples (paired and unpaired) is shown in Supplementary Figure S1. H-scores in tumor tissue (black) were compared to paired h-scores in benign tissue (gray) from the same biopsy block using a paired Wilcoxon signed rank test. The *P*-values and number of pairs are separately shown for each stain (C). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 H&E slides were assessed every 50 slides over 150 slides of each TMA slide and assessed for tumor or benign pathology

Concordance	Partial (2 levels)	Full (3 levels)
Tumor	64	122
Benign	74	107
Total	138/448 (30.80%)	229/448 (51.12%)

Concordance was judged as partial when it agreed at two levels or full when all three levels exhibited the same pathology. Pathology concordance across the TMA. H&E slides were assessed every 50 slides over 150 slides of each TMA slide and assessed for tumor or benign pathology. Concordance was judged as partial when it agreed at two levels or full when all three levels exhibited the same pathology.

difference between the paired h-scores for PSMA, p63, AMACR, and MSMB in both subgroups ($P < 0.01$). PSA was the exception: although there was a significant h-score difference in the "lower" Likert subgroup ($P = 0.0023$), there was no similar difference in the "higher" Likert subgroup ($P = 0.0945$).

Different Likert scores were considered for each group (benign or malignant) separately (Supplementary Figure S1). There was no significant h-score difference in benign tissue with different Likert scores assigned to the prostate area of origin for any biomarker ($P > 0.1$). This was also the case for malignant tissue, although AMACR reached the level of marginal statistical significance ($P = 0.03872$), suggesting that there could be a difference in AMACR h-scores between malignant tissues from prostatic areas with different Likert scores.

4 | CONCLUSION

Despite the large number of emerging genomic models the current predictive models for stratifying prostate cancer patients for treatment remain based on clinico-pathological variables such as age, serum PSA levels, disease stage, and Gleason grade.[15] Using refined classification schemes which utilize biomarkers provides a route to increased predictive ability and personalized patient management.[16] Using additional immunohistochemical markers at diagnosis is a simple and cheap approach that does not require any additional infrastructure, allowing rapid implementation in a pathology laboratory. However, all novel markers require validation on large numbers of representative patient samples before widespread use and TMAs are a useful way of examining expression in large numbers of tissue samples simultaneously. However, the majority of TMAs are derived purely from radical prostatectomy tissue that are more likely to be of a lower stage and Grade and are not sampled in an unbiased manner. As a result most TMAs do not accurately represent the tissue biopsies used for routine diagnosis and bias any subsequent biomarker validation studies.

Utilising archival biopsy tissue for routine biomarker validation we have constructed tissue-selective microarrays from vertically re-arranged prostate needle biopsy samples for the purposes of parallel IHC and radiological biomarker validation (Figure 1). Numerous strategies have been proposed for maximising needle biopsy TMA performance, but a simple and productive approach is the vertical re-

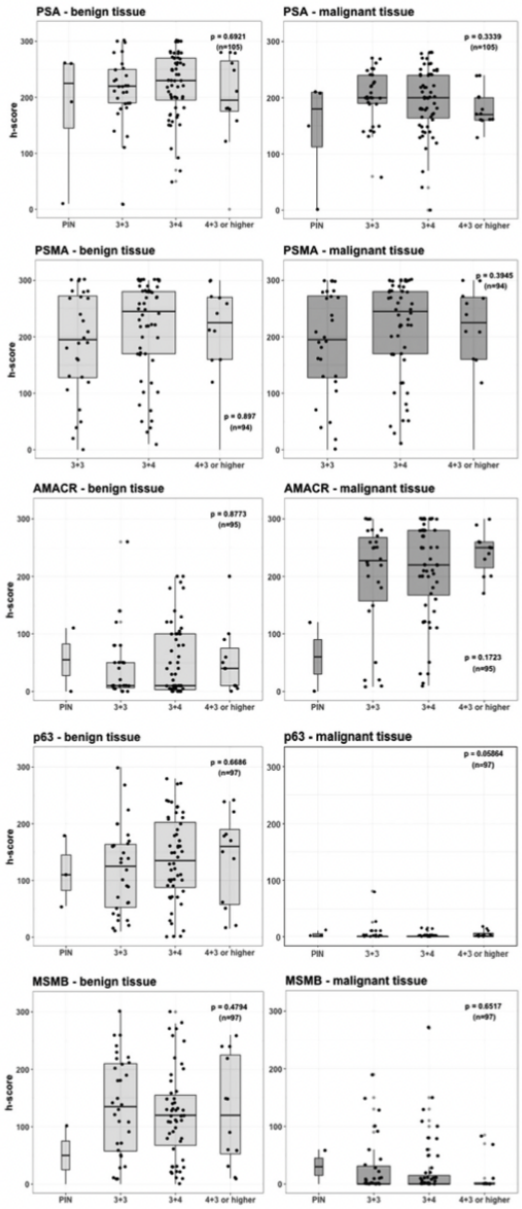

FIGURE 3 IHC h-scores versus Gleason grade: All IHC was analyzed using h-score method that takes into account staining intensity and the number of positively stained cells. H-scores for paired samples only in tumor tissue (black) and benign tissue (gray) are shown. All associations were tested within either benign or tumor groups using Kruskal-Wallis analysis of variation. The *P*-values and number of pairs are separately shown for each stain

orientation of biopsy cores for the construction of high-density arrays.[9,10] Although this approach has previously worked well in prostate tissue, the inherent tumor heterogeneity, and low tumor content in TRUS biopsy samples results in TMAs where tumor content

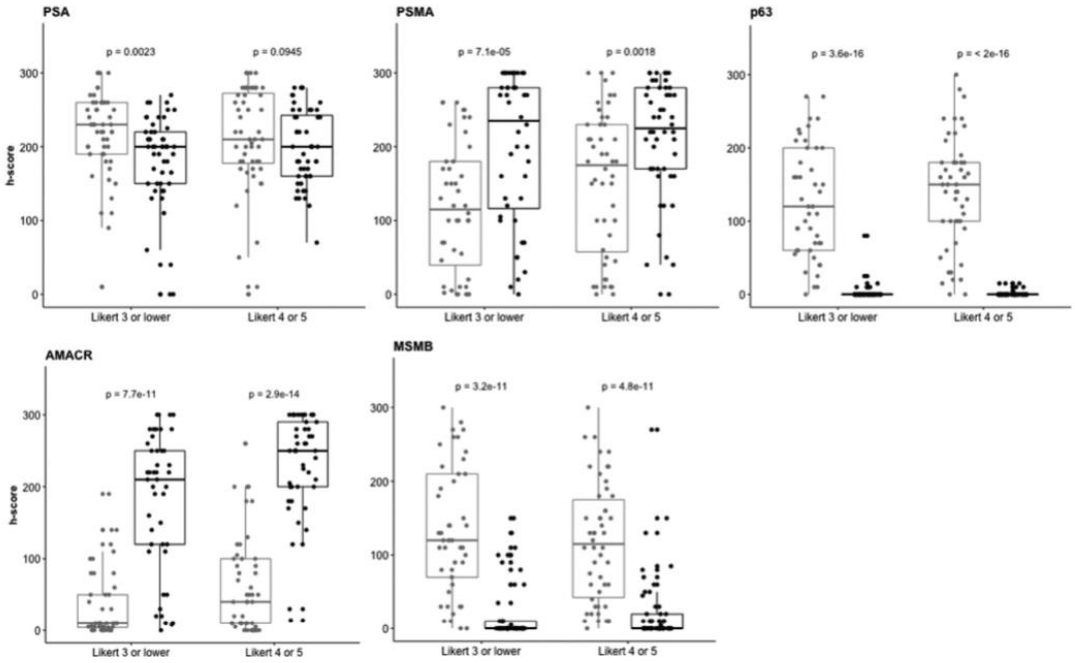

FIGURE 4 TMA IHC h-scores versus appearance on prostate mpMRI: All IHC was analyzed using h-score method that takes into account staining intensity and the number of positively stained cells. H-scores for paired samples only in tumor tissue (black) and benign tissue (gray) are shown. mpMRIs were graded using Likert score, a 5-point ordinal scalewhere Likert scores 1-2, 3, and 4-5 reflect a low, equivocal, and high probability of underlying clinically significant disease, respectively. For this analysis non-visible mpMRI areas were defined as Likert 1-3 and visible lesions as Likert 4/5. A detailed breakdown of paired and unpaired h-scores combined against all Likert scores is provided in Supplementary Figure S2. H-scores were compared using a Wilcoxon test

is often low or missing. In addition, true TMA performance is not routinely reported, at least meticulously.[11,12]

The TMA we describe here contains only patient-matched MRI-characterized tissue cores containing the highest disease burden from 5 mm TPM biopsies or adjacent benign tissue. By using 2 mm core segments rather than entire needle biopsy cores we were able to ensure a significant degree of homogeneity and produce high-quality TMA measures (Table 2). Excluding missing/not assessable cores or cores containing only stroma, the IHC and H&E concordance rate was greater than 90% for all stains. This uggests that, in instances where a core section ispresent for h-scoring and contains epithelial tissue, the scorer can be fairly confident that it contains benign or tumor tissue as originally intended in the H&E cutting plan. The numbers of missing, un-assessable, stroma-containing, concordant, or discordant cores were very similar between the five stains suggesting inter-slide reproducibility. We also demonstrated that tissue consistency was, on the whole, preserved along the entire TMA block, with concordance at two or more levels reaching almost 82%. TMA performance metrics are generally under-reported and our method of measuring performance could be widely adopted to facilitate comparisons between different needle biopsy TMA construction methods.

To demonstrate the tissue within the TMA is suitable for IHC it was used to assess expression of five widely used prostate biomarkers PSA, PSMA, p63, AMACR, and MSMB.[17–20] The differential expression of PSA, PSMA, p63, AMACR, and MSMB (as represented by h-scores) differed considerably between malignant and paired, neighboring benign tissue (Figure 2C) although h-scores within each of the benign or tumor groups were not associated with either Likert score or Gleason grade (Figure 3 and Supplementary Figure S2). However, when Likert score was grouped into lower risk (3 or lower) and higher risk (Likert 4/5) significant differences were seen for PSMA, p63, AMACR, and MSMB, but not PSA in both risk groups (Figure 4).

Although the tissue was obtained through extensive TPM biopsies outside the standard of care we have demonstrated the feasibility, reproducibility, and effectiveness of this TMA construction method and propose that it is possible reproduce similar results with standard TURP or image-guided biopsies. This TMA represents a unique paired tissue, high quality, resource with clinical, and radiological data which will allow validation of novel biomarkers correlated with imaging using a large number of biologically relevant patient samples.

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CONFLICTS OF INTEREST

The authors certify that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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