Predictive value of AZGP1 following radical prostatectomy for prostate cancer: a cohort study and meta-analysis

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jclinpath-2019-205940).

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Received 30 April 2019
Revised 3 June 2019
Accepted 7 June 2019

To cite: Kristensen G, Berg KD, Toft BG, *et al*. *J Clin Pathol* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jclinpath-2019-205940

ABSTRACT

Aims Zinc-alpha 2-glycoprotein (AZGP1) is a promising tissue biomarker to predict outcomes in men undergoing treatment for localised prostate cancer (PCa). We aimed to examine the association between AZGP1 expression and the endpoints: risk of biochemical failure (BF), initiating castration-based treatment, developing castration-resistant PCa (CRPC) and PCa-specific mortality following radical prostatectomy (RP).

Methods The study included a prospective cohort of 302 patients who underwent RP for PCa from 2002 to 2005. AZGP1 expression was analysed using immunohistochemistry on tissue microarray RP specimens and was scored semiquantitively as low or high expression. Risk of all endpoints was analysed using stratified cumulative incidences and cause-specific Cox regression, and validated with receiver operating curves, calibration and discrimination in competing-risk analyses. A meta-analysis was performed including previous studies investigating AZGP1 expression and risk of BF following RP.

Results Median time of follow-up was 14.0 years. The cumulative incidence of all endpoints was significantly higher in patients with low AZGP1 expression compared with patients with high AZGP1 expression (p<0.001). In a multivariate analysis, low AZGP1 expression increases the risk of BF (HR 2.7; 95% CI 1.9 to 3.8; p<0.0001), castration-based treatment (HR 2.2; 95% CI 1.2 to 4.2; p=0.01) and CRPC (HR 2.3; 95% CI 1.1 to 5.0; p=0.03). Validation showed a low risk of prediction error and a high model performance for all endpoints. In a meta-analysis, low AZGP1 was associated with BF (HR 1.7; 95% CI 1.2 to 2.5).

Conclusions Low AZGP1 expression is associated with the risk of aggressive time-dependent outcomes in men undergoing RP for localised PCa.

INTRODUCTION

Biomarkers that reflect the malignant potential of the tumour could refine prognostication of prostate cancer (PCa). Currently, a combination of clinical, biochemical and pathological variables is used[1–3]; however, these variables lack accuracy in predicting PCa outcomes on a patient-based level. Many potential biomarkers for PCa aggressiveness have been reported in the literature, but only few have entered clinical practice despite a clear clinical need for optimisation of prognostication. This may be due to the fact that most biomarkers have been tested for surrogate endpoints including biochemical failure (BF), whereas long-term endpoints such as time to metastatic disease and death are rarely investigated. A valid biomarker could change prognostication after RP and also define subgroups of patients for individualised follow-up or candidates for adjuvant therapies.

Zinc-alpha 2-glycoprotein (AZGP1) is a secretory protein that is encoded by the *AZGP1* gene located on chromosome 7q22.1. AZGP1 is important in lipid metabolism, glucose utilisation and regulation of insulin sensitivity and is found in most body fluids and secretory epithelial cells in several organs, including the prostate. The production of AZGP1 is regulated by different hormones including androgens.[4] Several studies have found that low AZGP1 expression is associated with BF following radical prostatectomy (RP).[5–11] However, the predictive value of AZGP1 as a marker for later and more adverse outcomes has received limited attention.

The primary goal of the present study was to assess the predictive value of AZGP1 expression in tumour tissue for the risk of initiating castration-based treatment, development of castration-resistant prostate cancer (CRPC) and PCa-specific mortality following RP. Furthermore, we aimed to investigate the association between AZGP1 expression and BF following RP and compare our results with previous studies in a meta-analysis.

MATERIALS AND METHODS

Cohort study

Patients

The study includes a historical consecutive series of patients who underwent RP for PCa at the Department of Urology, Rigshospitalet, Copenhagen University Hospital, Denmark between 1 January 2002 and 31 December 2005. We used tissue microarrays (TMAs) including malignant and benign prostate tissue from the patient's formalin-fixed, paraffin-embedded RP specimens. The collection of clinicopathological data and production of the TMAs have been described previously in detail.[12] The study was approved by the Danish National Committee on Health Research Ethics (Journal.no. H-6-2014-111) and The Danish Data Protection Agency (file#2006-1-6256).

RP Gleason Score (GS) was reclassified according to the 2005 International Society of Urological

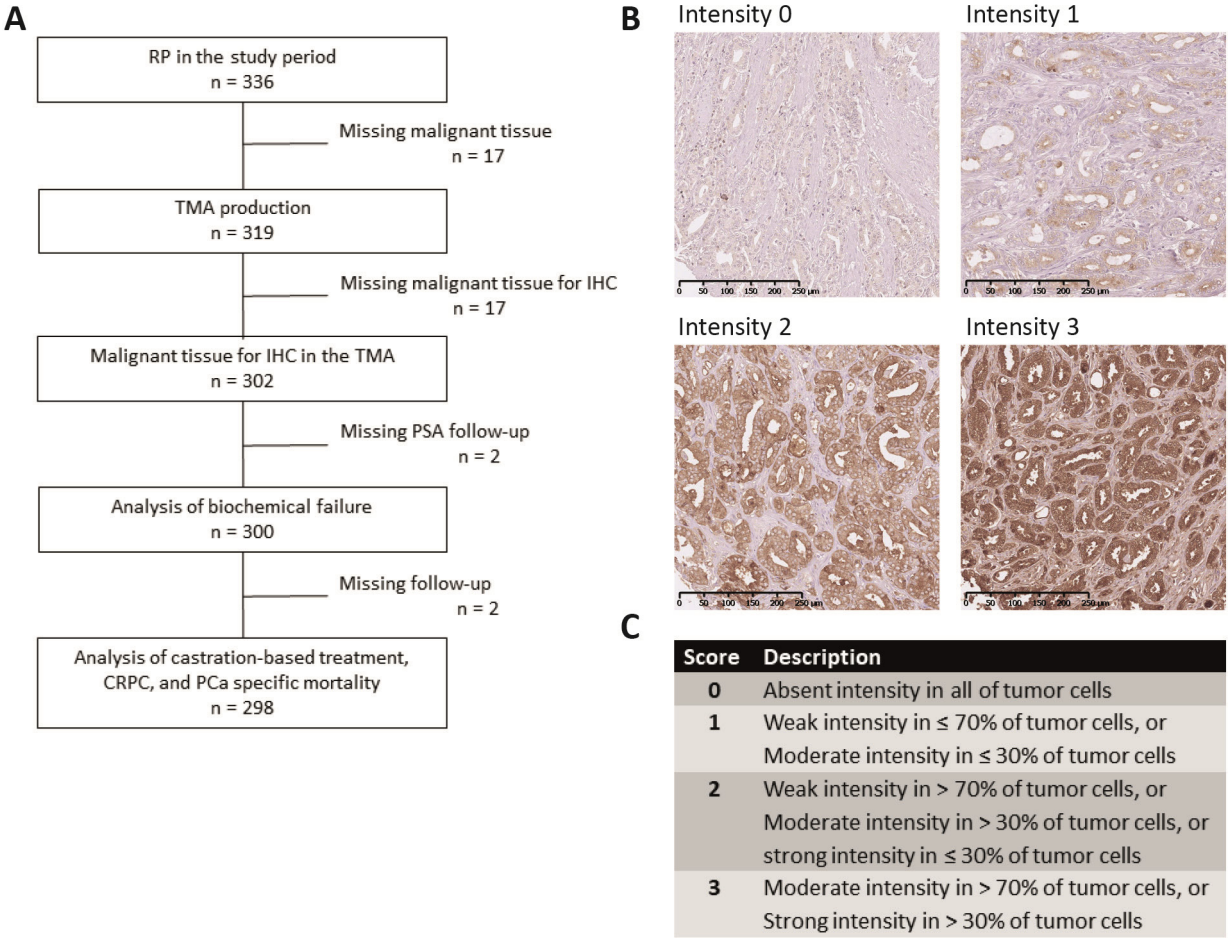

Figure 1 (A) Flowchart of patients included in the cohort study. (B) Immunohistochemical AZGP1 staining in representative prostate cancer samples showing absent, weak, moderate and strong intensity. (C) AZGP1 score table. CRPC, castration-resistant prostate cancer; IHC, immunohistochemistry; PCa, prostate cancer; PSA, prostate specific antigen; RP, radical prostatectomy; TMA, tissue microarray.

Pathology[13] Gleason grading system.[13] The study endpoints (BF, castration-based treatment, CRPC[14] and PCa-specific mortality) were updated in October 2018. Patients were excluded if malignant tissue was not present in the TMA for analysis of the biomarker status (figure 1A).

Immunohistochemistry

TMA tissue sections were used for immunohistochemical (IHC) staining of AZGP1 (1:500 dilution, anti-AZGP1: HPA012582; Sigma Aldrich) as described previously.[10] The stained slides were digitalised using the Hamamatsu Nano ZoomerXR at a magnification equivalent to ×20. Evaluation of the AZGP1-stained slides was done by using the Hamamatsu NDP.view V.2.6.13 viewing software. Whole field inspection of each core was carried out by one observer (GK) to evaluate the presence of cancer and benign glands as well as AZGP1 immunoreactivity. Each core on the TMA was given a score from 0 to 3 based on the cytoplasmic staining intensity (absent, weak, moderate and strong) and the fraction of positive tumour cells (figure 1B–C).[15] The AZGP1 scoring was checked on random cores by an experienced uropathologist (BGT). As each patient had multiple cores, a mean AZGP1 score (∑score for each core/number of cores) was calculated for each patient. This score was then dichotomised, and the patients were classified as having low AZGP1 expression (mean AZGP1 score ≤1.5) or high AZGP1 expression (mean AZGP1 score >1.5). Scoring of AZGP1 immunoreactivity was performed blinded to study endpoints.

Statistics

Association of AZGP1 expression in tumour tissue and clinicopathological variables was analysed using χ^2 test for categorical variables and Mann-Whitney U test for continuous variables. Median follow-up time was calculated using the reverse Kaplan-Meier method.[16] Follow-up was calculated until the latest follow-up date.

Cumulative incidences of study endpoints were analysed using the Aalen-Johansen method for competing risks. Death before BF, castration-based treatment and CRPC were treated as competing events when analysing risk of BF, castration-based treatment and CRPC, respectively. Other cause mortality was treated as a competing event when analysing risk of PCa-specific mortality. Gray's test was used to assess differences in the cumulative incidence of the endpoints between different AZGP1 expression.[17]

Univariate and multivariate cause-specific Cox proportional-hazards regression models were performed for risk of BF, castration-based treatment, CRPC and PCa-specific mortality, with results presented as HRs and 95% CIs. The analyses included age at RP, log2-transformed prostate specific antigen, pT-stage, pN-stage, RP GS, margin status and AZGP1 expression. The sensitivity and specificity of the models with and without AZGP1 expression were analysed using receiver operating characteristic curves and quantified using the areas under the curve (AUC) for selected time points. Changes in the predicted risk of BF, castration-based treatment and CRPC due to adding AZGP1 expression to the final models were assessed by reclassification diagrams and calibration plots

Table 1 Baseline characteristic

	Study population n=302	AZGP1 low n=128	AZGP1 high n=174	P value
Age at baseline, years, median (IQR)	62.9 (59.4–66.5)	63.2 (60.7–66.4)	62.3 (59.1–66.6)	0.2
Neoadjuvant treatment, n (%)				0.3
No	294 (97.4%)	126 (98.4%)	168 (96.6%)	
Yes	8 (2.6%)	2 (1.6%)	6 (3.4%)	
PSA, µg/L, median (IQR)	10.0 (6.8–15.0)	11.0 (7.4–16.0)	9.4 (6.1–14.0)	0.04
Clinical T-stage, n (%)				0.1
cT1	150 (49.7%)	55 (43.0%)	95 (54.6%)	
cT2a/b/c	145 (48.0%)	70 (54.7%)	75 (43.1%)	
cT3a/b	7 (2.3%)	3 (2.3%)	4 (2.3%)	
Biopsy Gleason Score, n (%)				*0.4*
≤6	203 (75.7%)	88 (72.1%)	115 (78.8%)	
3+4	46 (17.2%)	22 (18.0%)	24 (16.4%)	
4+3	5 (1.9%)	3 (2.5%)	2 (1.4%)	
8–10	14 (5.2%)	9 (7.4%)	5 (3.4%)	
Missing	34	6	28	
Biopsies, n (%)				0.8
<6	7 (2.4%)	3 (2.4%)	4 (2.4%)	
6–9	238 (81.5%)	103 (82.4%)	135 (80.8%)	
10–12	31 (10.6%)	11 (8.8%)	20 (12.0%)	
>12	16 (5.5%)	8 (6.4%)	8 (4.8%)	
PPB, %, median (IQR)	33.3 (16.7–50.0)	33.3 (16.7–66.7)	33.3 (16.7–50.0)	0.09
Pathological T-stage, n (%)				0.004
pT2	193 (63.9%)	70 (54.7%)	123 (70.7%)	
pT3	109 (36.1%)	58 (45.3%)	51 (29.3%)	
N-stage, n (%)				0.04
N0/x	296 (98.0%)	123 (96.1%)	173 (99.4%)	
N1	6 (2.0%)	5 (3.9%)	1 (0.6%)	
RP Gleason Score, n (%)				<0.0001
≤6	118 (39.1%)	31 (24.2%)	87 (50.0%)	
3+4	108 (35.8%)	47 (36.7%)	61 (35.1%)	
4+3	49 (16.2%)	34 (26.6%)	15 (8.6%)	
8–10	27 (8.9%)	16 (12.5%)	11 (6.3%)	
Margin status, n (%)				0.2
R–	125 (41.4%)	48 (37.5%)	77 (44.3%)	
R+	177 (58.6%)	80 (62.5%)	97 (55.7%)	

P value: Mann-Whitney U test for continuous variables and χ^2 test for categorical variables.
PPB, percent positive biopsies; PSA, prostate specific antigen; RP, radical prostatectomy.

for selected time points.[18] The change in discrimination ability (c-index) and prediction error (Brier score) due to adding AZGP1 expression were assessed throughout the study period.[19]

All tests were two-sided and p values <0.05 were considered as statistically significant. All statistical analyses were performed using SPSS V.22 or R (R Development Core Team, Vienna, Austria).

Meta-analysis

Studies addressing the association between IHC-based AZGP1 protein expression in tumour tissue from RP specimen and risk of developing BF following RP were identified. Studies were identified by searching the PubMed database in February 2019. The systematic study selection process is summarised in online supporting figure 1. In brief, we identified 40 papers using the following search strategy: Zinc-alpha 2-glycoprotein or AZGP1 or ZAG and prostate or prostate cancer (excluding non-English papers and comments). We screened all 37 abstracts and reviewed 14 full articles for inclusion in the meta-analysis. We excluded two studies who only included RP specimens with positive surgical margins[20 21] and one study who only included patient with primary Gleason grade 4.[22] Data were extracted directly for the published material (n=6) and by contacting the corresponding author (n=1) by one investigator (GK). Data included study population, number of patients within each AZGP1 expression category (negative/absent/weak/low and moderate/high/strong), median time of follow-up, outcome data (HR with 95% CI and p value for multivariate Cox regression analysis) and covariates adjusted for in the studies. Results are presented in a forest plot.

RESULTS

Cohort study

The study included 302 patients who had available clinicopathological data and AZGP1 expression (figure 1). The median time of follow-up after RP was 14.0 years (95% CI 13.9 to 14.2). The association between clinicopathological variables and AZGP1 expression are outlined in table 1. The median number of malignant tissue cores available for IHC analysis was 6 (IQR 4–7) per patient. IHC analysis of AZGP1 in the malignant tissue

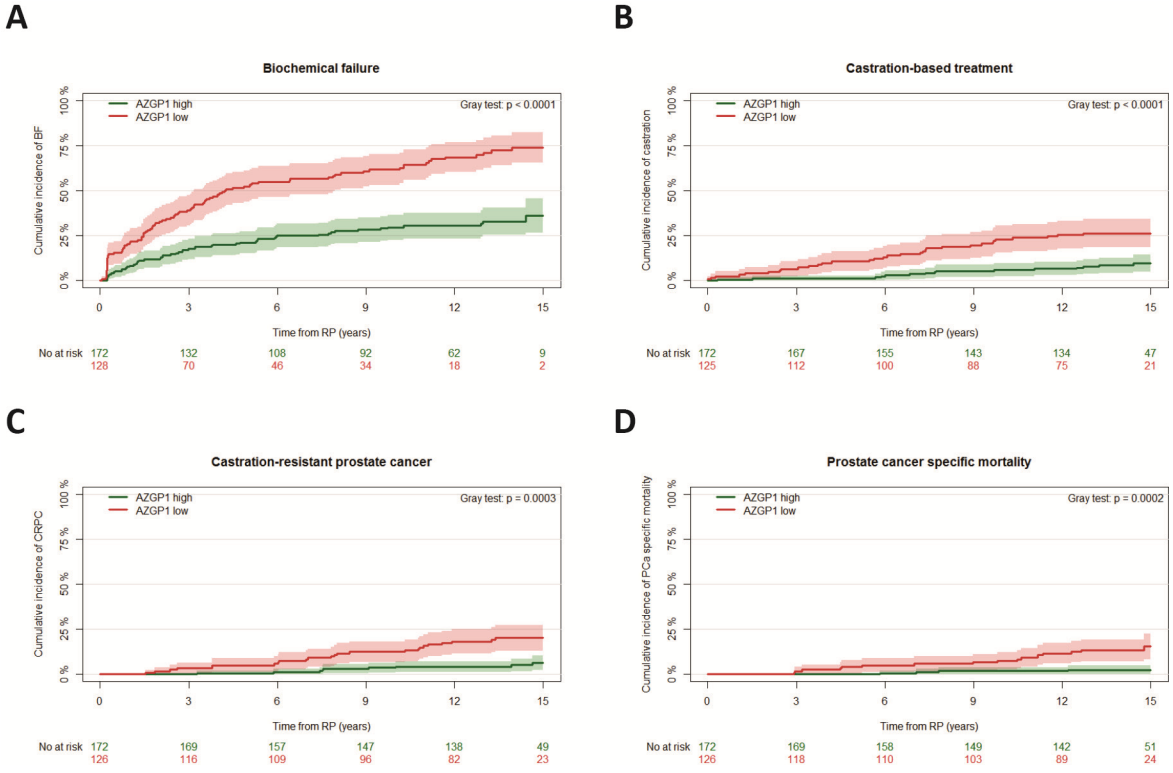

Figure 2 Cumulative incidence of (A) biochemical failure (BF), (B) castration-based treatment, (C) castration-resistant prostate cancer (CRPC) and (D) prostate cancer (PCa)–specific mortality following radical prostatectomy (RP). Competing event is (A) death without BF, (B) death without castration-based treatment, (C) death without CRPC and (D) death from other causes. Patients are stratified according to AZGP1 expression at RP. The p values for Gray's test are added.

Table 2 Univariate and multivariate cause-specific Cox proportional hazards of biochemical failure

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
AZGP1				
High	REF		REF	
Low	3.2 (2.3 to 4.6)	<0.0001	2.7 (1.9 to 3.8)	<0.0001
Age at RP				
For 5-year differences	1.0 (0.9 to 1.2)	0.7	0.9 (0.7 to 1.0)	0.09
PSA				
For 2-fold difference	1.5 (1.3 to 1.8)	<0.0001	1.3 (1.1 to 1.5)	0.007
Pathological T-stage				
pT2a/b/c	REF		REF	
pT3a/b	3.1 (2.2 to 4.3)	<0.0001	2.0 (1.4 to 2.9)	0.0002
N-stage				
N0/x	REF		REF	
N1	2.6 (1.0 to 7.1)	0.06	–	
RP Gleason Score				
≤6	REF		REF	
3+4	3.2 (2.0 to 5.0)	<0.0001	2.0 (1.3 to 3.2)	0.004
4+3	4.5 (2.7 to 7.4)	<0.0001	2.7 (1.6 to 4.5)	0.0003
8–10	5.7 (3.2 to 10.1)	<0.0001	2.9 (1.6 to 5.3)	0.0006
Margin status				
R–	REF		REF	
R+	2.4 (1.7 to 3.5)	<0.0001	1.6 (1.1 to 2.4)	0.02

PSA, prostate specific antigen; REF, reference; RP, radical prostatectomy.

showed that 128 (42.4%) had low AZGP1 expression and 174 (57.6%) had high AZGP1 expression. Furthermore, a total of 293 patients had benign prostate tissue available for analysis of AZGP1 expression. Of these, the majority (96.9%) had high AZGP1 expression while only few (3.1%) had low AZGP1 expression in adjacent benign prostatic epithelial cells.

Biochemical failure

The 10-year cumulative incidence of BF was 43.4% (95% CI 37.7 to 49.1). The 10-year cumulative incidence of BF was 61.6% (95% CI 53.1 to 70.2) in the AZGP1 low group compared with 29.9% (95% CI 22.6 to 36.6) in the AZGP1 high group (p<0.0001) (figure 2A). Multivariate analysis showed that low AZGP1 expression was an independent predictor of BF (HR 2.7; 95% CI 1.9 to 3.8; p<0.0001) (table 2). Evaluation of the cause-specific Cox regression model performance for BF with and without AZGP1 expression are shown in online supporting figure 2. In brief, including AZGP1 expression in the model showed improvement in the discriminatory accuracy of the model for predicting BF within 5 years compared with the model without AZGP1 expression (AUC 80.8% vs 77.9%; p=0.07).

Castration-based treatment, CRPC and prostate cancer-specific mortality

The 10-year cumulative incidence of castration-based treatment, CRPC and PCa-specific mortality was 13.1%, 7.3% and 4.1%, respectively. Overall, 48 initiated castration-based treatment, 32 developed CRPC and 21 died from PCa in the study cohort.

Table 3 Univariate and multivariate cause-specific Cox proportional hazards

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
(A) Initiation of castration-based treatment				
AZGP1				
High	REF		REF	
Low	3.5 (1.9 to 6.4)	<0.0001	2.2 (1.2 to 4.2)	0.01
Age at RP				
For 5-year differences	1.2 (0.9 to 1.6)	0.3	–	
PSA				
For 2-fold difference	1.4 (1.0 to 1.8)	0.03	1.0 (0.8 to 1.4)	0.7
Pathological T-stage				
pT2a/b/c	REF		REF	
pT3a/b	4.1 (2.2 to 7.4)	<0.0001	2.7 (1.4 to 5.0)	0.002
N-stage				
N0/x	REF		REF	
N1	8.3 (3.0 to 23.3)	<0.0001	–	
RP Gleason Score				
≤6	REF		REF	
7	7.8 (2.8 to 22.0)	0.0001	4.5 (1.5 to 13.4)	0.007
8–10	16.7 (5.1 to 54.8)	<0.0001	9.9 (2.9 to 33.7)	0.0003
Margin status				
R–	REF		REF	
R+	1.8 (1.0 to 3.3)	0.06	–	
(B) Development of castration-resistant prostate cancer				
AZGP1				
High	REF		REF	
Low	3.7 (1.8 to 7.8)	0.0005	2.3 (1.1 to 5.0)	0.03
Age at RP				
For 5-year differences	1.0 (0.7 to 1.4)	0.9	–	
PSA				
For 2-fold difference	1.4 (1.0 to 2.0)	0.07	–	
Pathological T-stage				
pT2a/b/c	REF		REF	
pT3a/b	7.0 (3.2 to 15.5)	<0.0001	4.8 (2.1 to 10.9)	0.0001
N-stage				
N0/x	REF		REF	
N1	7.2 (2.2 to 23.7)	0.001	–	
RP Gleason Score				
≤6	REF		REF	
7	7.1 (2.2 to 23.7)	0.001	3.6 (1.0 to 12.4)	0.04
8–10	13.6 (3.4 to 54.4)	0.0002	6.8 (1.6 to 27.8)	0.008
Margin status				
R–	REF		–	
R+	1.9 (0.9 to 4.1)	0.08	–	

PSA, prostate specific antigen; REF, reference; RP, radical prostatectomy.

Cumulative incidence curves demonstrated that low AZGP1 expression compared with high AZGP1 expression was significantly associated with castration-based treatment (p<0.0001), CRPC (p=0.0003) and PCa-specific mortality (p=0.0002) (figure 2B–D).

Modified multivariate cause-specific Cox regression models showed that low AZGP1 expression was an independent predictor of castration-based treatment (HR 2.2; 95% CI 1.2 to 4.2; p=0.01) and CRPC (HR 2.3; 95% CI 1.1 to 5.0; p=0.03) (table 3). The risk reclassification plot showed higher risk of castration-based treatment for patients with low AZGP1 expression within 5 years compared with patients with high AZGP1 expression (figure 3A). Calibration of the model including AZGP1 expression showed high agreement between predicted and observed probabilities of castration-based treatment within 5 years (figure 3B). We found a lower prediction error when AZGP1 expression was included in the model throughout the study period (figure 3C). Likewise, including AZGP1 expression in the model improved the discriminatory accuracy of the model for predicting initiation of castration-based treatment within 5 years compared with the model without AZGP1 expression (AUC 83.0% vs 78.0%; p=0.002) (figure 3D). Moreover, including AZPG1 expression in the model showed a higher change in discriminatory ability compared with the model without AZGP1

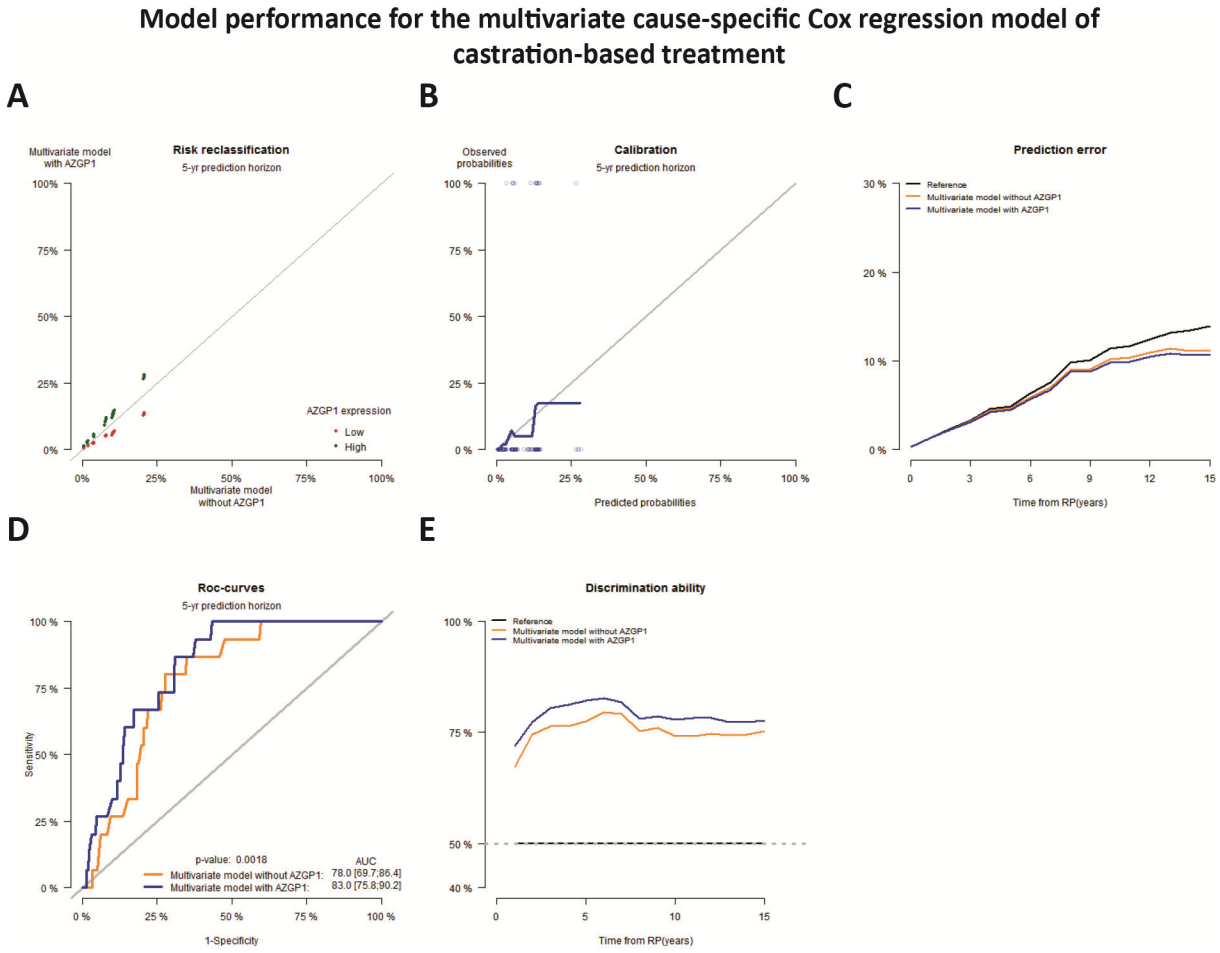

Figure 3 Evaluation of the model performance for the multivariate cause-specific Cox regression model of castration-based treatment with and without AZGP1 expression. (A) The effect of AZGP1 for predicting risk of castration-based treatment. The scatterplot shows the predicted risk of castration-based treatment within 5 years following radical prostatectomy (RP) for low and high AZGP1 expression. (B) The calibration of the model with AZGP1 expression showing the agreement between predicted and observed probabilities of castration-based treatment within 5 years following RP. (C) Prediction error for the model with and without AZGP1 expression throughout the study period. (D) Receiver operating characteristic (ROC) curves for the models for predicting castration-based treatment within 5 years following RP. Area under the ROC curve (AUC) and p values for the comparative test are added. (E) discriminatory ability (c-index) for the model with and without AZGP1 expression throughout the study period.

expression throughout the study period (figure 3E). Evaluation of the cause-specific Cox regression model performance for CRPC are shown in online supporting figure 3.

In univariate cause-specific Cox proportional hazard analysis, low AZGP1 expression was associated with PCa-specific mortality (HR 6.4; 95% CI 2.2 to 19.1; p=0.0008). Furthermore, a low AZGP1 expression was an independent predictor of PCa-specific mortality (HR 5.7; 95% CI 1.9 to 17.2; p=0.002) in a multivariate model including AZGP1 expression and RP GS (RP GS ≤7 vs RP GS 8–10).

Meta-analysis

A total of eight cohort studies including the present were identified for inclusion in the meta-analysis. Characteristic of the included cohort studies are presented in online supporting table 1. The prevalence of low AZGP1 expression in the RP specimen across the cohorts was 56.5%. The HR from the eight cohorts, for the association between AZGP1 expression (high AZGP1 vs low AZGP1) and BF, are presented in figure 4. A total of 11 384 patients were included in the meta-analysis. Low AZGP1 expression was associated with BF (HR 1.7; 95% CI 1.2 to 2.5) across the eight cohorts included, when weighted by number of patients and median time of follow-up (figure 4).

DISCUSSION

Despite comprehensive research activity concerning IHC-based tissue biomarkers in PCa, none of the proposed markers have been implemented in clinical practice. This may be explained by lack of meaningful validation studies, which are mandatory to evaluate the true value of the biomarker.[23] Furthermore, the lack of consensus in the selection of primary antibodies hampers validation for proposed biomarkers. Finally, few studies of PCa biomarkers used for prognostication of early staged disease include hard endpoints such as metastasis and PCa-specific mortality.

In the present study, we found that AZGP1 protein expression in tumour tissue is an independent predictive marker of BF following RP. We demonstrated that inclusion of AZGP1 in the predictive model with known predictive variables improves the accuracy of the model. This finding is in accordance with several previous studies all having demonstrated the predictive value of AZGP1 expression assessed by IHC as a marker

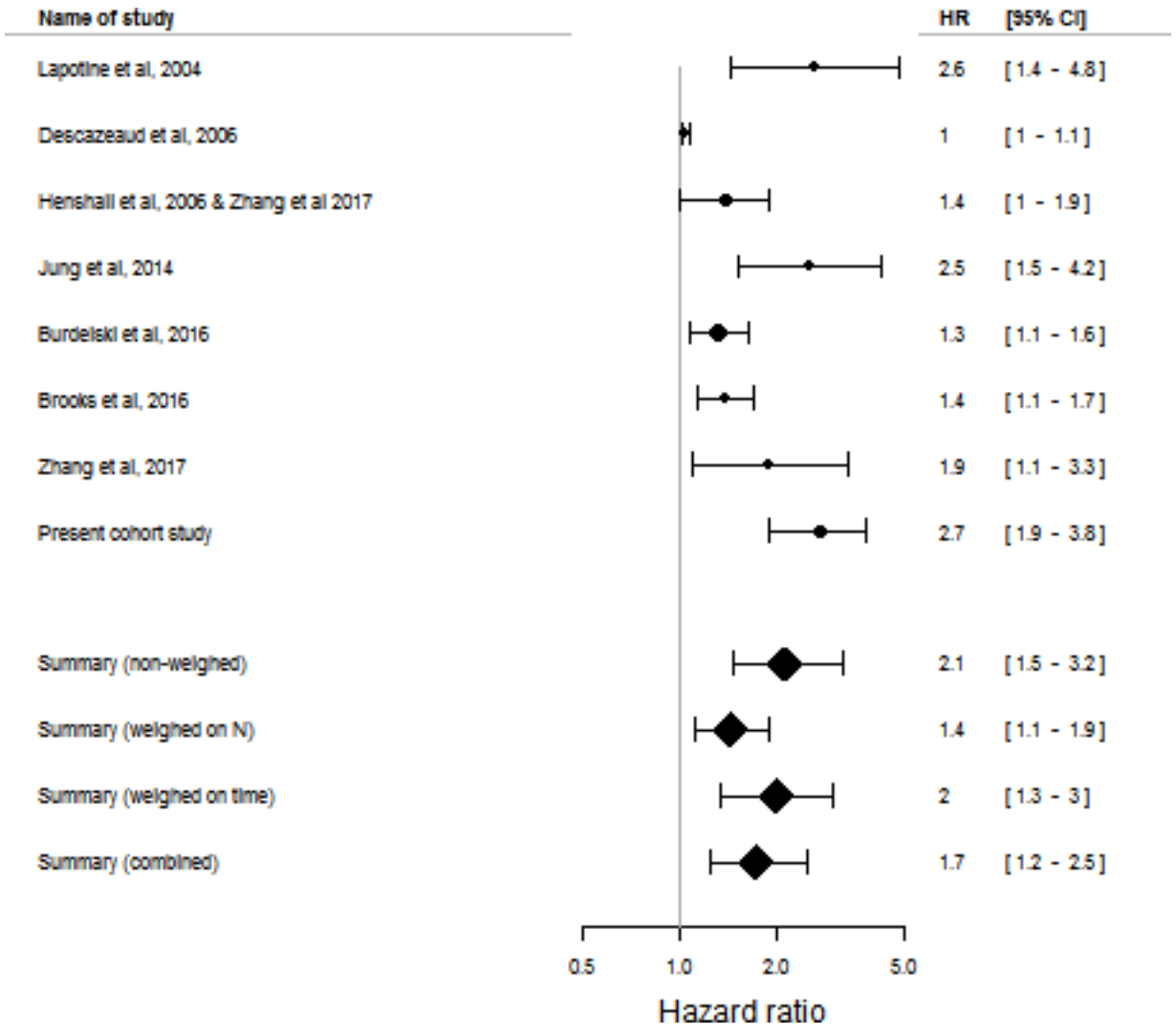

Figure 4 Data on AZGP1 expression and the risk of developing biochemical failure following radical prostatectomy.

for BF following RP.[5–11] As our cohort has accumulated long-term follow-up, we were able to investigate disease-relevant PCa outcomes. We found that AZGP1 is an independent predictor of initiating castration-based treatment and development of CRPC. Moreover, we found that AZGP1's ability to predict initiation of castration-based treatment and CRPC outperformed its ability to predict BF. Finally, our data support that AZGP1 is a predictor of PCa-specific mortality following RP.

Most studies use prediction of adverse pathology or BF as endpoints, which can both be influenced by technical features of surgery such as positive surgical margins that correlate only modestly with progression to metastasis and prostate cancer–specific death. Use of BF restricts clinical application of any biomarker, as a large proportion of patients with BF will not develop metastatic disease nor die from PCa within 10–15 years after BF, even without receiving additional treatment.[24] Recently, Zhang *et al*[11] published the first prospective multicentre validation study of AZGP1 demonstrating that low AZGP1 expression was an independent predictive marker of BF. Furthermore, they updated the follow-up of their previously published retrospective study[7 11] to a median follow-up time of 15.8 years and found that AZGP1 was also an independent predictor of metastatic disease.

The production of AZGP1 in tumour tissue has been shown to be associated with tumour differentiation.[25] The association between AZGP1 and cancer development and progression are not fully understood; however, several hypotheses have been suggested. First, it has been shown that AZGP1 RNA increases with androgen stimulation of the LNCaP cell line[26] indicating that loss of AZGP1 is associated with aggressive, androgen-independent PCa. Second, AZGP1 expression is found to be prognostic in several different adenocarcinomas[27–30]; thus, AZGP1 may play a role in carcinogenesis more broadly. Third, AZGP1 is known to be important in lipid metabolism, glucose metabolism and regulation of insulin sensitivity,[4] and one could speculate that AZGP1 plays a role in the link between lipid metabolism and cancer development.[31]

AZGP1 has been investigated in several RP cohorts, all showing that AZGP1 is an independent predictor of PCa outcomes.[5–11] No other single IHC-based marker, except PTEN, has shown to perform this well.[32] While IHC-based tissue biomarkers are subject to less standardisation than commercially available gene expression assays, it has been shown that PTEN has similar discrimination of PCa outcomes compared with the cell-cycle progression score in a model that incorporates the CAPRA-S score.[33] As prognostic testing with IHC has lower cost

compared with gene expression assays, it will be worth testing directly whether AZGP1 IHC will perform similarly to those gene expression assays.

The present study has limitations in addition to the ones associated with a retrospective study design. First, PCa is known to display intratumoural and intraprostatic heterogeneity[34] and, as with all TMA-based studies, we only investigated a small fraction of the total tumour tissue from the RP specimen for AZGP1 expression, and this sampling error could influence the reliability of the biomarker status of the patients in this study. However, four malignant cores per patient have been shown to be sufficient to analyse biomarker status to predict PCa outcome,[35] and we used a median of six malignant cores per patient. Second, we used a polyclonal antibody for IHC analysis of AZGP1 expression while acknowledging that a monoclonal antibody could have a higher specificity to the same epitope of an antigen. Finally, AZGP1 scoring of the IHC staining's was done semi-quantitatively by one observer. However, interobserver agreement has previously shown to be high for IHC-based assessment of AZGP1.[11]

Given the growing evidence that AZGP1 is a strong, independent predictor of outcome following RP for PCa, further work should focus on correlation of AZGP1 expression in biopsies with that found at RP, and testing in other clinical contexts, such as patients treated with radiation therapy and those managed with active surveillance. Furthermore, AZGP1 have been isolated in blood and urine from patients with PCa,[36 37] but the clinical utility of AZGP1 level as a blood-based or urine-based biomarker for PCa aggressiveness needs to be elucidated.

CONCLUSIONS

AZGP1 expression in tumour tissue at RP is an independent predictor for clinically relevant outcomes following RP for localised PCa. We demonstrate that AZGP1 is a better marker for predicting adverse outcomes compared with its ability to predict BF, likely reflecting a correlation of loss of expression with aggressive biological features.

Take home messages

- Immunohistochemical analysis of malignant tissue showed that 42.4% of patients had low AZGP1 expression while 57.6% had high AZGP1 expression.
- Low AZGP1 expression is associated with biochemical failure (BF), castration-based treatment, castration-resistant prostate cancer (CRPC) and prostate cancer–specific mortality.
- AZGP1 expression is an independent predictor of developing BF, initiating castration-based treatment and development of CRPC.
- AZGP1's ability to predict initiation of castration-based treatment and CRPC outperformed its ability to predict BF.
- Low AZGP1 expression was associated with BF in a meta-analysis of eight cohort studies.

Handling editor Dhirendra Govender.

Contributors GK, KDB, JDB, KB and MAR designed the study. GK drafted the manuscript which was critically reviewed by all other authors. BGT, JDB, KB and MAR provided study supervision. KDB and MAR contributed to data collection. GK, HVS and RN performed data analysis and all authors contributed to data interpretation.

Funding This study is sponsored by grants from the Danish Cancer Society and Beckett-Foundation.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Danish National Committee on Health Research Ethics (journal no. H-6-2014-111) and the Danish Data Protection Agency (file no. 2006-1-6256)

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

REFERENCES

1 D'Amico AV*et al*. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA* 1998;280:969–74.
2 Cooperberg MR, Pasta DJ, Elkin EP, *et al*. The University of California, San Francisco Cancer of the Prostate Risk Assessment score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. *J Urol* 2005;173:1938–42.
3 Cooperberg MR, Hilton JF, Carroll PR. The CAPRA-S score: a straightforward tool for improved prediction of outcomes after radical prostatectomy. *Cancer* 2011;117:5039–46.
4 Wei X, Liu X, Tan C, *et al*. Expression and function of Zinc-α2-glycoprotein. *Neurosci Bull*.
5 Lapointe J, Li C, Higgins JP, *et al*. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. *Proc Natl Acad Sci* 2004;101:811–6.
6 Descazeaud A, de la Taille A, Allory Y, *et al*. Characterization of ZAG protein expression in prostate cancer using a semi-automated microscope system. *Prostate* 2006;66:1037–43.
7 Henshall SM, Horvath LG, Quinn DI, *et al*. Zinc-alpha2-glycoprotein expression as a predictor of metastatic prostate cancer following radical prostatectomy. *J Natl Cancer Inst* 2006;98:1420–4.
8 Jung WY, Sung CO, Han SH, *et al*. AZGP-1 immunohistochemical marker in prostate cancer: potential predictive marker of biochemical recurrence in post radical prostatectomy specimens. *Appl Immunohistochem Mol Morphol* 2014;22:652–7.
9 Burdelski C, Kleinhans S, Kluth M, *et al*. Reduced AZGP1 expression is an independent predictor of early PSA recurrence and associated with ERG-fusion positive and *PTEN* deleted prostate cancers. *Int J Cancer* 2016;138:1199–206.
10 Brooks JD, Wei W, Pollack JR, *et al*. Loss of expression of AZGP1 is associated with worse clinical outcomes in a multi-institutional radical prostatectomy cohort. *Prostate* 2016;76:1409–19.
11 Zhang AY, Grogan JS, Mahon KL, *et al*. A prospective multicentre phase III validation study of AZGP1 as a biomarker in localized prostate cancer. *Ann Oncol* 2017;28:1903–9.
12 Kristensen G, Røder MA, Berg KD, *et al*. Predictive value of combined analysis of pro-NPY and ERG in localized prostate cancer. *APMIS* 2018;126:804–13.
13 Epstein JI, Allsbrook WC, Amin MB, *et al*. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. *Am J Surg Pathol* 2005;29:1228–42.
14 Cornford P, Bellmunt J, Bolla M, *et al*. EAU-ESTRO-SIOG guidelines on prostate cancer. Part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. *Eur Urol* 2017;71:630–42.
15 Simon R, Mirlacher M, Sauter G. Immunohistochemical analysis of tissue microarrays. *Methods Mol Biol* 2010;664:113–26.
16 Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials* 1996;17:343–6.
17 Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat* 1988;16:1141–54.
18 Gerds TA, Kattan MW, Schumacher M, *et al*. Estimating a time-dependent concordance index for survival prediction models with covariate dependent censoring. *Stat Med* 2013;32:2173–84.
19 Steyerberg EW. *Clinical prediction models: a practical approach to development, validation, and updating*. New York, NY: Springer-Verlag, 2009.
20 Yip PY, Kench JG, Rasiah KK, *et al*. Low AZGP1 expression predicts for recurrence in margin-positive, localized prostate cancer. *Prostate* 2011;71:1638–45.
21 Bruce HM, Stricker PD, Gupta R, *et al*. Loss of AZGP1 as a superior predictor of relapse in margin-positive localized prostate cancer. *Prostate* 2016;76:1491–500.
22 Mills J, Oliver A, Sherwin JC, *et al*. Utility of RhoC and ZAG protein expression as biomarkers for prediction of pSa failure following radical prostatectomy for high grade prostate cancer. *Pathology* 2012;44:513–8.
23 Huber F, Montani M, Sulser T, *et al*. Comprehensive validation of published immunohistochemical prognostic biomarkers of prostate cancer—what has gone wrong? A blueprint for the way forward in biomarker studies. *Br J Cancer* 2015;112:140–8.
24 Antonarakis ES, Feng Z, Trock BJ, *et al*. The natural history of metastatic progression in men with prostate-specific antigen recurrence after radical prostatectomy: long-term follow-up. *BJU Int* 2012;109:32–9.
25 Hale LP, Price DT, Sanchez LM, *et al*. Zinc alpha-2-glycoprotein is expressed by malignant prostatic epithelium and may serve as a potential serum marker for prostate cancer. *Clin cancer Res* 2001;7:846–53.

26 Nelson PS, Clegg N, Arnold H, *et al*. The program of androgen-responsive genes in neoplastic prostate epithelium. *Proc Natl Acad Sci U S A* 2002;99:11890–5.
27 Huang Y, Li L-Z, Zhang C, *et al*. Decreased expression of zinc-alpha2-glycoprotein in hepatocellular carcinoma associates with poor prognosis. *J Transl Med* 2012;10.
28 Huang C-yu, Zhao J-jing, Lv L, *et al*. Decreased expression of AZGP1 is associated with poor prognosis in primary gastric cancer. *PLoS ONE* 2013;8:e69155.
29 Parris TZ, Kovács A, Aziz L, *et al*. Additive effect of the AZGP1, Pip, S100A8 and UBE2C molecular biomarkers improves outcome prediction in breast carcinoma. *Int J Cancer* 2014;134:1617–29.
30 Xue Y, Yu F, Yan D, *et al*. Zinc-α-2-glycoprotein: a candidate biomarker for colon cancer diagnosis in Chinese population. *Int J Mol Sci* 2014;16:691–703.
31 Allott EH, Masko EM, Freedland SJ. Obesity and prostate cancer: weighing the evidence. *Eur Urol* 2013;63:800–9.
32 Lotan TL, Wei W, Morais CL, *et al*. PTEN loss as determined by clinical-grade immunohistochemistry assay is associated with worse recurrence-free survival in prostate cancer. *Eur Urol Focus* 2016;2:180–8.
33 Leapman MS, Nguyen HG, Cowan JE, *et al*. Comparing prognostic utility of a single-marker immunohistochemistry approach with commercial gene expression profiling following radical prostatectomy. *Eur Urol* 2018;74:668–75.
34 Andreoiu M, Cheng L. Multifocal prostate cancer: biologic, prognostic, and therapeutic implications. *Hum Pathol* 2010;41:781–93.
35 Rubin MA, Dunn R, Strawderman M, *et al*. Tissue microarray sampling strategy for prostate cancer biomarker analysis. *Am J Surg Pathol* 2002;26:312–9.
36 Katafigiotis I, Tyritzis SI, Stravodimos KG, *et al*. Zinc α2-glycoprotein as a potential novel urine biomarker for the early diagnosis of prostate cancer. *BJU Int* 2012;110:E6 88–E693.
37 Bondar OP, Barnidge DR, Klee EW, *et al*. LC-MS/MS quantification of Zn- 2 glycoprotein: a potential serum biomarker for prostate cancer. *Clin Chem* 2007;53:673–8.