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Thioredoxin and tetraspanin 30 (CD63) as potential biomarkers for angioinvasion in papillary thyroid cancer

Angelika Buczyńska[1,4]✉, Maria Kościuszko[2,4], Iwona Sidorkiewicz[3,4], Agnieszka Adamska[2,4], Katarzyna Siewko[2,4], Janusz Dzięcioł[1,2,3,4], Małgorzata Szelachowska[2,4], Anna Popławska-Kita[2,4,5] & Adam Jacek Krętowski[1,2,4,5]

Papillary thyroid cancer (PTC) is a heterogeneous malignancy in which current prognostic markers often fail to distinguish between indolent and aggressive disease. The need for reliable biomarkers is especially urgent in cases of angioinvasion, where improved risk stratification could guide decisions such as extended surgery and early radioiodine therapy. This study evaluates thioredoxin (TRX), a redox-regulating protein, and tetraspanin 30 (CD63), a mediator of tumor-endothelial interactions, as potential non-invasive biomarkers of angioinvasive PTC. We analyzed serum samples from 90 patients—45 with histologically confirmed angioinvasion and 45 with low-risk, non-angioinvasive PTC. Both TRX and CD63 levels were significantly elevated in the angioinvasive group ($p < 0.001$). Logistic regression confirmed their strong association with angioinvasion, and ROC analysis showed high diagnostic performance: TRX (AUC = 0.85), CD63 (AUC = 0.83), and a combined model (AUC = 0.93). These findings support TRX and CD63 as promising biomarkers for detecting angioinvasion and guiding more individualized treatment in PTC.

Keywords Papillary thyroid cancer, Angioinvasion, Thioredoxin, CD63

Thyroid cancer is the fifth most common malignancy worldwide, with over 62,000 new cases annually[1]. Differentiated thyroid carcinomas (DTC) constitute approximately 90% of all thyroid malignancies, with papillary thyroid carcinoma (PTC) being the predominant histological subtype[2]. Although PTC generally has a favorable prognosis, some cases exhibit aggressive behavior with angioinvasion and metastasis[3–5]. Consequently, a comprehensive understanding of the mechanisms underlying angioinvasion, along with the identification of reliable biomarkers for its detection, is imperative for PTC clinical management[6,7]. Given the heterogeneous nature of PTC, current prognostic markers often fail to accurately differentiate between indolent and aggressive cases, leading to overtreatment or delayed intervention[8,9]. Additionally, traditional markers such as thyroglobulin (TGB) and thyroglobulin antibodies (TGBAb) have limitations in certain clinical scenarios, particularly in patients with persistent disease, lobectomy, or interfering autoantibodies underscoring the urgent need for novel, noninvasive indicators capable of early detection of aggressive PTC[10–13]. Furthermore, angiogenesis and oxidative stress play key roles in tumor progression yet circulating biomarkers reflecting these processes remain insufficiently explored[14–16]. Identifying novel, non-invasive serum markers could enhance early detection, improve risk stratification, and aid in personalized treatment decisions for radioiodine (RAI) treatment qualification for PTC patients[17].

Recent studies have increasingly emphasized the role of novel biomarkers in predicting tumor aggressiveness and guiding therapy, not only in thyroid cancer but also across multiple malignancies and other diseases (e.g., YBX family in pan-cancer and HCC, GLO1 in breast cancer lymph node metastasis, and exosome-related markers in cardiovascular disease[18–20]. These findings highlight the need to explore additional circulating biomarkers also in PTC clinical management. Among emerging biomarkers for PTC angioinvasion, thioredoxin (TRX) and tetraspanin 30 (CD63) stand out to their complementary roles in redox signaling and exosome-

[1]Clinical Research Centre, Medical University of Bialystok, 15-276 Bialystok, Poland. [2]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, 15-276 Bialystok, Poland. [3]Clinical Research Support Centre, Medical University of Białystok, ul. Waszyngtona 17, 15-276 Białystok, Poland. [4]Department of Human Anatomy, Medical University of Bialystok, 15-276 Bialystok, Poland. [5]Anna Popławska-Kita and Adam Krętowski contributed equally to this work. ✉email: Angelika.buczynska@umb.edu.pl

mediated vascular invasion[21,22]. The selection of these molecules as candidate biomarkers of angioinvasion is supported by several lines of evidence. First, TRX, a central component of the cellular redox system, is overexpressed in various malignancies (e.g., gastric and lung cancers) and released into the circulation, where its levels correlate with tumor growth rate and metastatic risk[23–26]. In context of DTC, literature data indicated that TRX expression correlated with more aggressive outcomes in thyroid tissue. Importantly, recent studies suggest that TRX can modulate CD63-mediated vesicle trafficking and exosome formation, creating a feedback loop that amplifies endothelial activation and tumor cell intravasation[27]. Exosomal CD63 + vesicles are significantly enriched in serum samples from patients with metastatic PTC compared to those without vascular invasion[21–30]. Moreover, CD63 facilitates the assembly of integrin - VEGFR2 complexes on endothelial cell surfaces, thereby enhancing angiogenesis[27]. Furthermore, TRX directly scavenges reactive oxygen species (ROS) and maintains intracellular redox homeostasis, enabling PTC cells to survive and proliferate under increased oxidative stress[31]. Therefore, elevated ROS levels stimulate exosome biogenesis and release—processes critically dependent on CD63 - thereby linking oxidative stress to enhanced CD63-mediated vesicle trafficking and vascular invasion[32]. Given the established correlation between oxidative stress and poor prognosis in angioinvasive PTC[6,14,16,33], TRX and CD63 may represent promising biomarkers for angioinvasion[22,27]. To our knowledge, this is the first study evaluating serum TRX and CD63 in a PTC cohort stratified by angioinvasion. Our hypothesis is that combined measurement of TRX and CD63 will outperform existing markers in detecting early angioinvasion following their synergistic interplay of redox signaling and exosome-mediated angiogenic activation.

This study aims to determine the potential screening value of combined serum TRX and CD63 levels in detecting angioinvasion in PTC. The findings may facilitate earlier identification of aggressive PTC phenotypes and informing therapeutic strategies, thus enabling personalized therapeutic decisions and potentially reducing overtreatment.

Materials and methods

This study was conducted at the Department of Endocrinology, Diabetology, and Internal Diseases at the Medical University of Bialystok, Poland. All procedures received approval from the Local Ethics Committee of the Medical University of Bialystok (APK.002.7.2024), and written informed consent was obtained from all participants.

Studied population

In this comprehensive study, 300 patients diagnosed with PTC were initially considered. To fulfill the specific objectives of this study, only individuals with histopathologically confirmed angioinvasion/or unequivocally angioinvasion-free PTC, thereby preserving maximal cohort homogeneity. All selected patients were free from any medication regimens or underlying conditions that could influence peripheral concentration of detected factors. Therefore, before enrollment, participants confirmed they had not followed restrictive diets, used tobacco, consumed alcohol, or engaged in excessive physical activity. Individuals were excluded if they presented with any of the following severe comorbid conditions: decompensated heart failure (NYHA class III–IV), recent myocardial infarction or unstable angina (< 6 months), uncontrolled hypertension, chronic kidney disease stage IV–V, severe hepatic impairment (Child–Pugh B–C), active systemic infection, autoimmune disease requiring immunosuppressive therapy, or a concurrent malignancy. From the initial cohort of 300 PTC patients, we deliberately selected equal-sized groups to ensure maximal homogeneity and statistical power. This case-control design was not intended to reflect the natural prevalence of angioinvasion, but rather to provide a robust comparison for biomarker evaluation. Vascular invasion was defined according to the WHO 2022 criteria, requiring unequivocal presence of tumor cells within endothelial-lined vascular spaces attached to the vessel wall. All cases were reviewed independently by two pathologists, and discrepancies were resolved by consensus (Fig. 1). As a result, the study group comprised 45 patients with histopathologically confirmed angioinvasive PTC. In contrast, the reference group consisted of 45 patients with very low-risk PTC (Table 1). All participants were enrolled post-thyroidectomy. Fasting morning venous blood (5.5 ml) was drawn into serum tubes and allowed to clot for 60 min at room temperature. Samples were centrifuged at 1000 × g for 10 min at 25 °C within 60 min of collection. Serum was aliquoted (1500 µL) into polypropylene tubes and stored at − 80 °C. No freeze-thaw cycles were permitted before analysis. Hemolyzed/lipemic/icteric samples were excluded using predefined thresholds. Plate order was randomized; operators were blinded to group allocation. Following an interdisciplinary evaluation, all patients in the study group were deemed eligible for radioiodine therapy (RAI), whereas those in the reference group were managed through active surveillance in accordance with the guidelines of the American Thyroid Association (ATA) and local protocols[34,35].

Biochemical measurement

Serum concentrations of triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), cholesterol (CHOL), and C-reactive protein (CRP) were determined using an enzymatic colorimetric assay on the Roche C111 analyzer (Roche Diagnostics, Basel, Switzerland). Meanwhile, thyroid-related parameters, including thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), TGB, and TGBAb, were measured via electrochemiluminescence (ECLIA) using the Roche E411 analyzer (Roche Diagnostics, Sussex, UK).

Serum concentrations of TRX (BlueGene Biotech, E01T0336, Shanghai, China) and CD63 (Invitrogen, EEL135, Vienna, Austria) were measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocols. All measurements were performed in duplicate with acceptance CV ≤ 10%. Intra- and inter-assay precision, spike-recovery (target 80–120%), and serial dilution linearity ($r^2 \geq 0.98$) were established in pooled patient sera. LoD was defined as mean blank + 3SD; LoQ as mean blank + 10SD. Calibration was performed using standard curves with 4-PL fit.

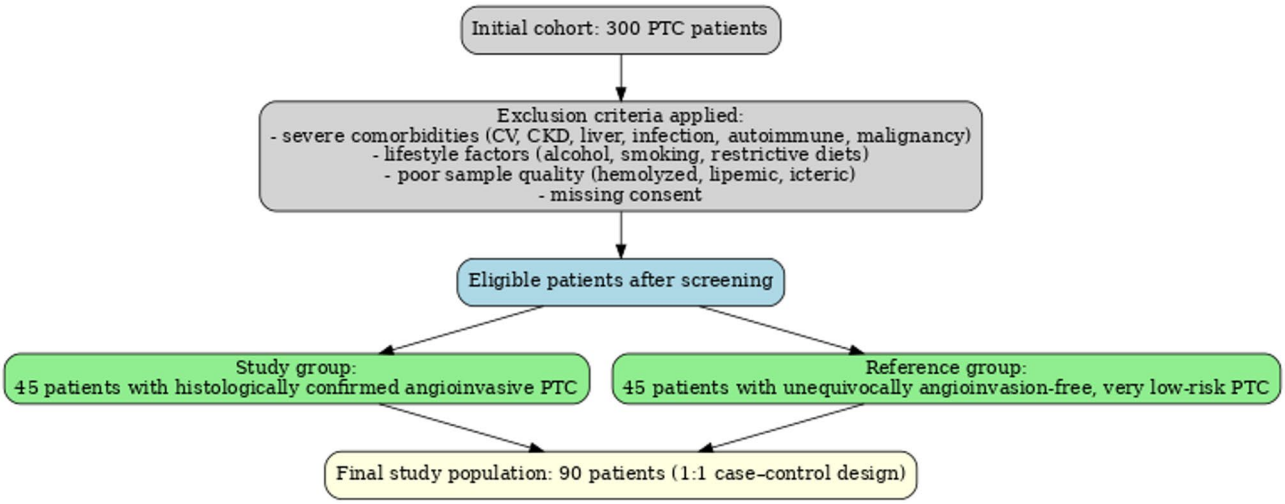

Fig. 1. Flow chart presenting screening for the study; CV—cardiovascular disease, CKD—chronic kidney disease.

	Study group	Reference group
Number of patients	45	45
Median age (upper and lower quartiles)	52 (50.41; 66.32)	54 (50.61; 66.11)
Sex	M: 12	M: 16
	F: 29	F: 33
Menopausal status		
Premenopausal	5	6
Postmenopausal	28	23
Stage (TNM)	pT1a(m):11 pT1b: 13 pT1b(m): 6 pT2: 10 pT3/pT4: 5	pT1a:32 pT1b:13
Patients diagnosed with angioinvasion	45	0
Patients without angioinvasion	0	45

Table 1. Characteristics of patients with PTC. F, female; M, male; (m), multifocal; p, pathological; TNM, cancer tumor-node-metastasis classification (based on the characteristics of primary tumor site (pT)); pT1a, Tumor size ≤ 1 cm in greatest dimension limited to the thyroid,; pT1b, Tumor > 1 cm but ≤ 2 cm in greatest dimension, limited to the thyroid; pT2, Tumor size > 2 cm but ≤ 4 cm, limited to the thyroid; T3/pT4, Tumor size > 4 cm, with gross extrathyroidal extension; SE, standard error.

Statistical analysis

The statistical analyses were conducted using GraphPad Prism 10.0 (GraphPad Software Inc., San Diego, CA, USA) and MedCalc Statistical Software version 22.017 (MedCalc Software Ltd, Ostend, Belgium). The normality of the investigated parameters was assessed using the Shapiro–Wilk test, which indicated that the data did not follow a normal distribution. Consequently, nonparametric tests were applied for group comparisons, with results presented as median values and ranges. The Mann–Whitney U test was employed to evaluate significant differences in clinical parameters between the study groups, with a significance threshold set at $p < 0.05$.

Spearman's rank correlation coefficient was used to assess correlations between variables within the entire patient cohort. Correlation analyses between TRX, CD63, and lipid/thyroid-related parameters (TG, TGAb, HDL, LDL, TSH) were conducted to explore potential links between oxidative stress, lipid metabolism, and thyroid regulation—factors known to influence thyroid cancer biology. To control for false discovery, p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) method with q = 0.05. All reported p-values in the tables are FDR-adjusted.

To assess the diagnostic potential of TRX and CD63, univariable logistic regression models were initially constructed for each biomarker. Subsequently, a multivariable logistic regression model was developed, adjusting for age, sex, tumor size/stage, TSH level, multifocality, and lymph node status. Model discrimination was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) with 95% confidence intervals (CIs) was calculated. The optimal cut-off points were determined according to the Youden index, and corresponding sensitivity, specificity, PPV, and NPV values were derived.

Positive predictive value (PPV) and negative predictive value (NPV) were calculated based on sensitivity, specificity, and disease prevalence observed in the study cohort. PPV and NPV were derived from the confusion matrix at the optimal Youden's index threshold, using the standard formulas: PPV = TP/(TP + FP), NPV = TN/(TN + FN); where TP = true positives, FP = false positives, TN = true negatives, and FN = false negatives. Corresponding 95% confidence intervals for PPV and NPV were estimated using the Wilson score method

Model calibration was evaluated using the Hosmer–Lemeshow goodness-of-fit test and Brier score, while multicollinearity was assessed via variance inflation factors (VIF). To verify the model's clinical utility, Decision Curve Analysis (DCA) was performed, and the net benefit across threshold probabilities (0.2–0.8) was calculated to compare the combined TRX + CD63 model with single-marker and default "treat-all" and "treat-none" strategies. Finally, the explicit logistic regression formula for the combined TRX and CD63 model was derived to enable individual risk estimation: logit(p) = − 5.42 + 0.27×TRX + 0.23×CD63; where p represents the probability of angioinvasion.

Results

Biochemical characteristics of the PTC patients

The study group exhibited significantly higher concentrations of TGB, TGBAb, CHOL, and LDL compared to the reference group ($p < 0.05$; $p < 0.05$; $p < 0.05$; $p < 0.001$, respectively). In contrast, no statistically significant differences were identified between the groups for the remaining parameters ($p > 0.05$) (Table 2).

TRX and CD63 profiling among PTC patients

To investigate our hypothesis, we stratified the PTC patient cohort into distinct subgroups. The study group consisted of individuals with angioinvasive PTC. The following subgroup, serving as the reference group, consisted of patients with very low-risk PTC, as defined by the 2015 American Thyroid Association (ATA) guidelines. This classification enabled a systematic comparison of biomarker expression across different levels of disease severity.

Serum TRX and CD63 levels were significantly elevated in the angioinvasive group (both, $p < 0.001$) compared to the reference group (Table 3; Fig. 2).

Logistic regression profiling of the examined parameters

Additionally, in order to ascertain whether the TRX and CD63 markers stem from the cancer itself or result from systemic processes triggered by the disease, logistic regression analysis was conducted. It confirmed a strong association with angioinvasion, supporting their role in tumor vascular invasion. Based on simple logistic regression findings, we developed a logistic regression model using the simultaneous assessment of TRX and

Parameter	Study group Median (minimum-maximum)	Reference group Median (minimum-maximum)	*P*-value*
TSH (μIU/ml)	0.76 (0.05–3.83)	1.38 (0.09–3.71)	0.092
FT3 (pg/ml)	2.64 (1.36–3.76)	2.41 (1.0-6.27)	0.883
FT4 (ng/mL)	1.22 (0.54–2.14)	1.83 (0.42–1.82)	0.941
TGB (ng/ml)	6.41 (0.04–37.05)	0.39 (0.04–3.20)	**0.041**
TGBAb (IU/mL)	11.22 (0.6-132.4)	5.99 (0.00-45.10)	**0.048**
CHOL (mg/dl)	211.00 (160.00-361.00)	184.70 (99.00-262.00)	**0.027**
LDL (mg/dl)	155.50 (92.00-298.00)	109.10 (60.00-175.00)	**< 0.001**
TG (mg/dl)	134.00 (70.00-219.00)	128.50 (84.00-151.00)	0.921
HDL (mg/dl)	39.10 (29.00–56.00)	45.80 (40.00–77.00)	**0.043**
25-OH vit. D (ng/ml)	28.21 (10.5–51.2)	27.18 (12.2–62.6)	0.567
GLUCOSE (mg/dL)	96.05 (77–114)	96.44 (69–118)	0.518
CRP (mg/L)	2.15 (0.4–5.8)	3.4 (0.2–8.6)	0.557

Table 2. A comparison of the biochemical profiles between the study group and the reference group. Values marked in bold and underlined indicate statistically significant results at the level of $p < 0.05$. *U-Mann Whitney test. CHOL, cholesterol; CRP, C reactive protein; PTC, papillary thyroid cancer; fT3, free triiodothyronine; fT4, free thyroxine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; TGB, thyroglobulin; TGBAb, antithyroglobulin antibodies; TSH, thyroid-stimulating hormone; vit. D, vitamin D.

Parameter	Study group Median (minimum-maximum)	Reference group Median (minimum-maximum)	*P*-value*
TRX (ng/ml)	4.2 (1.9–10.7)	2.2 (0.4–3.7)	**<0.001**
CD63 (ng/mL)	2.5 (1.1–5.3)	1.4 (0.3–2.5)	**<0.001**

Table 3. Angioinvasion markers profiling. Values marked in bold and underlined indicate statistically significant results at the level of $p < 0.05$. **U-Mann Whitney test TAC, total antioxidant capacity; 3-NT – 3-nitrotyrosine; PLGF, placental growth factor; ITGAV, integrin subunit Alpha V; ITGαVβ3, integrin subunit alpha V beta 3.

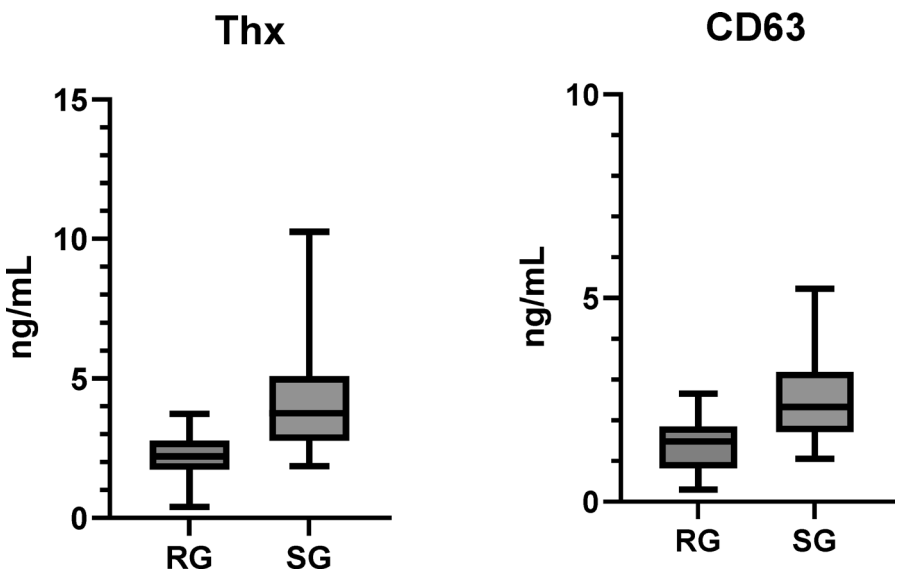

Fig. 2. Comparison of marker concentrations; RG- reference group, SG—study group, TRX—thioredoxin, CD63—tetraspanin 30.

Parameter	B	SE	*p*	OR (95% CI)
TRX (ng/ml)	1.458	0.399	**0.0003**	4.296 (2.19 to 10.64)
CD63 (ng/mL)	2.112	0.549	**0.0001**	8.266 (3.223 to 28.63)

Table 4. The angioinvasion logistic regression analysis. Values marked in bold and underlined indicate statistically significant results at the level of $p < 0.05$. TRX, thioredoxin; CD63, tetraspanin 30; B, beta; SE, standard error, *p*, *p*-value; OR, odds ratio.

CD63 creating multiple logistic regression model associated with angioinvasion occurrence. In multivariable logistic regression adjusted for age, sex, tumor size/stage, TSH, multifocality, and lymph node status, both TRX (OR = 1.32 per 1 ng/mL, 95% CI 1.14–1.55, $p = 0.001$) and CD63 (OR = 1.25 per 1 ng/mL, 95% CI 1.09–1.44, $p = 0.002$) remained independently associated with angioinvasion. The combined model including TRX and CD63 achieved the best discrimination (AUC = 0.93), with good calibration (Hosmer–Lemeshow $p = 0.48$; Brier score = 0.12) (Table 4). Nagelkerke R^2 was 0.58, and VIF for all predictors were < 2.0, indicating no relevant collinearity.

The clinical utility

To assess the significance of TRX and CD63 in angioinvasion screening in PTC patients, the ROC curve analysis was performed. The highest screening utility was presented for TRX (AUC = 0.85, 95% CI: 0.77–0.92) and CD63 (AUC = 0.83, 95% CI: 0.74–0.90), respectively (all $p < 0.05$) (Fig. 3). Using ROC curve analysis, the optimal cut-off points were determined according to the Youden index. For TRX, the optimal threshold was 6.0 ng/mL, yielding a Youden's J of ~ 0.60, with sensitivity = 0.80 (95% CI: 0.67–0.89) and specificity = 0.81 (95% CI: 0.68–0.90).

For CD63, the optimal threshold was 5.0 ng/mL, corresponding to a Youden's J of ~ 0.55, with sensitivity = 0.78 (95% CI 0.64–0.88) and specificity = 0.79 (95% CI 0.65–0.88).

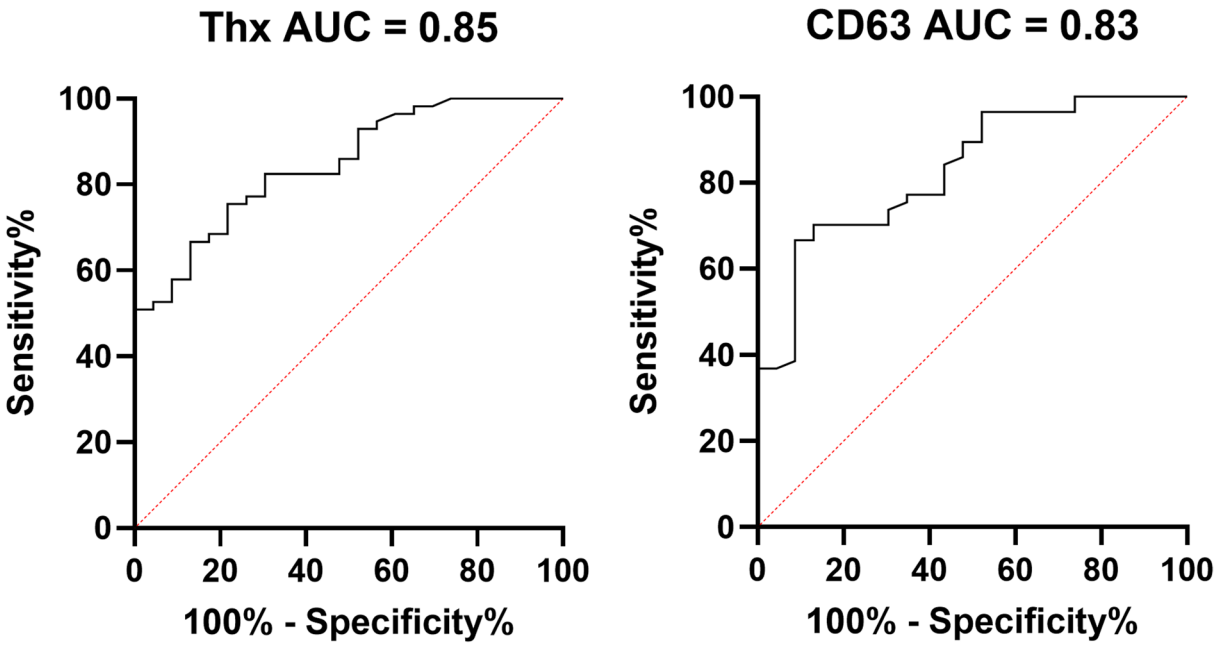

Fig. 3. The angioinvasion screening utility of markers compared to AUC = 0.5; TRX—Thioredoxin, CD63—Tetraspanin 30.

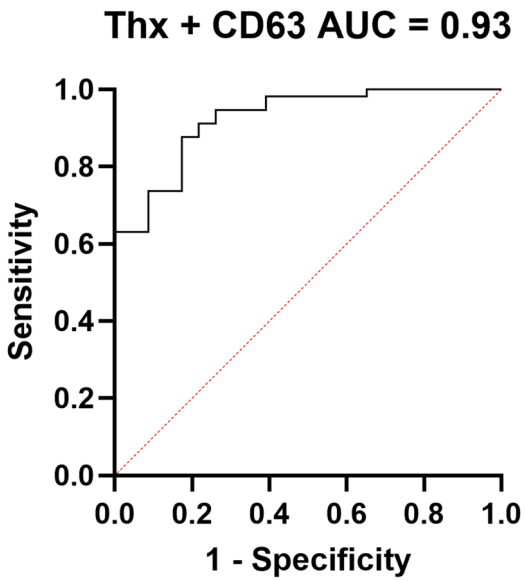

Fig. 4. The angioinvasion screening utility of proposed screening model compared to AUC = 0.5; TRX—Thioredoxin, CD63—Tetraspanin 30.

Furthermore, a logistic regression model incorporating assessments of TRX and CD63 was constructed, demonstrating the highest screening utility (AUC = 0.93, 95% CI 0.87–0.98, $p < 0.01$) (Fig. 4).

To further enhance clinical interpretability, we present the explicit logistic regression formula for the combined TRX and CD63 model predicting angioinvasion in PTC: $\text{logit}(p) = -5.42 + 0.27 \times \text{TRX} + 0.23 \times \text{CD63}$, where p represents the probability of angioinvasion.

The optimal probability threshold derived from ROC analysis was 0.50, corresponding to the maximum Youden's index (~0.75). At this threshold, the model achieved sensitivity = 0.87 (95% CI: 0.74–0.94), specificity = 0.84 (95% CI: 0.70–0.92), PPV = 0.86 (95% CI: 0.73–0.94), and NPV = 0.85 (95% CI: 0.72–0.93). This formula enables clinicians to estimate the individual probability of angioinvasion by substituting serum TRX and CD63 concentrations (in ng/mL), thus supporting its practical application in personalized risk assessment (Table 5).

Biomarker/model	AUC	Optimal Cut-off	Youden's J	Sensitivity (%)	Specificity (%)	NPV (95% CI)	PPV (95% CI)
TRX	0.85	~6.0 ng/mL	~0.60	~80	~80	0.80 (0.67–0.89)	0.81 (0.69–0.90)
CD63	0.83	~5.0 ng/mL	~0.55	~75–80	~75–80	0.78 (0.64–0.88)	0.79 (0.66–0.88)
TRX + CD63 model	0.93	~0.5 probability	~0.75	>85	>85	0.86 (0.73–0.94)	0.85 (0.72–0.93)

Table 5. The cutoff of studied parameters. RX, thioredoxin; CD63, tetraspanin 30; AUC, area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value.

	TRX		CD63	
Parameter	*P*-value	*R*	*P*-value	*R*
CHOL	**0.04**	**− 0.45**	0.05	0.19
TG	0.53	− 0.06	0.38	0.09
LDL	0.67	0.04	0.11	0.16
HDL	0.90	− 0.01	0.78	− 0.03
GLUCOSE	0.65	0.04	0.52	0.06
CRP	0.88	0.01	0.78	0.03
25-OH VIT D	0.77	− 0.03	0.06	0.18
TGBAb	**0.04**	**0.34**	**0.03**	**0.32**
TGB	0.45	0.07	**0.02**	**0.35**
TSH	**<0.01**	**− 0.49**	**0.02**	**− 0.50**
FT3	**<0.01**	**0.37**	**0.01**	**− 0.50**
FT4	0.11	0.16	**<0.01**	**− 0.74**

Table 6. The correlation of studied parameters. Values marked in bold and underlined indicate statistically significant results at the level of $p < 0.05$. TRX, thioredoxin; CD63, tetraspanin 30; CHOL, cholesterol; CRP, C reactive protein; PTC, papillary thyroid cancer; fT3, free triiodothyronine; fT4, free thyroxine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; TGB, thyroglobulin; TGBAb -antithyroglobulin antibodies; TSH, thyroid-stimulating hormone; vit. D, vitamin D.

Model calibration and clinical usefulness were evaluated using the Hosmer–Lemeshow test, Brier score, and DCA. The combined TRX + CD63 model showed excellent calibration (Hosmer–Lemeshow $p = 0.48$; Brier score = 0.12), indicating strong agreement between predicted and observed probabilities of angioinvasion. In the DCA, the combined model provided the greatest net benefit within the clinically relevant threshold range (0.2–0.8), surpassing both individual biomarkers and default "treat-all" or "treat-none" strategies.

Correlations

Spearman's rank correlation coefficient was employed to analyze all correlations within the entire patient cohort, aiming to discern relationships with thyroid cancer occurrences. The reported p-values are FDR-adjusted; only these corrected values are presented in Table 6. After correction, significant positive correlations between TRX and TGBAb ($r = 0.34$, $p = 0.04$) and between CD63 and TGBAb ($r = 0.32$, $p = 0.03$) and between CD63 and TGB ($r = 0.35$, $p = 0.02$) were observed. Conversely, significant negative correlations were identified between TRX and CHOL ($r = -0.45$, $p = 0.04$), and between TRX and TSH ($r = -0.49$, $p < 0.01$). Furthermore, significant negative correlations were found between CD63 and TSH ($r = -0.50$, $p = 0.02$), between CD63 and FT3 and FT4 ($r = -0.50$, $p = 0.01$; $r = -0.74$, $p < 0.01$, respectively). Moreover, significant correlation was found between TRX and FT3 ($r = 0.37$, $p < 0.01$). Additionally, significant negative correlations were observed between 25-OH vit D levels and concentrations of TGB and TGBAb ($r = -0.52$, $r = -0.52$; all $p < 0.05$, respectively). Furthermore, significant negative correlations were found between TGBAb levels and concentrations of HDL ($r = -0.58$, $p = 0.02$). Conversely, a significant positive correlation was noted between TGB levels and glucose ($r = 0.55$, $p = 0.01$) and CRP ($r = 0.54$, $p < 0.01$). Additionally, significant positive correlations were identified between TSH levels and concentrations of CHOL ($r = 0.67$, $p < 0.01$), LDL ($r = 0.64$, $p = 0.01$), HDL ($r = 0.44$, $p = 0.03$) (Table 6; Fig. 5).

Discussion

Thyroid cancer tissue profiling enables the identification of biomarkers for improved risk assessment and treatment strategies[36]. Studies performed by Lincoln et al. reveled that the expression of TRX in thyroid cancer tissues have demonstrated their increased presence in the cytoplasm and nuclei of cancer cells compared to healthy tissue. Interestingly, strong TRX expression was correlated with increased tumor cell proliferation, suggesting their role as autocrine stimulators of cancer growth[22,37,38]. TRX was also identified as potential medical target in cancer therapy[39]. Interestingly, TRX is secreted into the extracellular environment, serving as a potential marker of cancer progression, as previously demonstrated in hepatocellular, gastric, breast, and lung cancers[37,38,40–42]. Similarly, the tetraspanin protein family plays a pivotal role in regulating cellular

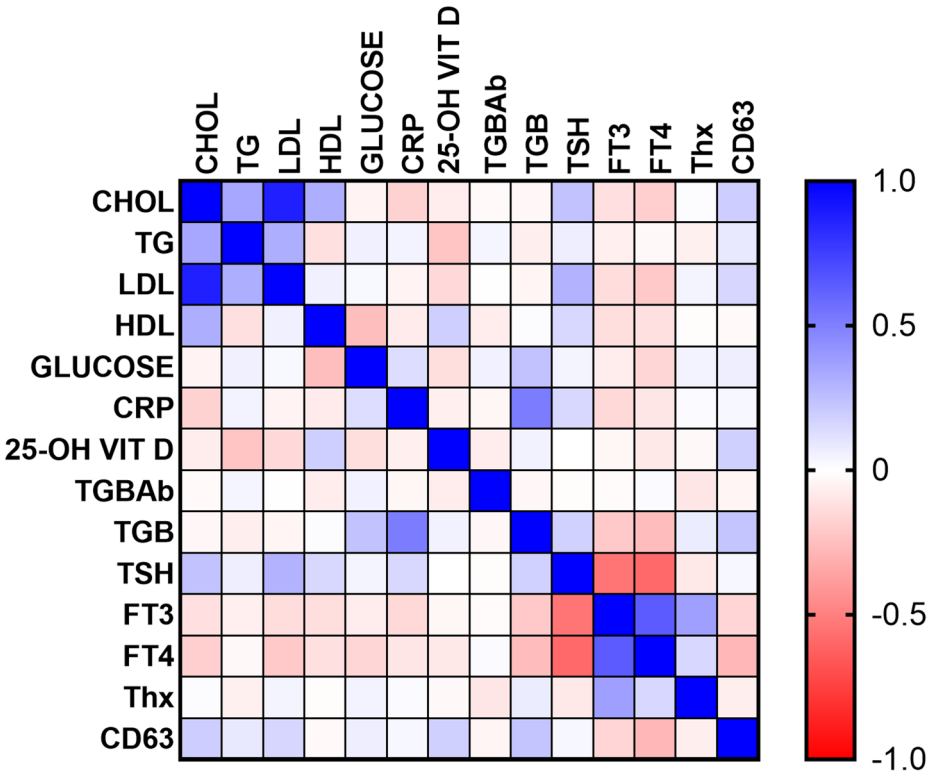

Fig. 5. Heat map of correlation coefficient. CHOL—cholesterol, CRP—C reactive protein, PTC—papillary thyroid cancer, fT3—free triiodothyronine, fT4—free thyroxine, HDL—high-density lipoprotein, LDL—low-density lipoprotein, TG—triglyceride, TGB—thyroglobulin, TGBAb—antithyroglobulin antibodies, TSH—thyroid-stimulating hormone, vit. D—vitamin D, TRX—thioredoxin, CD63—tetraspanin 30.

processes, including involvement in tumorigenesis, epithelial-mesenchymal transition, thrombosis, tumor stem cell regulation, and exosome-mediated signaling[43]. Studies on CD63 concentration evaluation in thyroid cancer have not established a direct association with thyroid carcinogenesis[44]. Nevertheless, exosome-mediated signaling pathways have been investigated in the context of PTC pathogenesis and cancer progression[28,45,46]. Several in vivo studies support the role of the TSP/CD63 system in these processes, simultaneously underscoring the significance of CD63-mediated signaling in the suppression of pathological angiogenesis and its function as a critical regulator within the tumor microenvironment. Moreover, these findings highlight the therapeutic potential of targeting the CD63 antiangiogenic pathway as a promising strategy for cancer treatment, particularly in limiting tumor vascularization and metastasis[47–49]. Previous studies suggest that TRX and CD63 may participate in pathways linking oxidative stress and exosome-mediated angiogenesis in PTC. While our findings are consistent with this hypothesis, our study does not provide direct mechanistic evidence. The pursuit of novel biomarkers is reinforced by both our previous research and existing literature, highlighting the necessity for more precise diagnostic tools in the monitoring and risk stratification of PTC[7,33,50]. While prior studies from our group have focused on markers of oxidative stress and endothelial damage, the current study highlights TRX and CD63 as potential serum biomarkers of angioinvasion. The role of oxidative signaling and exosome-mediated angiogenesis in tumor progression remains a hypothesis to be tested in future functional studies. Current evidence suggests that integrating conventional diagnostic methods with novel metabolic markers such as TRX and CD63 may significantly enhance the accuracy of disease progression assessment, supporting a more comprehensive and personalized approach in PTC patient management[6,14].

Our study demonstrated that serum concentrations of TRX and CD63 were significantly elevated in patients with angioinvasive PTC. Subsequent logistic regression analysis confirmed a strong association between both biomarkers and the presence of angioinvasion, reinforcing their potential role in vascular invasion. The combined assessment of TRX and CD63 allowed for the identification of angioinvasion with high diagnostic accuracy (AUC = 0.93). The good calibration and positive decision curve results further support the robustness and clinical applicability of the combined TRX + CD63 model as a reliable tool for individualized angioinvasion risk prediction in PTC. Moreover, observed correlations between TRX, CD63, and thyroid-related parameters provided valuable insights into their potential clinical significance. The positive associations of TRX and CD63 with TGB and TGBAb further support their utility as biomarkers by demonstrating consistency with routine clinical markers, which may serve as an additional validation of their potential clinical relevance. Since TGB and TGBAb are widely utilized for assessing the completeness of thyroid cancer cell resection during PTC surveillance[51–53], the observed correlations further support the relevance of TRX and CD63 in postoperative monitoring and disease management. These findings underscore the potential utility of TRX and CD63 as complementary biomarkers

alongside existing markers, warranting future studies to validate their clinical relevance and establish their role in improving the accuracy of treatment response evaluation and recurrence detection in PTC[54–57]. Furthermore, their clinical utility may be particularly relevant in patients who have undergone lobectomy rather than total thyroidectomy, where TGB and TGBAb markers evaluation is not applicable due to the presence of remaining thyroid tissue, which continues to produce thyroglobulin and may lead to falsely elevated TGB levels unrelated to cancer recurrence. This limitation highlights the need for alternative biomarkers, such as TRX and CD63, which are independent of thyroid tissue volume and may reflect tumor-specific processes[58]. Additionally, the positive correlations of TGB with glucose and CRP suggest that metabolic and inflammatory disturbances may interfere with disease activity assessment, reinforcing the need for the development of novel, comprehensive monitoring tools beyond standard oncologic markers. As it was proved by Preissner et al., who demonstrated that heterophile antibody (HAB) interference in automated TG assays can cause falsely elevated Tg levels, potentially leading to unnecessary radioiodine treatment decisions, underscoring how immunoassay artifacts and unrecognized inflammatory states may distort the interpretation of thyroid cancer monitoring markers[59]. Nevertheless from a clinical perspective, this highlights the potential role of metabolic dysregulation and chronic inflammation in promoting an aggressive tumor microenvironment, facilitating processes such as endothelial dysfunction and oxidative stress. Exosome-mediated signaling has been proposed in the literature, but was not directly evaluated in our study. Therefore, identifying and validating alternative biomarkers such as TRX and CD63, which reflect these underlying biological mechanisms, could improve risk stratification and therapeutic decision-making, particularly in patients with complex metabolic profiles or disturbed immunological processes[56,60–62]. One of the notable findings of our study is the negative correlation of TRX and TSH with CHOL, as well as CD63 and TSH, which suggests a potential link between oxidative stress, metabolic alterations, and thyroid hormone levels in PTC[63]. The observed correlation between TRX and CD36 expression levels and serum TSH concentration supports the rationale for TSH suppression as a strategy to reduce the risk of potential angioinvasion and subsequent metastasis in these patients. Furthermore, the observed associations between TGB, TGBAb, and lipid profiles suggest that metabolic dysregulation, particularly involving cholesterol and lipoproteins, may contribute to the progression and recurrence of PTC. These findings highlight the importance of identifying biomarkers such as TRX and CD63, which may reflect underlying tumor-promoting processes, including oxidative stress, angiogenesis, and lipid metabolism dysregulation, thus serving as potential indicators of aggressive disease and metastatic risk[64]. Since higher cholesterol levels are often associated with hypothyroidism, the observed inverse relationship with TRX, TGB, TGBAb concentrations underscores the importance of lipid profile monitoring in PTC patients, particularly those with abnormal TSH levels[65]. As thyroid hormones regulate lipid metabolism, ensuring adequate thyroid hormone levels could mitigate the risk of dyslipidemia, which is particularly relevant given the cardiovascular risks associated with PTC treatments[66]. The observed correlations in this study further emphasize the systemic nature of PTC, linking thyroid cancer markers, lipid metabolism, inflammation, and oxidative homeostasis[65]. The observed negative correlations between CD63 and thyroid hormones (TSH, FT3, FT4) suggest that fluctuations in thyroid function may modulate exosome activity, creating a microenvironment conducive to vascular invasion and metastasis. These findings support the hypothesis that CD63, as a marker of extracellular vesicle dynamics, reflects tumor-promoting processes driven by altered thyroid hormone signaling, highlighting its potential as a diagnostic tool in PTC[67]. These findings suggest that maintaining suppression status in PTC patients could have a broader impact beyond endocrine homeostasis, potentially influencing tumor behavior and response to treatment[68]. Another important finding of our study concerns the interplay between vitamin D status and thyroid cancer monitoring markers. Lower 25-OH vitamin D levels were associated with higher TGB and TGBAb concentrations, indicating that vitamin D deficiency may contribute to an unfavorable tumor microenvironment characterized by persistent inflammation and impaired immune surveillance, which in turn could promote PTC progression and recurrence. These observations highlight the potential benefits of vitamin D supplementation in modulating the immune response and reducing the risk of disease persistence, particularly in patients with elevated TGBAb levels[69].

To date, studies evaluating TRX and CD63 in PTC have primarily focused on tissue expression or extracellular vesicles, with limited data on their circulating levels in the bloodstream. While direct comparisons with other serum-based studies are therefore challenging, existing literature supports the role of TRX and CD63 in tumor progression, angiogenesis, and metastatic potential across various malignancies. Our findings align with this evidence and build upon previous research identifying oxidative stress and endothelial dysfunction markers, such as TAC, 8-OHdG, and sortilin, as potential indicators of angioinvasion in PTC[7,70]. Our previous studies demonstrated significant correlations between oxidative stress markers and lipid parameters in PTC patients, highlighting the relevance of lipid peroxidation in disease progression. Oxidized lipids may contribute to endothelial damage and promote angioinvasion, providing a mechanistic link to the current findings. The involvement of TRX and CD63 in oxidative stress and exosome-mediated pathways further supports their potential as biomarkers reflecting tumor-promoting mechanisms, including vascular invasion[6,71].

Despite the lack of direct studies on TRX and CD63 in PTC, their well-established functions in other malignancies highlight the need for further research to determine their potential role as diagnostic or prognostic markers in PTC. However, certain limitations must be acknowledged, primarily the relatively small number of analyzed cases. To confirm these findings on a larger scale, a multicenter study is recommended. A limitation of this study is that we did not assess TRX and CD63 expression directly in tumor tissues. However, previous reports demonstrated their upregulation in thyroid carcinoma tissues, particularly in aggressive phenotypes. Future studies are warranted to correlate circulating TRX and CD63 levels with their tissue expression and the extent of vascular invasion. Another limitation is the lack of a healthy control group, which prevents us from establishing baseline TRX and CD63 reference ranges. Future studies including healthy volunteers will be essential to validate the diagnostic cut-offs for clinical application. Our strict inclusion/exclusion criteria and 1:1 case–control sampling maximized internal validity for biomarker testing but limit generalizability to

broader PTC populations with common comorbidities. Future external validation in less-selected, multicenter cohorts and with survival outcomes to establish true prognostic value is warranted. The present study evaluates serological association, and does not provide mechanistic proof of a TRX–CD63 interaction in PTC; this remains a hypothesis to be validated in tissue and functional studies. Nevertheless, a key strength of this study is the extensive collection of clinical data, allowing for a comprehensive analysis of multiple factors influencing PTC progression. We also minimized the risk of type I error from multiple testing by applying FDR correction; thus, the associations reported as significant represent robust findings rather than chance correlations. Thus, the numerous significant correlations observed directly relate to the nature of PTC and the role of angiogenesis in tumor development. These findings provide a strong foundation for future research, emphasizing the relevance of TRX and CD63 in refining risk assessment and supporting the development of basic research exploring the potential use of CD63 and TRX inhibition as a novel therapeutic approach for PTC. By elucidating their roles in tumor angiogenesis and progression, future studies may pave the way for innovative treatment strategies targeting these pathways in modern PTC therapy.

In line with our findings, recent pan-cancer research has underscored the value of biomarkers reflecting tumor–microenvironment interactions. Yuan et al. identified YBX family members, particularly YBX2, as prognostic markers and potential therapeutic targets, with a YBXs score predicting immune activation and therapy responsiveness across multiple cancers. While their approach was based on transcriptomic profiling, our study demonstrates that circulating proteins such as TRX and CD63 can also capture key aspects of tumor aggressiveness, including oxidative stress and exosome-mediated vascular invasion. The convergence of these findings suggests that integrating genomic and proteomic biomarkers could enhance risk stratification and inform precision treatment strategies, not only in papillary thyroid cancer but across tumor types[20]. Our findings also resonate with recent studies emphasizing tumor–microenvironment remodeling as a driver of aggressive disease phenotypes. For instance, Xie et al. demonstrated that GLO1 potentiates lymph node metastasis in breast cancer by orchestrating paracrine TME alterations, including expansion of myCAFs, accumulation of APOE + macrophages, and CD8 + T cell exhaustion[19]. Similar to our observation that TRX and CD63 reflect oxidative stress and exosome-mediated vascular invasion in PTC, their study highlights how metabolic reprogramming and redox balance can facilitate metastatic spread through immune and stromal modulation. Together with the YBX pan-cancer analysis identifying YBX2 as a biomarker and therapeutic target in liver cancer, these findings support a broader paradigm in which both genomic and proteomic biomarkers capture dynamic TME interactions. Incorporating such markers may enable earlier identification of high-risk patients and inform precision therapeutic strategies across tumor types. Moreover, Xu and colleagues reviewed the role of exosomes as biomarkers and therapeutic vehicles in cardiovascular diseases. Exosomes, by transferring proteins, lipids, and nucleic acids, influence key processes such as inflammation, oxidative stress, and endothelial dysfunction. Evidence links them to coronary artery disease, heart failure, cardiomyopathy, and atrial fibrillation, suggesting strong potential as non-invasive biomarkers and drug delivery tools. However, their clinical use remains immature due to challenges in standardization and validation[18]. The study highlights exosomes as universal mediators of pathology, supporting the relevance of exosome-related markers such as CD63 in cancer progression, as shown in our PTC cohort. Thus, our findings are consistent with emerging evidence from other tumor and non-tumor contexts that emphasize the central role of tumor microenvironment remodeling and exosome biology in disease progression.

Conclusions

The identification of angioinvasion as a screening factor in PTC is crucial for optimizing patient management and treatment strategies. These findings emphasize that TRX and CD63 are not merely passive indicators but may reflect underlying biological processes such as oxidative stress and lipid peroxidation. Their possible relationship with exosome-mediated endothelial disruption has been suggested in previous studies, but our results should be considered hypothesis-generating. This suggests that combining TRX and CD63 profiling could improve early identification of aggressive disease phenotypes, allowing for tailored treatment approaches. The potential role of lipid peroxidation products and immune disturbances in modulating the PTC microenvironment highlights the importance of integrated biomarker panels that can capture this complexity. Future studies should validate these markers in larger, prospective cohorts and investigate their utility in guiding therapeutic decisions, particularly in patients with metabolic disturbances or after lobectomy, where standard monitoring tools are limited.

Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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References

1. Lee, K., Anastasopoulou, C., Chandran, C. & Cassaro, S. Thyroid Cancer. *StatPearls* (2023).
2. Limaiem, F., Rehman, A., Mazzoni, T., Anastasopoulou, C. & Mazzoni, T. Papillary Thyroid Carcinoma. *StatPearls*.
3. Wang, H et al. Global, regional, and national under-5 mortality, adult mortality, age-specific mortality,and life expectancy, 1970–2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet* **390**(10100), 1084–1150 (2017).
4. Haigh, P. I., Urbach, D. R. & Rotstein, L. E. Extent of thyroidectomy is not a major determinant of survival in low- or high-risk papillary thyroid cancer. *Ann. Surg. Oncol.* **12**(1), 81–89 (2005).
5. Bergdorf, K. et al. Papillary thyroid carcinoma behavior: Clues in the tumor microenvironment. *Endocr. Relat. Cancer*. **26**(6), 601–614 (2019).

6. Buczyńska, A. et al. Clinical significance of oxidative stress markers as angioinvasion and metastasis indicators in papillary thyroid cancer. *Sci. Rep.* **13**(1). https://doi.org/10.1038/s41598-023-40898-9 (2023).
7. Buczyńska, A. et al. Enhancing angioinvasion assessment in papillary thyroid cancer via a biomarker panel involving TAC, 8-OHdG and sortilin. *J. Clin. Endocrinol. Metab.* https://doi.org/10.1210/CLINEM/DGAE007 (2024).
8. Hong, S. et al. Distinct molecular subtypes of papillary thyroid carcinoma and gene signature with diagnostic capability. *Oncogene* **41**(47), 5121–5132 (2022).
9. Krajewska, J. et al. Early diagnosis of Low-Risk papillary thyroid cancer results rather in overtreatment than a better survival. *Front. Endocrinol. (Lausanne)*. **11**, 571421 (2020).
10. Spencer, C. & Fatemi, S. Thyroglobulin antibody (TgAb) methods – Strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer. *Best Pract. Res. Clin. Endocrinol. Metab.* **27**(5), 701–712 (2013).
11. Chiovato, L. et al. Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. *Ann. Intern. Med.* **139**(5 I). https://doi.org/10.7326/0003-4819-139-5_PART_1-200309020-00010 (2003).
12. Latrofa, F. et al. Thyroglobulin autoantibodies in patients with papillary thyroid carcinoma: comparison of different assays and evaluation of causes of discrepancies. *J. Clin. Endocrinol. Metab.* **97**(11), 3974–3982 (2012).
13. Miao, S. et al. **9**(3), 653–660 (2020).
14. Buczyńska, A. et al. The relationship between oxidative status and radioiodine treatment qualification among papillary thyroid cancer patients. *Cancers 2023*. **15**(9), 2436 (2023).
15. Buczyńska, A. et al. Oxidative stress and radioiodine treatment of differentiated thyroid cancer. **11**(1) (2021).
16. Kościuszko, M., Buczyńska, A., Krętowski, A. J. & Popławska-Kita, A. Could oxidative stress play a role in the development and clinical management of differentiated thyroid cancer? *Cancers (Basel)*. **15**(12), 3182 (2023).
17. Buczyńska, A. et al. The relationship between oxidative status and radioiodine treatment qualification among papillary thyroid cancer patients. *Cancers (Basel)*. **15**(9). https://doi.org/10.3390/CANCERS15092436 (2023).
18. Xu, Y. et al. Exosomes and their derivatives as biomarkers and therapeutic delivery agents for cardiovascular diseases: situations and challenges. *J. Transl Int. Med.* **11**(4), 341–354 (2023).
19. Xie, J. et al. Paracrine orchestration of tumor microenvironment remodeling induced by GLO1 potentiates lymph node metastasis in breast cancer. *Adv. Sci. (Weinh)*. **12**(32). https://doi.org/10.1002/ADVS.202500722 (2025).
20. Yuan, Z. et al. Comprehensive pan-cancer analysis of YBX family reveals YBX2 as a potential biomarker in liver cancer. *Front. Immunol.* **15** https://doi.org/10.3389/FIMMU.2024.1382520 (2024).
21. Delcorte, O., Degosserie, J. & Pierreux, C. E. Role of extracellular vesicles in thyroid physiology and diseases: implications for diagnosis and treatment. *Biomedicines* **10**(10), 2585 (2022).
22. Lincoln, D. T. et al. Thioredoxin and thioredoxin reductase expression in thyroid cancer depends on tumour aggressiveness.
23. Dunn, L. L., Buckle, A. M., Cooke, J. P. & Ng, M. K. C. The emerging role of the thioredoxin system in angiogenesis. *Arterioscler. Thromb. Vasc Biol.* **30**(11), 2089–2098 (2010).
24. Hou, R. et al. Thioredoxin1 inactivation mediates the impairment of Ischemia-Induced angiogenesis and further injury in diabetic myocardium. *J. Vasc Res.* **57**(2), 76–85 (2020).
25. Dey, S., Basu, S. & Ranjan, A. Revisiting the role of CD63 as Pro-Tumorigenic or Anti-Tumorigenic tetraspanin in cancers and its theragnostic implications. *Adv. Biol.* **7**(7). https://doi.org/10.1002/ADBI.202300078 (2023).
26. Shao, S. et al. The role of tetraspanins in digestive system tumor development: Update and emerging evidence. *Front. Cell. Dev. Biol.* **12**, 1343894 (2024).
27. Tugues, S. et al. Tetraspanin CD63 promotes vascular endothelial growth factor receptor 2-β1 integrin complex formation, thereby regulating activation and downstream signaling in endothelial cells in vitro and in vivo. *J. Biol. Chem.* **288**(26), 19060–19071 (2013).
28. Li, G. et al. Exosome-mediated delivery of miR-519e-5p promotes malignant tumor phenotype and CD8 + T-Cell exhaustion in metastatic PTC. *J. Clin. Endocrinol. Metab.* **109**(6), 1601–1617 (2024).
29. Delcorte, O., Degosserie, J. & Pierreux, C. E. Role of extracellular vesicles in thyroid physiology and diseases: Implications for diagnosis and treatment. *Biomedicines*. **10**(10), 2585 (2022).
30. Jia, M. et al. Navigating thyroid cancer complexity: The emerging role of EV-derived non-coding RNAs. *Cell. Death Discov.* **11**(1), 142 (2025).
31. Lu, J. & Holmgren, A. The thioredoxin antioxidant system. *Free Radic Biol. Med.* **66**, 75–87 (2014).
32. Chiaradia, E. et al. Extracellular vesicles under oxidative stress conditions: biological properties and physiological roles. *Cells 2021*. **10**(7), 1763 (2021).
33. Buczyńska, A., Kościuszko, M., Krętowski, A. J. & Popławska-Kita, A. Exploring the clinical utility of angioinvasion markers in papillary thyroid cancer: A literature review. *Front. Endocrinol. (Lausanne)* **14**, 1261860 (2023).
34. Jarząb, B. et al. Zembala-Nozyńska E, Diagnosis and treatment of thyroid cancer in adult patients - recommendations of polish scientific societies and the national oncological strategy. *Endokrynol. Pol.* **73**(2), 173–300 (2022).
35. Nguyen, Q. T. et al. *Diagnosis and Treatment of Patients with Thyroid Cancer* 30–38 (Engage Healthcare Communications, Inc., 2015).
36. Guo, M., Sun, Y., Wei, Y., Xu, J. & Zhang, C. Advances in targeted therapy and biomarker research in thyroid cancer. *Front. Endocrinol. (Lausanne)* **15**, 1372553 (2024).
37. Shang, W. et al. Increased Thioredoxin-1 expression promotes cancer progression and predicts poor prognosis in patients with gastric cancer. *Oxid. Med. Cell. Longev.* **2019**, 9291683 (2019).
38. Li, J. et al. Serum thioredoxin is a diagnostic marker for hepatocellular carcinoma. *Oncotarget* **6**(11), 9551 (2015).
39. Seitz, R. et al. Exploring the thioredoxin system as a therapeutic target in cancer: Mechanisms and implications. *Antioxid. 2024*. **13**(9), 1078 (2024).
40. Liu, X., Dong, X., Hu, Y. & Fang, Y. Identification of thioredoxin-1 as a biomarker of lung cancer and evaluation of its prognostic value based on bioinformatics analysis. *Front. Oncol.* **13**, 1080237 (2023).
41. Lee, Y. J. et al. The blood level of thioredoxin 1 as a supporting biomarker in the detection of breast cancer. *BMC Cancer*. **22**(1), 1–14 (2022).
42. Ye, S. et al. Thioredoxin reductase as a novel and efficient plasma biomarker for the detection of non-small cell lung cancer: A large-scale, multicenter study. *Sci. Rep.* **9**(1), 1–12 (2019).
43. Zhang, H. et al. Tspan protein family: Focusing on the occurrence, progression, and treatment of cancer. *Cell Death Discov.* **10**(1), 1–14 (2024).
44. Chen, Z. et al. CD82, and CD63 in thyroid cancer. *Int. J. Mol. Med.* **14**(4), 517–527 (2004).
45. Paskeh, M. D. A. et al. Emerging role of exosomes in cancer progression and tumor microenvironment remodeling. *J. Hematol. Oncol.* **15**(1), 83 (2022).
46. Zhang, S. et al. Exploring exosomes: novel diagnostic and therapeutic frontiers in thyroid cancer. *Front. Pharmacol.* **15**, 1431581 (2024).
47. Simantov, R. & Silverstein, R. L. CD36: a critical anti-angiogenic receptor. *Front. Biosci.* **8**(SUPPL.). https://doi.org/10.2741/1168 (2003).
48. Laís Justo, B., Galvonas Jasiulionis, M., Smetana, K. & Kolář, M. Characteristics of TIMP1, CD63, and β1-Integrin and the functional impact of their interaction in cancer. *Int. J. Mol. Sci. 2021*. **22**(17), 9319 (2021).

49. Feng, W. W., Zuppe, H. T. & Kurokawa, M. The role of CD36 in cancer progression and its value as a therapeutic target. ;**12**(12), 1605. (2023).
50. Rogucki, M. et al. Expression profile and diagnostic significance of MicroRNAs in papillary thyroid cancer. *Cancers (Basel)* **14**(11). https://doi.org/10.3390/CANCERS14112679 (2022).
51. Xu, S. et al. Predictive value of serum thyroglobulin for structural recurrence following lobectomy for papillary thyroid carcinoma. *Thyroid* **31**(9), 1391–1399 (2021).
52. Spencer, C. A. & LoPresti, J. S. Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. *Nat. Clin. Pract. Endocrinol. Metab.* **4**(4), 223–233 (2008).
53. Whitley, R. J. & Ain, K. B. Thyroglobulin a specific serum marker for the management of thyroid carcinoma. *Clin. Lab. Med.* **24**(1), 29–47 (2004).
54. Prpić, M., Franceschi, M., Romić, M., Jukić, T. & Kusić, Z. Thyroglobulin as a tumor marker in differentiated thyroid cancer—clinical considerations. *Acta Clin. Croat*. **57**(3), 518 (2018).
55. Algeciras-Schimnich, A. Thyroglobulin measurement in the management of patients with differentiated thyroid cancer. *Crit. Rev. Clin. Lab. Sci.* **55**(3), 205–218 (2018).
56. Spencer, C., Petrovic, I. & Fatemi, S. Current thyroglobulin autoantibody (TgAb) assays often fail to detect interfering TgAb that can result in the reporting of falsely low/undetectable serum Tg IMA values for patients with differentiated thyroid cancer. *J. Clin. Endocrinol. Metab.* **96**(5), 1283–1291 (2011).
57. Landenberger, G. M. C., De Souza Salerno, M. L., Golbert, L. & De Souza Meyer, E. L. Thyroglobulin antibodies as a prognostic factor in papillary thyroid carcinoma patients with indeterminate response after initial therapy. *Horm. Metab. Res.* **53**(2), 94–99 (2021).
58. Kwon, H. et al. A comparison of lobectomy and total thyroidectomy in patients with papillary thyroid microcarcinoma: A retrospective individual risk factor-matched cohort study. *Eur. J. Endocrinol.* **176**(4), 371–378 (2017).
59. Preissner, C. M., O'Kane, D. J., Singh, R. J., Morris, J. C. & Grebe, S. K. G. Phantoms in the assay tube: Heterophile antibody interferences in serum thyroglobulin assays. *J. Clin. Endocrinol. Metab.* **88**(7), 3069–3074 (2003).
60. Aronson, D. et al. Association between fasting glucose and C-reactive protein in middle-aged subjects. *Diabet. Med.* **21**(1), 39–44 (2004).
61. Berlińska, A. & Świątkowska-Stodulska, R. Clinical use of thyroglobulin: Not only thyroid cancer. *Endocrine* **84**(3), 786 (2024).
62. Spencer, C. A., Petrovic, I., Fatemi, S. & LoPresti, J. Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer using sensitive (second-generation) immunometric assays can be disrupted by False-Negative and False-Positive serum thyroglobulin autoantibody misclassifications. *J. Clin. Endocrinol. Metab.* **99**(12), 4589 (2014).
63. Tronci, L. et al. Crosstalk between metabolic alterations and altered redox balance in PTC-Derived cell lines. *Metabolites* **9**(2). https://doi.org/10.3390/METABO9020023 (2019).
64. Jiang, N. et al. Plasma lipidomics profiling reveals biomarkers for papillary thyroid cancer diagnosis. *Front. Cell. Dev. Biol.* **9**, 682269 (2021).
65. Zwara, M. et al. The role of lipid metabolism disorders in the development of thyroid cancer. *Int. J. Mol. Sci.* **25**(13), 7129 (2024).
66. Tsai, M. C. et al. Association between thyroid cancer and cardiovascular disease risk: A nationwide observation study. *Sci. Rep.* **12**(1). https://doi.org/10.1038/s41598-022-22462-z (2022).
67. Furuno, T., Teshima, R., Kitani, S., Sawada, J. I. & Nakanishi, M. Surface expression of CD63 antigen (AD1 antigen) in P815 mastocytoma cells by transfected IgE receptors. *Biochem. Biophys. Res. Commun.* **219**(3), 740–744 (1996).
68. Hannoush, Z. C. & Weiss, R. E. Thyroid hormone replacement in patients following thyroidectomy for thyroid cancer. *Rambam Maimonides Med. J.* **7**(1), e0002 (2016).
69. Koljević Marković, A. & Ristić-Medić, D. Evaluation of vitamin D3 levels and dietary management in patients with papillary thyroid cancer. *Hell J. Nucl. Med.* **26**(3), 194–200 (2023).
70. Buczyńska, A. et al. Clinical significance of oxidative stress markers as angioinvasion and metastasis indicators in papillary thyroid cancer. *Sci. Rep.* **13**(1), 1–10 (2023).
71. Buczyńska, A. et al. Oxidative stress and radioiodine treatment of differentiated thyroid cancer. *Sci. Rep.* **11**(1). https://doi.org/10.1038/S41598-021-96637-5 (2021).

Author contributions

Conceptualization, A.B. and A.PK; Methodology, A.B. A.A, K.S and M. Sz; Software, I.S., M.K. and A. A; Validation, A.B., I.S and J.DZ.; Formal Analysis, A.B.; Investigation, A.B., A.PK; Resources, A.PK.; Data Curation, A.B. I.S and M.K.; Writing—Original Draft Preparation, A.B.; Writing—Review and Editing, I.S, A.J.K. and A.PK; Visualization, A.B.; Supervision, A.PK.; Project Administration, A.B and A.PK.; Funding Acquisition, A.B. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical University of Bialystok, Poland (APK.002.7.2024).

Informed consent

Written informed consent has been obtained from the patients to publish this paper.

Additional information

Correspondence and requests for materials should be addressed to A.B.

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