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Evaluating the Diagnostic Potential of Biomarker Panels in Breast Cancer and Prostate Adenocarcinoma

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Received: 23 December 2024 | **Revised:** 27 March 2025 | **Accepted:** 16 April 2025

Funding: This study was supported by the Shota Rustaveli National Science Foundation of Georgia (FR-19-24458).

Keywords: biomarkers | blood samples | cancer | machine learning | panel

ABSTRACT

Background: Noninvasive diagnostic methods are essential for early cancer detection and improved patient outcomes. Circulating biomarkers, measurable indicators of pathological processes, offer a promising avenue, yet optimal panels for reliable cancer diagnosis remain undefined. This study evaluates the diagnostic performance of selected plasma biomarkers in distinguishing breast cancer and prostate adenocarcinoma patients from healthy individuals, using statistical analysis and machine learning.

Materials and Methods: We analyzed blood samples from 162 participants (73 cancer patients: 51 with breast cancer and 22 with prostate adenocarcinoma; 89 healthy controls). Levels of 12 cancer-associated biomarkers—including Ki67, DNMT1, BRCA1, and MPO—were quantified using enzyme-linked immunosorbent assays (ELISA). Statistical analyses, including the Mann–Whitney U test and machine learning models (random forest), were employed to assess the predictive accuracy of these biomarkers in distinguishing between cancerous and healthy states.

Results: Biomarkers such as Ki67, DNMT1, and MPO were significantly elevated in cancer groups. Random forest models using selected combinations (e.g., BRCA1-CTA-TP53) achieved perfect classification accuracy (AUC = 1.00). However, high inter-marker correlations suggested potential redundancy, underscoring the need for biomarker panel optimization.

Conclusion: Our findings support the potential of biomarker panels for accurate, noninvasive cancer diagnostics. Further validation in larger, more diverse cohorts is warranted to establish clinical utility and generalizability.

1 | Introduction

Cancer has seen remarkable advancements, with breast and prostate cancers among the most frequently diagnosed malignancies in women and men, respectively [1, 2]. Despite advances in treatment, early detection remains a critical determinant of prognosis and survival. Current diagnostic methods often rely on imaging or tissue biopsies, which, while effective, are invasive, time-consuming, and may delay treatment initiation [3, 4]. In recent years, circulating biomarkers—measurable molecules in blood or other body fluids—have emerged as a promising alternative for noninvasive cancer diagnostics [5–7]. Biomarkers, such as Ki67, BRCA1, and DNMT1 are associated with tumor proliferation, DNA repair dysfunction, and epigenetic changes that are central to oncogenesis [8–10]. However, many individual

Abbreviations: AUC-ROC, area under the receiver operating characteristic curve; BRCA1, breast cancer type 1 susceptibility protein; CA15.3, cancer antigen 15-3; CEA, carcinoembryonic antigen; CTA, cancer/testis antigen; DNMT1, DNA methyltransferase 1; ELISA, enzyme-linked immunosorbent assay; FOXP3, forkhead box P3; Ki67, a cellular marker for proliferation; miRNA, microRNA; MPO, myeloperoxidase; NIR, no information rate; PDCD1LG2, programmed cell death 1 ligand 2; SD, standard deviation; TP53, tumor protein 53; TP63, tumor protein 63.

Summary

- Question
  - Can specific biomarker panels reliably distinguish between cancer patients (breast cancer and prostate adenocarcinoma) and healthy individuals?
- Findings
  - Biomarkers such as Ki67, DNMT1, and MPO were significantly elevated in cancer patients compared to healthy controls, highlighting their diagnostic relevance; high correlations between certain biomarkers (e.g., Ki67 and DNMT1) suggest potential redundancy, emphasizing the need for selecting complementary biomarkers for diagnostic panels.
- Meaning
  - Carefully selected biomarker panels hold significant potential for noninvasive, accurate cancer diagnostics.

biomarkers, lack sufficient sensitivity or specificity when used alone [11], and existing blood-based assays (e.g., CA15.3, CEA) provide limited diagnostic value for early-stage cancer [12]. Given cancer's complexity, combining multiple biomarkers into diagnostic panels offers a more comprehensive approach. However, identifying optimal biomarker combinations remains a challenge. Machine learning, particularly random forest classifiers, has shown promise in analyzing complex biological data and optimizing diagnostic accuracy through multi-marker analysis [13–15]. This study was undertaken to evaluate the diagnostic potential of 12 selected plasma biomarkers in distinguishing between patients with breast cancer, prostate adenocarcinoma, and healthy individuals. The biomarkers were chosen based on their documented roles in cancer biology, including proliferation, immune regulation, epigenetic alteration, and tumor suppression. We hypothesized that a machine learning approach using these biomarkers would yield accurate and reliable diagnostic models for both cancer types. Moreover, we aimed to explore potential redundancy between markers to refine future biomarker panel development.

2 | Materials and Methods

2.1 | Study Population

This cross-sectional study was conducted from March 1, 2021 to January 31, 2022 and included 162 participants: 73 cancer patients (51 with histologically confirmed breast cancer and 22 with prostate adenocarcinoma) and 89 healthy controls (Table 1). All cancer patients were newly diagnosed and treatment-naïve at the time of sample collection. Clinical data, including cancer stage and subtype, were collected where available. Among breast cancer cases, 60.8% were stage II, 27.5% stage III, and 11.7% stage I. Molecular subtype data were partially available, with luminal A and B subtypes comprising the majority. For prostate cancer patients, all cases were localized (stage II–III) adenocarcinoma. The control consisted of healthy volunteer blood donors [15] (61 males, 28 females), aged 20–60 years, with no history of malignancy or chronic illness. Ethical approval was obtained from the Bioethics International Committee of the Petre Shotadze Tbilisi Medical Academy (IRB45765021/9), and written informed consent was secured from all participants.

2.2 | Biomarker Selection and Measurement

Twelve biomarkers were selected based on prior literature supporting their relevance to cancer detection, progression, and prognosis. These include markers of cell proliferation (Ki67), DNA methylation (DNMT1), oxidative stress (MPO), tumor suppression (TP53, TP63, BRCA1), immune modulation (PDCD1LG2, FOXP3), and known cancer-associated proteins (CEA, CA15.3, CTA). The list was refined based on pilot data and assay availability. Each biomarker's relevance is supported by previous studies:

Ki67 and TP53: widely used markers of proliferation and genomic instability in breast and prostate cancers [16–18].

DNMT1: epigenetic regulator involved in methylation-driven tumorigenesis [19, 20].

MPO: linked to inflammation and oxidative stress pathways in cancer [21].

BRCA1: central to DNA repair; mutations elevate breast and prostate cancer risk [22].

FOXP3 and PDCD1LG2: immune checkpoint markers relevant for tumor microenvironment modulation [23].

miRNA: specifically, miR-21 was analyzed, as it is among the most upregulated oncogenic miRNAs in both breast and prostate cancers [24, 25].

Quantification was performed via enzyme-linked immunosorbent assay (ELISA) kits from Wuhan Fine Biotech Co. Ltd.

TABLE 1 | Demographic summary of study participants by diagnosis.

		Age		Sex	
Diagnosis	***N***	**Mean**	**SD**	**Males**	**Females**
All cancer cases	73	57	13	22	51
Breast cancer	51	50	7	0	51
Prostate adenocarcinoma	22	75	7	22	0
Healthy controls	89	37	11	61	28

(Wuhan, Hubei, China), following the manufacturer's protocol. Absorbance was measured at 450 nm using a microplate reader. Sample concentrations were calculated based on standard curves using CurveExpert Basic software (Hyams Development, https://www.curveexpert.net).

2.3 | Statistical Analysis

To ensure the robustness of our findings, we first assessed the normality of biomarker distributions using the Shapiro–Wilk test. Unsurprisingly, most biomarkers did not follow a normal distribution, necessitating the use of non-parametric methods for further analysis. Specifically, the Mann-Whitney U test was employed to compare biomarker levels between cancer patients and healthy controls. Additionally, Levene's test was used to assess the equality of variances across the different groups, further justifying our choice of non-parametric tests due to the unequal variances observed.

2.4 | Machine Learning Analysis

We turned to machine learning, specifically the random forest algorithm, to build predictive models. This algorithm was chosen for its ability to handle complex interactions between variables and its effectiveness in classification tasks. The data set was divided into training (70%) and testing (30%) sets via stratified sampling, ensuring that both cancer patients and healthy controls were proportionally represented in each set. The random forest model, implemented using the "randomForest" package in R, comprised 500 trees to strike a balance between accuracy and overfitting. Tables 2 and 3 summarize the performance of these models. We evaluated the model's performance using metrics such as accuracy, sensitivity, specificity, and the area under the receiver operating characteristic curve (AUC-ROC). Furthermore, we conducted an analysis of variable importance, helping to identify which biomarkers were most influential in the predictive models.

3 | Results

3.1 | Participant Characteristics

A total of 162 individuals were enrolled in this study, including 73 cancer patients and 89 healthy controls. Among cancer patients, 51 (69.9%) were diagnosed with breast cancer (all female) and 22 (30.1%) with prostate adenocarcinoma (all male). The mean age for breast cancer patients was 50 years (SD±7), and for prostate adenocarcinoma patients, 75 years (SD±7). The control group had a mean age of 37 years (SD±11), comprising 61 males and 28 females. Clinical data revealed that 60.8% of breast cancer cases were stage II, 27.5% stage III, and 11.7% stage I. Molecular subtype data were partially available, indicating predominance of luminal A and B subtypes. All prostate cancer cases were confirmed as adenocarcinoma, predominantly stage II or III.

3.2 | Biomarker Distribution

Statistical analysis using the Mann-Whiney U test (Table 4) identified significant differences in biomarker levels between cancer patients and healthy individuals. Ki67, DNMT1, and MPO were markedly elevated in both cancer types ($p < 0.001$ for all comparisons). In particular:

- Ki67 levels in breast patients averaged 4.4 ng/mL, compared to 0.1 ng/mL in healthy females.
- DNMT1 levels in prostate cancer patients averaged 2.8 ng/mL, versus 0.1 ng/mL in healthy males.

TABLE 2 | Random forest models using single biomarkers for breast cancer and prostate.

	Breast cancer					Prostate adenocarcinoma				
Biomarker	**Acc.**	***P***	**Sens.**	**Spec.**	**AUC**	**Acc.**	***P***	**Sens.**	**Spec.**	**AUC**
All	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00
BRCA1	0.89	0.01	0.92	0.86	0.92	0.87	0.03	0.67	1.00	0.97
CA15.3	0.58	0.76	0.50	0.71	0.45	0.73	0.22	0.67	0.78	0.62
CEA	0.74	0.24	0.67	0.86	0.82	0.67	0.40	0.67	0.67	0.56
CTA	0.84	0.04	0.92	0.71	0.96	0.53	0.79	0.50	0.56	0.60
DNMT1	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00
FOXP3	0.74	0.24	0.58	1.00	0.79	1.00	0.00	1.00	1.00	1.00
Ki67	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00
miRNA	0.47	0.95	0.50	0.43	0.48	0.73	0.22	0.83	0.67	0.63
MPO	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00
PDCD1LG2	0.89	0.01	0.92	0.86	0.98	0.87	0.03	1.00	0.78	0.94
TP53	0.95	0.00	1.00	0.86	0.99	0.73	0.22	1.00	0.56	0.96
TP63	0.95	0.00	0.92	1.00	0.95	0.93	0.01	0.83	1.00	1.00

Note: Adenocarcinoma from healthy controls. Metrics: Accuracy (Acc.), *p*-value of accuracy vs. No Information Rate (*P*), Sensitivity (Sens.), Specificity (Spec.), and Area Under the Curve (AUC). NIR for the breast cancer group was 0.63, and 0.6 for prostate adenocarcinoma

TABLE 3 | Random forest models using selected biomarker combinations.

Model	Accuracy	*P*	Sensitivity	Specificity	AUC
Breast cancer					
BRCA1-CTA-TP53	1.00	0.00	1.00	1.00	1.00
BRCA1-CTA	1.00	0.00	1.00	1.00	1.00
BRCA1-TP53	1.00	0.00	1.00	1.00	1.00
TP53-CTA	0.89	0.01	1.00	0.71	1.00
Prostate adenocarcinoma					
BRCA1-CA15.3-TP53	1.00	0.00	1.00	1.00	1.00
BRCA1-CA15.3	0.80	0.09	1.00	0.67	0.94
BRCA1-TP53	1.00	0.00	1.00	1.00	1.00
TP53-CA15.3	1.00	0.00	1.00	1.00	1.00

Note: Adenocarcinoma from healthy controls. Metrics: Accuracy (Acc.), *p*-value of accuracy vs. No Information Rate (*P*), Sensitivity (Sens.), Specificity (Spec.), and Area Under the Curve (AUC).

TABLE 4 | Summary statistics and Mann–Whitney U test results for biomarkers in cancer and control groups.

	Breast cancer			Healthy females			Mann–Whitney	
	Mean	Median	SD	Mean	Median	SD	U	*P*
BRCA1 (pg/mL)	18	18	1	13	12	4	1211	< 0.001
CA15.3 (U/mL)	30	20	33	16	17	7	863	0.128
CEA (ng/mL)	2.0	0.6	6	1.2	1.2	1	547	0.09
CTA (pg/mL)	1	1	1	0	0	1	1195	< 0.001
DNMT1 (ng/mL)	3.0	2.7	1	0.1	0.1	0	1428	< 0.001
FOXP3 (ng/mL)	3.0	0.0	6.8	0.1	0.1	0.0	500	0.025
Ki67 (ng/mL)	4.4	3.7	2.1	0.1	0.1	0.2	1428	< 0.001
miRNA (ng/mL)	0.2	0.1	0.2	0.1	0.1	0.1	928	0.029
MPO (ng/mL)	4.4	4.0	1.7	0.1	0.1	0.0	1428	< 0.001
PDCD1LG2 (ng/mL)	0.5	0.5	0.1	0.1	0.1	0.1	1409	< 0.001
TP53 (ng/mL)	1.3	1.4	0.6	0.1	0.1	0.2	1389	< 0.001
TP63 (ng/mL)	36	28	33	0	0	0	1276	< 0.001
	Prostate adenocarcinoma			**Healthy males**			**Mann–Whitney**	
	Mean	**Median**	**SD**	**Mean**	**Median**	**SD**	**U**	***P***
BRCA1 (pg/mL)	18	18	1	11	11	4	1279	< 0.001
CA15.3 (U/mL)	25	20	18	14	14	6	881	0.030
CEA (ng/mL)	1.2	1.0	1	1.4	1.4	1	546	0.197
CTA (pg/mL)	1	1	0	0	0	1	1096	< 0.001
DNMT1 (ng/mL)	2.8	2.6	1	0.1	0.1	0	1342	< 0.001
FOXP3 (ng/mL)	1.1	0.0	3.8	0.1	0.1	0.1	485	0.054
Ki67 (ng/mL)	3.8	3.6	1.2	0.1	0.1	0.0	1342	< 0.001
miRNA (ng/mL)	0.2	0.2	0.1	0.1	0.1	0.1	936	0.006
MPO (ng/mL)	3.9	3.7	0.9	0.1	0.1	0.0	1342	< 0.001
PDCD1LG2 (ng/mL)	0.5	0.5	0.2	0.1	0.1	0.1	1298	< 0.001
TP53 (ng/mL)	1.3	1.4	0.6	0.1	0.1	0.2	1306	< 0.001
TP63 (ng/mL)	44	26	82	0	0	0	1342	< 0.001

- MPO levels were significantly higher in both cancer groups compared to respective controls (mean 4.4 ng/mL in breast cancer; 3.9 ng/mL in prostate cancer, both $p < 0.001$).

Conversely, some markers such as CA15.3 and CEA did not show statistically significant differences between patients and controls, limiting their diagnostic value in this cohort.

3.3 | Machine Learning Model Performance

Random forest classifiers demonstrated strong performance in distinguishing cancer patients from healthy controls.

- Single biomarker models: certain biomarkers achieved high classification metrics on their own. For both breast and prostate cancer Ki67, DNMT1, MPO, and TP53 reached 100% accuracy, sensitivity, and specificity, with AUCs of 1.00.
- Multi-biomarker models:
  - In breast cancer, combinations like BRCA1-CTA-TP53 and BRCA1-TP53 achieved perfect classification (Accuracy = 1.00; AUC = 1.00).
  - In prostate cancer, BRCA1-CA15.3-TP53 and TP53-CA15.3 combinations also reached 100% classification accuracy.

Through our variable importance analysis, we identified Ki67, DNMT1, and MPO as some of the most influential biomarkers in these models (Figure 1), as indicated by their high Mean Decrease Gini scores. This suggests that these biomarkers are critical for accurate classification and should be considered key components of any future diagnostic panels (Figure 2).

Analysis of variable importance (based on Mean Decrease Gini scores) identified Ki67, DNMT1, and MPO as the most influential features in predictive models (Figures 2 and 3). However, correlation heatmaps revealed strong inter-marker correlations ($r < 0.9$) among these three biomarkers, indicating potential redundancy. For instance, combining Ki67 and DNMT1 did not improve model accuracy beyond individual performance, suggesting that only one may be required in a diagnostic panel to minimize overlap.

4 | Discussion

Our findings provide strong evidence for the diagnostic utility of selected plasma biomarkers in distinguishing cancer patients with breast cancer or prostate adenocarcinoma and healthy individuals. The application of random forest models enabled accurate classification, with certain biomarker combinations (e.g., BRCA1-TP53-CTA) achieving 100% sensitivity and specificity. This underscores the potential of machine learning-enhanced multi-marker panels as powerful noninvasive tools for early cancer detection. Ki67, DNMT1, and MPO emerged as the most diagnostically informative markers in both cancer types. Ki67 is a well-established proliferation marker correlated with tumor aggressiveness, while DNMT1 plays a central role in DNA methylation and epigenetic dysregulation in cancer. MPO, a pro-oxidative enzyme linked to inflammation and carcinogenesis, also demonstrated high discriminatory power. However, the observed strong correlations among these markers indicate possible functional redundancy. This finding aligns with existing research suggesting that combining highly correlated markers may not enhance model performance and can reduce cost-effectiveness in clinical implementation [26]. Importantly, our study addresses two clinically distinct but high-incidence cancers—breast and prostate— providing a gender-complementary model for exploring plasma-based diagnostics. While these cancers differ in their hormonal and molecular etiology, the overlap in biomarker expression patterns observed here suggests potential for shared diagnostic panels or at least shared analytical frameworks. This supports the notion advanced by recent multi-cancer early detection research, such as [27], which advocates for pan-cancer blood tests using multiplexed biomarker detection. Compared to previous biomarker studies, our work distinguishes itself by integrating machine learning with biomarker selection, as opposed to evaluating single markers in isolation. While prior studies [28] have identified predictive signatures using transcriptomic approaches, few have explored ELISA-based plasma biomarker panels combined with machine learning classifiers in real-world clinical samples. Furthermore, our use of treatment-naïve patients strengthens the potential for diagnostic application rather than treatment monitoring alone. This study also contributes to the growing body of evidence emphasizing the diagnostic value of immune-related markers, such as PDCD1LG2 and FOXP3, which were included due to their roles in immune checkpoint regulation. Recent work [29] has highlighted how such markers may enhance early detection when added to conventional panels, especially in cancers with immunosuppressive microenvironments. Despite its promising results, our study is not without limitations. The sample size, particularly for prostate cancer, is relatively small and may affect model stability and generalizability. Also, although biomarker levels differed significantly between groups, we lacked complete molecular subtype data, particularly for breast cancer, which may influence biomarker expression. Additionally, although miR-21 was chosen due to its known oncogenic role, broader miRNA profiling may identify more sensitive noncoding RNA candidates.

5 | Limitations

Despite the encouraging results, this study has several limitations. First, the sample size, particularly for prostate cancer cases, limits the statistical power and may affect the reproducibility of the machine learning models in larger populations. Second, whole we included only newly diagnosed, treatment-naïve patients, detailed molecular subtype data—especially for breast cancer—were only partially available, limiting stratified analysis. Third, although we focused on clinically relevant biomarkers, the high correlation between several of them (e.g., Ki67, DNMT1, MPO) suggests potential redundancy, which may limit panel efficiency in real-world applications. Finally, the miRNA measurement in this study was restricted to miR-21 based on a priori data, but broader miRNA profiling could reveal more comprehensive insights. Future studies should aim to include larger, more diverse cohorts, longitudinal follow-up, and multi-cancer comparisons to strengthen diagnostic validity and explore potential prognostic applications.

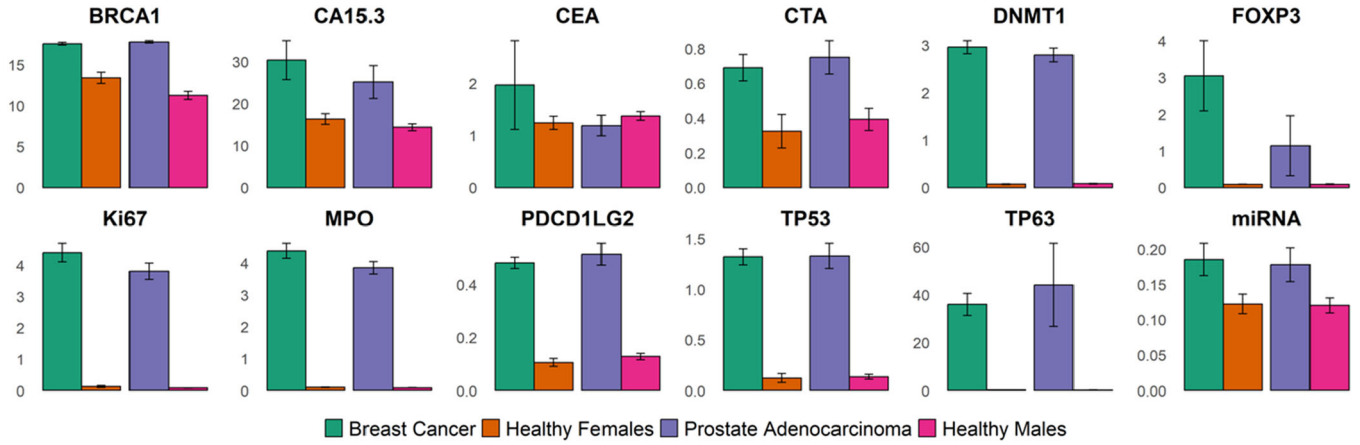

FIGURE 1 | Mean biomarker levels across groups. This figure illustrates the mean levels of 12 biomarkers in four groups: breast cancer, healthy females, prostate adenocarcinoma, and healthy males. Error bars indicate the standard error of the mean.

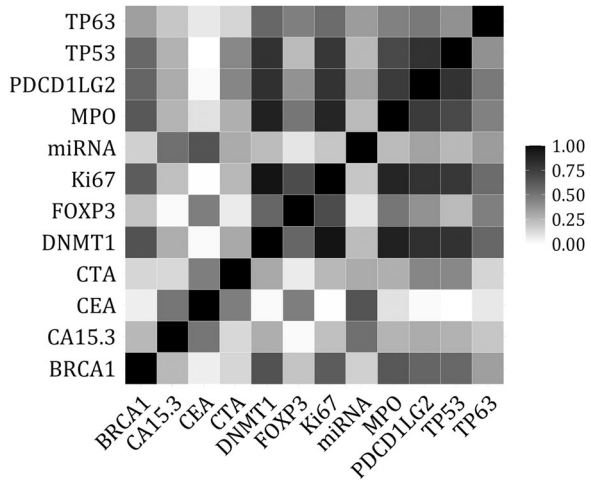

FIGURE 2 | Correlation heatmap of biomarkers. *Note:* Negative correlations are not displayed as they were not present.

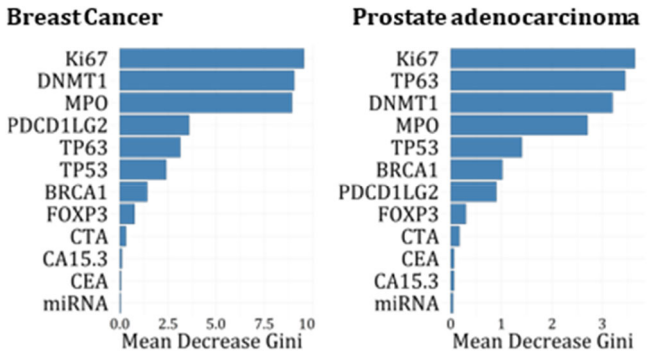

FIGURE 3 | Variable importance plot for all biomarkers models. *Note:* Higher Mean Decrease Gini values correspond to higher level of importance.

6 | Conclusion

This study demonstrates the diagnostic potential of selected plasma biomarkers, both individually and in combination, for distinguishing patients with breast cancer and prostate adenocarcinoma from healthy individuals. Using a machine learning-based approach, we identified highly accurate models—notably those incorporating BRCA1, TP53, and Ki67—capable of achieving perfect classification in our sample. These findings support the feasibility of developing noninvasive, blood-based diagnostic panels that could complement or reduce reliance on more invasive procedures. However, the presence of strong correlations among key biomarkers such as ki67, DNMT1, and MPO highlights the importance of optimizing panel composition to avoid redundancy and enhance clinical practicality. While promising, our findings require validation in larger, multicenter cohorts with detailed clinical annotation to ensure robustness, particularly across cancer subtypes and stages. By integrating machine learning with targeted biomarker assessment, this study contributes to the growing field of precision diagnostics and lays the groundwork for future development of scalable, cost-effective cancer screening tools.

Author Contributions

Kldiashvili Ekaterina: conceptualization, writing – original draft, writing – review and editing, and supervision. **Iordanishvili Saba:** investigation and formal analysis. **Adamia Sophia:** supervision, writing – review and editing. **Abiatari Ivane:** conceptualization, and writing – original draft. **Zarnadze Maia:** investigation and formal analysis.

Acknowledgments

We would like to express our sincere gratitude to all the participants who generously contributed to this study. We acknowledge the financial support provided by the Shota Rustaveli National Science Foundation of Georgia for the project FR-19-24458: Discovery and understanding role of epigenetic alterations in patients with hematological malignancies residing in Country of Georgia a region affected by Chernobyl explosion, which enabled the preparation of the present paper.

Ethics Statement

This study received approval from the Bioethics International Committee (IRB45765021/9, January 20, 2021) of the Petre Shotadze Tbilisi Medical Academy and adhered to the principles of the Helsinki Declaration (revised in 2013). All participants were informed about the study's objectives and design, and they provided written informed consent for inclusion and anonymous data publication.

Consent

All procedures adhered to the Helsinki Declaration of 1975, revised in 2013, with participants receiving comprehensive study information and providing written informed consent before inclusion.

During the preparation of this paper the authors used ChatGPT (https://chat.openai.com/) for stylistic purposes, checking grammar and spelling. After using this tool, the authors reviewed and edited the content; they take full responsibility for the content of the publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article; the data sets are available from the corresponding author on reasonable request.

Transparency Statement

The lead author Kldiashvili Ekaterina affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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