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Predictive Value of [−2]proPSA-Related Indices for Prostate Cancer Detection Years Before Prostate-Specific Antigen Elevation: Insights From a Five-Year Longitudinal Screening Cohort

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ABSTRACT

Background: [-2]proPSA(p2PSA)-related indices have demonstrated diagnostic value for detecting prostate cancer (PC) in males with modest increases in prostate-specific antigen levels (PSA) of < 10 ng/mL. However, the "leak point" of p2PSA level in the blood circulation before developing screen-detectable PC remains uncertain. Therefore, this study evaluated the ability of p2PSA-related indices in the years before considering conventional biopsy indications.

Methods: This case-control study analyzed database and serum bank information from a population-based screening cohort in Gunma Prefecture. The case group included 58 males diagnosed with PC due to increased PSA (4–10 ng/mL) followed serially for 5 years. The control group comprised 58 males without PC based on biopsy who were matched to the case group by adjusted PSA levels (± 2 ng/mL) and age (± 5 years) at the time of biopsy. p2PSA, free PSA, and PSA were measured from frozen serum samples from 0 to 5 years before biopsy.

Results: p2PSA-to-free PSA ratio (%p2PSA) and prostate health index (phi) were significantly higher in the case group than in the control group from five, three, and 3 years before biopsy, respectively. The areas under the receiver operating characteristic curve for predicting PC were 0.437 for PSA, 0.637 for phi, 0.663 for %p2PSA, and 0.618 for free PSA-to PSA ratio (%fPSA) at 3 years before biopsy.

Conclusions: p2PSA-related indices showed group-level differences between future PC cases and matched controls before PSA exceeded conventional cut-offs. These findings should be regarded as hypothesis-generating and require validation in prospective studies before clinical application to risk-stratified screening.

1 | Introduction

Prostate-specific antigen (PSA) is widely used for the early detection of prostate cancer (PC) and remains a key tumor marker in both screening and clinical practice [1, 2]. However, its limited specificity is a major limitation, as PSA levels may also be elevated in benign conditions such as benign prostatic hyperplasia and prostatitis. Consequently, false-positive findings can lead to unnecessary diagnostic procedures and biopsies, as well as overdiagnosis of clinically insignificant PC [2, 3]. Although the European Randomized Study of Screening for Prostate Cancer demonstrated that PSA-based screening can reduce PC mortality [1], optimizing the balance between benefits and harms remains a critical challenge. Minimizing missed clinically

significant PC (csPC) while reducing unnecessary biopsies and overdiagnosis is essential.

In this context, especially in males with PSA levels of ≤ 10 ng/mL, making biopsy decisions based on PSA alone may increase the number of unnecessary biopsies. Thus, more precise PSA-related biomarkers are needed to guide decision-making. Among such potential clinical markers, indices based on [-2]proPSA (p2PSA), including prostate health index (phi) and p2PSA/free PSA ratio (%p2PSA), have attracted attention. Previous studies in males with PSA levels ≤ 10 ng/mL have demonstrated the superior diagnostic accuracy of phi over PSA and free PSA-to PSA ratio (%fPSA), particularly for the detection of csPC [4–11]. Additionally, a meta-analysis demonstrated that phi has superior diagnostic performance compared with %fPSA and may help reduce unnecessary biopsies [12]. Moreover, a prospective study of males in Japan with PSA levels above the age-specific cutoff and < 10 ng/mL also showed that phi outperformed conventional markers and avoided some unnecessary biopsies while maintaining sensitivity [13].

Although phi values are known to be elevated at the time of PC diagnosis, it remains unclear at what point during PC development p2PSA begins to leak into the blood; that is, when phi and %p2PSA values start to rise before conventional PSA-based diagnosis. One report showed that males later diagnosed with PC had higher p2PSA levels as early as 4 years before their diagnosis [14]. However, within the framework of population-based PC screening, few studies have examined at what point along the prediagnostic timeline changes in p2PSA-related indices may distinguish males who will subsequently develop PC.

Therefore, in the present study, we analyzed serum samples collected during a long-term population-based PC screening program to investigate whether phi and %p2PSA can identify future PC cases years before biopsy or overt PSA elevation. Although a prior longitudinal report demonstrated that p2PSA itself begins to rise several years before clinical PC diagnosis [14], the prediagnostic longitudinal behavior of phi has not been characterized in a population-based screening setting. Through this analysis, we aimed to clarify the prediagnostic behavior of phi and %p2PSA to inform the future development of risk-stratified screening strategies.

2 | Materials and Methods

2.1 | Study Design and Participants

The present retrospective, nested case–control study analyzed data from the Gunma Prefecture PC screening database and samples from the Gunma University serum bank. The PC screening program covered Gunma Prefecture between 1992 and 2023, and was conducted by the Gunma Health Promotion Foundation on behalf of the municipalities. The study population comprised male participants aged ≥ 20 years who had undergone annual PC screening for a minimum of six consecutive years. Residual frozen serum samples obtained during this routine screening were stored with comprehensive participant consent for research use. The case cohort comprised 58 males who had undergone annual PC screening for a minimum of five consecutive years before prostate biopsy, had a total PSA level of 4–10 ng/mL at the time of biopsy, and were subsequently diagnosed with PC on prostate biopsy. The control cohort comprised 58 males who had undergone a minimum of five consecutive annual screening procedures, with a PSA level of 4–10 ng/mL at the corresponding screening visit. However, these males were not diagnosed with PC on prostate biopsy. The controls were matched to the case subjects for PSA level (± 2 ng/mL) and age (± 5 years) at the time of biopsy. In each instance, a single control was selected for the case subject. The PSA range was set at 4–10 ng/mL in this study because, in Japan, insurance coverage for phi testing is currently available for patients with PSA levels of 4–10 ng/mL, patients aged 50–64 with PSA levels of 3.0–10.0 ng/mL, and men aged 65–69 with PSA levels of 3.5–10.0 ng/mL. Furthermore, in Japanese PC screening guidelines, the PSA threshold is defined as either the aforementioned age-specific cutoff values or 4.0 ng/mL [15].

2.2 | Laboratory Measurements

Whole blood samples were collected at each annual screening visit from 5 years before biopsy to the year of biopsy (years −5 through 0). These samples were stored at 4°C, typically centrifuged and separated into serum within 3 h, and stored at −70°C at the Gunma University serum bank. The frozen serum samples were then retrospectively analyzed for total PSA, free PSA, and [−2]proPSA levels using the Access Hybritech assay (Beckman Coulter, Brea, CA, USA) on a UniCel DxI800 instrument (Beckman Coulter) at Mishima Laboratory (Shizuoka, Japan). The following serum indices were calculated at each time point from year −5 to year 0: total PSA, %fPSA = free PSA/total PSA, %p2PSA = [−2]proPSA ÷ 10/free PSA, and phi = [−2]proPSA/free PSA × √total PSA.

2.3 | Statistical Analysis

Statistical comparisons of serum indices between the case and control cohorts at each time point were performed using the Mann–Whitney *U*-test. The discriminatory ability of each index was evaluated using the area under the receiver operating characteristic curve (AUC-ROC). All statistical analyses were performed using IBM SPSS Statistics for Windows, version 29.0 (IBM Corp, Armonk, NY, USA), with statistical significance set at $p < 0.05$.

2.4 | Institutional Ethics Approval

The present study was approved by the Ethics Committee of Gunma University (approval no. 2097), which waived the requirement for written informed consent for participation owing to the opt-out consent process.

3 | Results

3.1 | Patient Characteristics

The clinical characteristics and serum PSA-related indices at the time of biopsy are summarized in Table 1. The case group

TABLE 1 | Clinical characteristics, serum PSA-related indices in the case and control cohorts.

	Case ($n = 58$)			Control ($n = 58$)			
	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	*p*-value
Age (years)	73.9 ± 5.5	74.5	70 to 78	72.8 ± 5.7	74	69 to 76	0.284
PSA (ng/mL)	5.75 ± 1.33	5.31	4.97 to 5.97	5.35 ± 1.14	5.19	4.71 to 5.84	0.117
%fPSA	13.5 ± 4.5	13.5	9.8 to 16.0	18.0 ± 7.6	17.1	12.9 to 22.1	0.001
%p2PSA	2.66 ± 1.08	2.54	1.85 to 3.23	2.05 ± 1.13	1.73	1.36 to 2.31	< 0.001
phi	62.9 ± 25.1	57.7	43.6 to 79.5	48.7 ± 29.3	38.7	30.8 to 54.4	< 0.001

Abbreviations: IQR, interquartile range; phi, prostate health index; PSA, prostate specific antigen; SD, standard deviation.

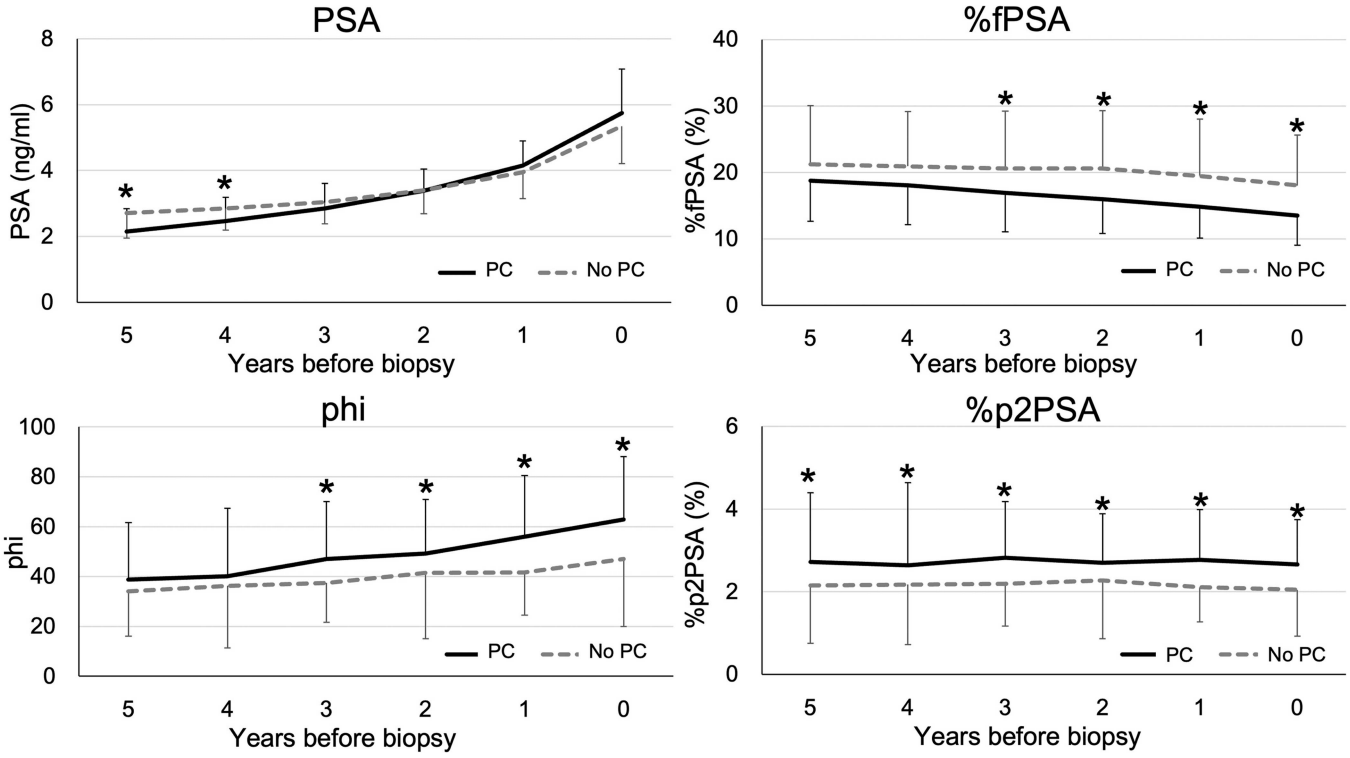

FIGURE 1 | Longitudinal changes in serum PSA, %fPSA, phi, and %p2PSA from 5 years before biopsy to the time of biopsy. Data are presented as mean values; error bars represent standard deviation. *$p < 0.05$. PC, prostate cancer, PSA, prostate specific antigen; %fPSA, free PSA/PSA ratio; phi, prostate health index; %p2PSA, p2PSA/free PSA ratio.

ranged in age from 63 to 86 years, whereas the control group ranged in age from 59 to 87 years. The case and control groups showed no significant differences in age (73.9 ± 5.5 vs. 72.8 ± 5.7 years, $p = 0.284$) or PSA level (5.75 ± 1.33 vs. 5.35 ± 1.14 ng/mL, $p = 0.117$). Conversely, the %fPSA levels were significantly lower in the case group compared with the control group (13.5 ± 4.5% vs. 18.0 ± 7.6%, $p = 0.001$). Similarly, %p2PSA (2.66 ± 1.08% vs. 2.05 ± 1.13%, $p < 0.001$) and phi (62.9 ± 25.1 vs. 48.7 ± 29.3, $p < 0.001$) levels were significantly higher in the case group than in the control group.

3.2 | Longitudinal Changes in PC Tumor Marker Levels

The longitudinal changes in serum PSA, %fPSA, phi, and %p2PSA from 5 years before biopsy to the biopsy year (year 0) are shown in Figure 1. PSA levels were significantly lower in the case group than in the control group at four and 5 years before biopsy. In contrast, one of the main findings of this study was that the %p2PSA levels in the case group were already significantly higher than those in the control group 5 years before biopsy, when total PSA levels had not yet reached the conventional biopsy threshold. phi levels were significantly elevated in the case group compared with the control group from 3 years before biopsy. Additionally, %fPSA was significantly lower in the case group from 3 years before biopsy.

3.3 | PC Prediction

The ROC curves for PSA, phi, and %p2PSA at year 0 and 3 years before biopsy are shown in Figure 2, and Table 2 summarizes the corresponding AUC values and false-positive rates at 90% sensitivity for each time point. At year 0, phi demonstrated the highest predictive performance (AUC = 0.716), followed by %p2PSA (AUC = 0.698), both substantially outperforming PSA alone (AUC = 0.584). The AUC values for all markers decreased

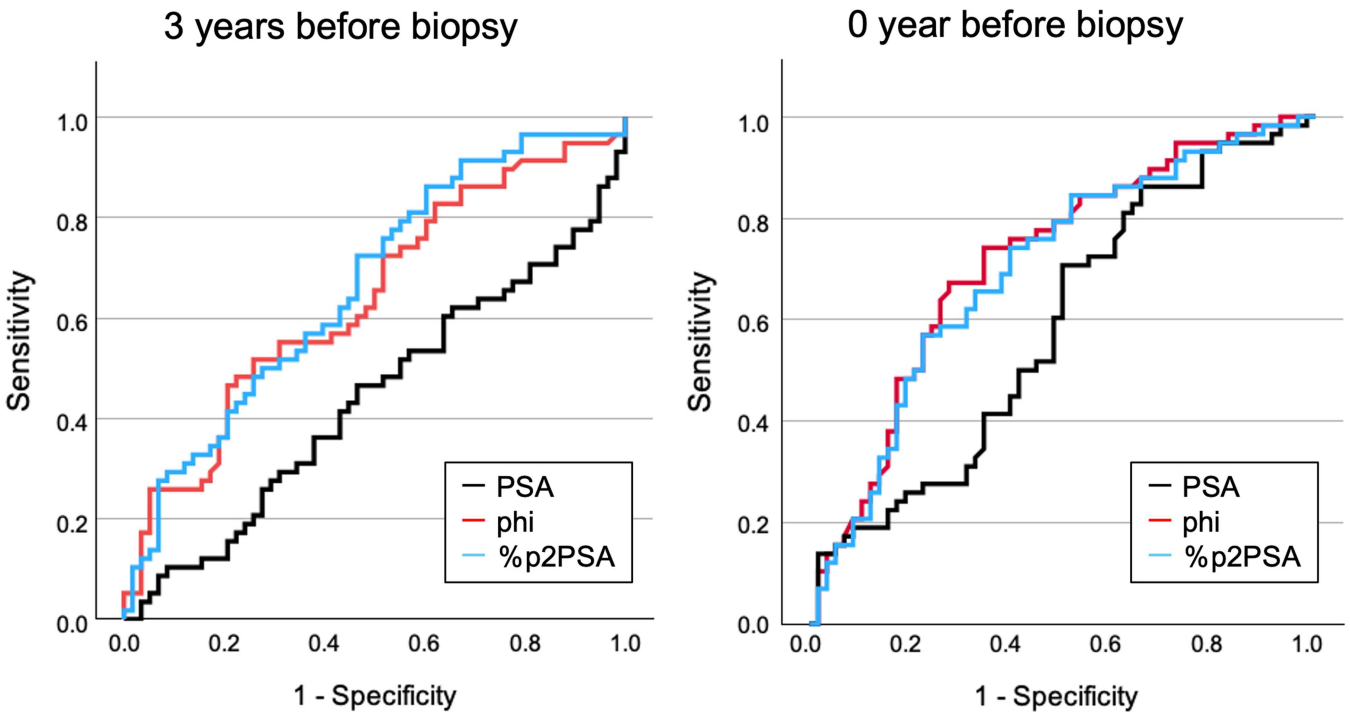

FIGURE 2 | Receiver operating characteristic curves for PSA, phi, and %p2PSA for predicting PC at 3 years before biopsy (left panel) and at the time of biopsy (0 years before biopsy; right panel). PSA, prostate specific antigen; phi, prostate health index; %p2PSA, p2PSA/free PSA ratio. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 | Longitudinal changes in AUC-ROC and FPR at 90% sensitivity.

Index	AUC-ROC	95% CI	FPR at 90% sensitivity	Cutoff value at 90% sensitivity
0 year				
PSA	0.584	0.480–0.689	0.776	4.574
phi	0.716	0.632–0.810	0.707	33.00
%p2PSA	0.698	0.602–0.794	0.724	1.439
−1 year				
PSA	0.555	0.450–0.660	0.828	3.227
phi	0.699	0.604–0.795	0.741	30.05
%p2PSA	0.680	0.583–0.777	0.776	1.447
−2 years				
PSA	0.505	0.399–0.611	0.931	2.233
phi	0.650	0.549–0.751	0.81	24.25
%p2PSA	0.647	0.546–0.749	0.862	1.26
−3 years				
PSA	0.437	0.332–0.542	0.983	1.75
phi	0.637	0.537–0.738	0.793	25.45
%p2PSA	0.663	0.564–0.761	0.672	1.58

Abbreviations: AUC, area under the curve; CI, confidence interval; FPR, false-positive rate; phi, prostate health index; PSA, prostate specific antigen; %p2PSA; p2PSA/free PSA ratio; ROC, receiver operating characteristic.

as the time interval from biopsy increased. However, phi and %p2PSA maintained good predictive performance even at 3 years before biopsy (AUC = 0.637 and 0.663, respectively), whereas PSA showed poor predictive performance at that time point (AUC = 0.437). For phi, the cutoffs at 90% sensitivity were 33 and 25.45 at year 0 and 3 years before biopsy, respectively.

4 | Discussion

The results of this retrospective case–control study using serum samples from a population-based screening program demonstrated that phi and %p2PSA may distinguish between males with and without PC even in the pre-stage leading up to PSA levels of 4–10 ng/mL. These findings suggest that p2PSA-related

markers may be more useful for early risk stratification than PSA alone.

In cross-sectional analysis at the time of biopsy, age and PSA did not differ between the case and control groups; however, the cancer group showed lower %fPSA and higher phi and % p2PSA levels. Previous studies have similarly demonstrated that, within the PSA range of 2–10 ng/mL, phi and %p2PSA outperform PSA alone or %fPSA for cancer detection [4, 7–10, 13, 16]. Furthermore, combining phi with magnetic resonance imaging (MRI) improved the detection of csPC [17, 18]. The present cross-sectional findings are also consistent with those of a population-based screening study [19] that reported that phi demonstrated superior predictive performance compared with PSA and %fPSA in a Japanese screening cohort. Although the study [19] evaluated phi cross-sectionally at the time of biopsy, the present study extends these findings by characterizing the prediagnostic longitudinal behavior of phi and % p2PSA during the 5 years before biopsy in a comparable population-based screening setting. Thus, the two studies may be regarded as complementary. The elevated phi levels in the cancer group, even after adjusting for age and PSA, support the utility of phi as an adjunct diagnostic marker in the PSA 4–10 ng/mL range.

In the longitudinal analysis, %p2PSA differed significantly between groups from 5 years before biopsy, whereas phi differed from 3 years prior, suggesting that p2PSA-related indices may change earlier than PSA. The higher PSA level in the control group 4–5 years before biopsy likely reflects the matching strategy, which selected controls with PSA levels similar to those in the case group at the time of biopsy. Consequently, controls may have had higher baseline PSA levels, whereas PSA in the cancer group likely increased more rapidly approaching diagnosis. These findings suggest that temporal changes in p2PSA-related indices may more sensitively reflect underlying pathological changes than PSA alone. p2PSA is a proPSA isoform strongly associated with PC, with increased expression in malignant tissue and elevations reported several years before diagnosis, as well as during pathological progression under active surveillance [14, 20–22]. Consistent with these observations, the increases in phi and %p2PSA observed in this study may reflect early tumor presence or progression. However, as we previously reported, the contribution of p2PSA-related indices appears limited in individuals with baseline PSA levels < 2.0 ng/mL [23], suggesting that their clinical value may be greatest during the early phase of PSA elevation. In addition, the increase in PSA levels observed in the control group four to 5 years before biopsy may reflect underlying biological differences in the prostate. The control group may have had higher baseline PSA levels, potentially due to benign prostatic hyperplasia or other non-malignant conditions, whereas PSA levels in the case group appeared to increase more rapidly as biopsy approached. Prostate volume is an important determinant of PSA-based biomarkers, and previous studies have shown that the diagnostic performance of phi and phi density may vary based on prostate volume, although phi density does not consistently provide a clinically meaningful advantage over phi alone [24, 25]. Because prostate volume data were not available in the present study, the contribution of prostate volume to these early PSA patterns could not be directly assessed. This interpretation should therefore be considered with caution.

In ROC analysis at 3 years before biopsy, phi and %p2PSA (AUC = 0.637 and 0.663, respectively) outperformed PSA (AUC = 0.437), indicating superior predictive performance even in the pre-diagnostic phase. However, discrimination remained modest compared with that at diagnosis [13, 26, 27]. At a phi cutoff of 25.45, corresponding to a sensitivity of 90%, specificity was only 20.7%, highlighting the limited predictive performance at this stage. Therefore, phi should not be used in isolation to determine biopsy indications, but rather as part of a broader risk stratification framework incorporating longitudinal trends and clinical variables. This approach may be particularly relevant in high-risk populations, including men with pathogenic BRCA1/2 variants or a family history of PC. In men with a family history, % p2PSA, phi, and phi-based models have outperformed conventional PSA-based parameters [28, 29], whereas prospective evidence in BRCA1/2 carriers is mainly derived from the PSA-based IMPACT study [30, 31]. Longitudinal monitoring of phi and % p2PSA, integrated with family history and genetic background, may further refine risk stratification in such populations; however, this hypothesis remains to be validated in dedicated prospective studies.

This study has six severe limitations. First, as this was a retrospective case–control study, it is subject to selection bias and unmeasured confounding. Second, because the data originated from a population-based screening database and detailed pathological information such as Gleason score was limited, we could not fully verify the ability to identify csPC at an early stage. The clinical relevance of the observed prediagnostic biomarker differences for csPC therefore cannot be directly established from these data. Third, variability in sample handling and storage conditions may have influenced p2PSA measurements [32]. Fourth, the study period extended over a span of more than three decades (1992–2023), during which the broader diagnostic landscape for PC has undergone substantial evolution, particularly with the introduction of MRI and the gradual shift from systematic to MRI-targeted biopsy. Despite the lack of change in the screening referral protocol, the diagnostic certainty associated with a negative biopsy in the control group differs between earlier and later years. Furthermore, a small number of earlier-era controls may include occult cancer that would have been detected with current imaging and biopsy techniques. Due to the limited number of cases and controls, a formal time-stratified sensitivity analysis with adequate statistical power could not be performed, and we are unable to fully rule out residual misclassification of control status. Fifth, individual-level data on key clinical covariates known to influence PSA-based diagnostic evaluation were not systematically documented for each case and control in this population-based screening database. These variables include prostate volume, family history of prostate cancer, prior biopsy history, digital rectal examination findings, MRI findings, and biopsy-related factors, such as the biopsy approach used (systematic *vs.* MRI-targeted biopsy) and the number of cores obtained. Because these covariates could not be incorporated into the analysis, multivariable adjustment for these factors and direct comparison with contemporary clinical risk prediction models were not feasible. Consequently, the observed between-group

differences may, at least in part, reflect unmeasured baseline differences, including differences in prostate volume. Sixth, the 58 cases and 58 controls examined in this study constitute merely a structurally constrained subset of all participants in the Gunma PC screening program during the 1992–2023 period. In order to qualify for the study, participants were required to have attended annual screening visits for a minimum of five consecutive years prior to undergoing biopsy. Additionally, residual frozen serum from each of these visits had to be available in the serum bank at Gunma University. These requirements substantially restricted the pool of available cases and controls; consequently, the 58 cases do not represent the total number of PC cases detected in the screening program during this period. A comprehensive recount of all program-detected PC cases and the corresponding distribution of PSA values at diagnosis was not feasible from the available data structure.

5 | Conclusions

p2PSA-related indices might help distinguish males with and without risk of developing PC before PSA levels rise above conventional thresholds, thus informing the development of risk-stratified screening strategies. Whether longitudinal monitoring of phi and %p2PSA alone—or integrated with imaging, genetic, and clinical information, including in high-risk populations such as males with a family history of PC or BRCA1/2 variants—can contribute to the early detection of csPC and the avoidance of unnecessary biopsies must be confirmed in large-scale prospective studies with pathological grade information.

Author Contributions

Yoshitaka Sekine, Kazuto Ito, and Kazuhiro Suzuki conceptualized and designed the study. Yuji Fujizuka, Syun Nakazawa, Yusuke Tsuji, Akira Ohtsu, Yoshiyuki Miyazawa, Seiji Arai, Masashi Nomura, Hidekazu Koike, and Hiroshi Matsui acquired the data. Yoshitaka Sekine performed most of the analyses, wrote the manuscript, and prepared the figures. Kazuto Ito and Kazuhiro Suzuki revised the manuscript and supervised the work.

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The authors have nothing to report.

Ethics Statement

This study was approved by the Institution Review Board of Gunma University (approval number: 2097).

Disclosure of AI Use

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) and Claude (Anthropic) to assist with literature search planning. The authors independently reviewed all retrieved references and verified all statements, citations, and conclusions. The authors are solely responsible for the final content of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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