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Liquid Biopsy–Based Biomolecular Alterations for the Diagnosis of Triple-Negative Breast Cancer in Adults: A Scoping Review

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Abstract

Background/Objectives: Triple-negative breast cancer (TNBC) is an aggressive subtype, with limited diagnostic options and no targeted early detection tools. Liquid biopsy represents a minimally invasive approach for detecting tumor-derived molecular alterations in body fluids. This scoping review aimed to comprehensively synthesize all liquid biopsy–derived molecular biomarkers evaluated for the diagnosis of TNBC in adults. **Methods:** This review followed the Arksey and O'Malley framework and PRISMA-ScR guidelines. Systematic searches of PubMed, Scopus, Embase, and Web of Science identified primary human studies evaluating circulating molecular biomarkers for TNBC diagnosis. Non-TNBC, non-human, hereditary, treatment-response, and non-molecular studies were excluded. Data on study design, patient characteristics, biospecimen type, analytical platforms, biomarker class, and diagnostic performance were extracted and synthesized descriptively by biomolecule class. **Results:** Thirty-two studies met inclusion criteria, comprising 15 protein-based, 11 RNA-based, and 6 DNA-based studies (one reporting both protein and RNA). In total, 1532 TNBC cases and 3137 participants in the comparator group were analyzed. Protein biomarkers were the most frequently studied, although only APOA4 appeared in more than one study, with conflicting results. RNA-based biomarkers identified promising candidates, particularly miR-21, but validation cohorts were scarce. DNA methylation markers showed promising diagnostic accuracy yet lacked replication. Most studies were small retrospective case–control designs with heterogeneous comparators and inconsistent diagnostic reporting. **Conclusions:** Evidence for liquid biopsy–derived biomarkers in TNBC remains limited, heterogeneous, and insufficiently validated. No biomarker currently shows reproducibility suitable for clinical implementation. Robust, prospective, and standardized studies are needed to advance liquid biopsy–based diagnostics in TNBC.

Keywords: triple-negative breast cancer; liquid biopsy; circulating biomarkers; protein biomarkers; RNA biomarkers; DNA methylation; microRNAs; diagnostic accuracy; non-invasive diagnostics; molecular oncology

1. Introduction

Breast cancer was the second most frequently diagnosed malignancy worldwide in 2022, second only to lung cancer, with an estimated 2.3 million new cases, representing 11.6% of all new cancer cases. It is the leading cause of cancer-related death in women, accounting for 15.4% of deaths

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(666,000 deaths) [1]. It is a heterogeneous disease with variable morphological and biological characteristics, resulting in different clinical behaviors and responses to therapy [2]. Immunophenotyping using immunohistochemistry allows for molecular classification based on the expression of nuclear estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor 2 (HER2), and Ki-67, which act as prognostic factors and have predictive value for therapy response. These markers allow breast cancer to be classified into the following subtypes: luminal A (ER and/or PR+, HER2−, Ki-67 low), luminal B (ER and/or PR+, HER2−, Ki-67 high) or (ER and/or PR+, HER2+), HER2-enriched (ER and PR−, HER2+), and triple-negative (ER−, PR−, HER2−). The triple-negative breast cancer (TNBC) subtype does not express any of the aforementioned markers and accounts for 15–20% of breast cancers. These tumors present a wide spectrum of morphologies, with most being high-grade and proliferation indices exceeding 80% [2,3]. They have a more aggressive clinical course, with earlier age of presentation, a higher potential risk of metastasis, a worse clinical outcome with more frequent relapses, and a lower survival rate. This subtype includes various entities with different genetic, transcriptional, histological, and clinical profiles [4]. Therefore, early diagnosis is fundamental to enable effective and timely treatment with curative aims. Currently, diagnosis is based on imaging studies, primarily mammography and ultrasound, the latter being indicated mainly in women under 40 years of age. In cases of suspicious findings, a biopsy is performed to obtain neoplastic tissue that allows for diagnosis and classification. In this regard, diagnosis can be limited by patient access, limitations in sample collection, and access to the tissue necessary for diagnosis. Therefore, the development of diagnostic techniques that facilitate access and allow for early diagnosis is necessary. Liquid biopsy is a potential diagnostic technique, involving the collection of a sample of bodily fluid (blood, urine, or saliva, for example) and has the potential to allow the evaluation of the presence of tumor derivatives such as DNA, RNA, circulating tumor cells (CTCs), proteins, or extracellular vesicles (EVs) [5].

Although liquid biopsy has been widely studied in breast cancer [6,7], TNBC-specific evidence is limited and largely restricted to reviews focused on selected biomarker types [8–10]. In this scoping review, our objective is to synthesize the available evidence on all types of liquid biopsy–derived biomarkers reported for the diagnosis of TNBC up to the end of 2024.

2. Materials and Methods

2.1. Protocol and Reporting

This scoping review was conducted in accordance with the methodological framework of Arksey and O'Malley and reported following the PRISMA-ScR checklist [11]. The protocol and eligibility criteria were pre-specified prior to study initiation.

2.2. Information Sources and Search

Five reviewers (O.N-F., J.R., E.M., V.H., and A.L.R.-C.) conducted systematic searches in Medline (via PubMed), Scopus, Embase, and Web of Science (WoS) up to October 9, 2024. The search strategies combined controlled vocabulary (MeSH, Emtree, and DeCS) with free-text terms related to "triple-negative breast cancer," "liquid biopsy," body fluids ("plasma," "serum," "blood," "saliva," "extracellular vesicles"), and molecular analytes (DNA, cfDNA, ctDNA, methylation, miRNA, lncRNA, mRNA, proteins/proteomics, lipids/lipidomics, and glycosylation), connected using Boolean operators (AND/OR/NOT). Reference lists of all included studies were also manually screened to identify additional relevant articles. Full search strategies are provided in Supplementary Table S1.

2.3. Eligibility Criteria

We included primary human studies in adults (≥18 years) that enrolled patients with triple-negative breast cancer (TNBC) and evaluated molecular alterations detectable in liquid biopsy

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(circulating tumor-derived biological material in body fluids) exclusively in relation to diagnosis, without language restrictions. Excluded: in vivo/in vitro/in silico-only work or methodological study, without a validation cohort; case reports, tissue-only analyses, no liquid biopsy (not reflecting tumor-derived liquid biopsy signals), not molecular biomarkers, or not diagnostic molecular biomarkers; reviews, editorials, and letters; studies without a TNBC subgroup, or data for TNBC could not be identified; analyses restricted to Circulating Tumor Cells (CTC) counts without molecular characterization; hereditary or constitutional/germline studies; and studies focused on metastatic disease, treatment response, or non-diagnostic TNBC focus.

2.4. Study Selection and Data Extraction

Two reviewers (O.N-F. and E.M.) independently screened titles and abstracts, with disagreements resolved by a third reviewer (A.L.R.-C.). Full-text assessment and data extraction were conducted by three reviewers (O.N-F., E.M., J.R.). Extracted data items included: DOI, authorship, year, country, study design, sample size and age, biospecimen type, analytical technique, biomarker type, biomarker name/gene symbol, alteration type (e.g., hypermethylation, up-/down-regulation), and diagnostic performance metrics (p-values, AUC, sensitivity, specificity when reported). Extracted datasets are presented in Supplementary Table S3 (Sheets A–E).

2.5. Synthesis of Results

Given heterogeneity across biomarkers, analytical platforms, comparators, and endpoints, results were synthesized descriptively and grouped by analyte or biomolecule class: DNA, RNA and Protein-based. Emphasis was placed on studies reporting diagnostic performance (AUC, sensitivity, specificity). Where available, pre-analytical and analytical factors were also noted.

3. Results

3.1. Study Selection and Characteristics

A total of 899 publications up to October 9, 2024 were identified (Figure 1). Duplicated articles (n=268) and those that did not meet the inclusion criteria during the initial screening of titles and abstracts (n=412), and full-text analysis (n=188) were excluded (Supplementary file, Sheet A-C). Additionally, 1 article was incorporated by hand searching by checking the reference lists of relevant studies (Supplementary file, Sheet D). Finally, 32 studies were examined in this scoping review (Supplementary file, Sheet E).

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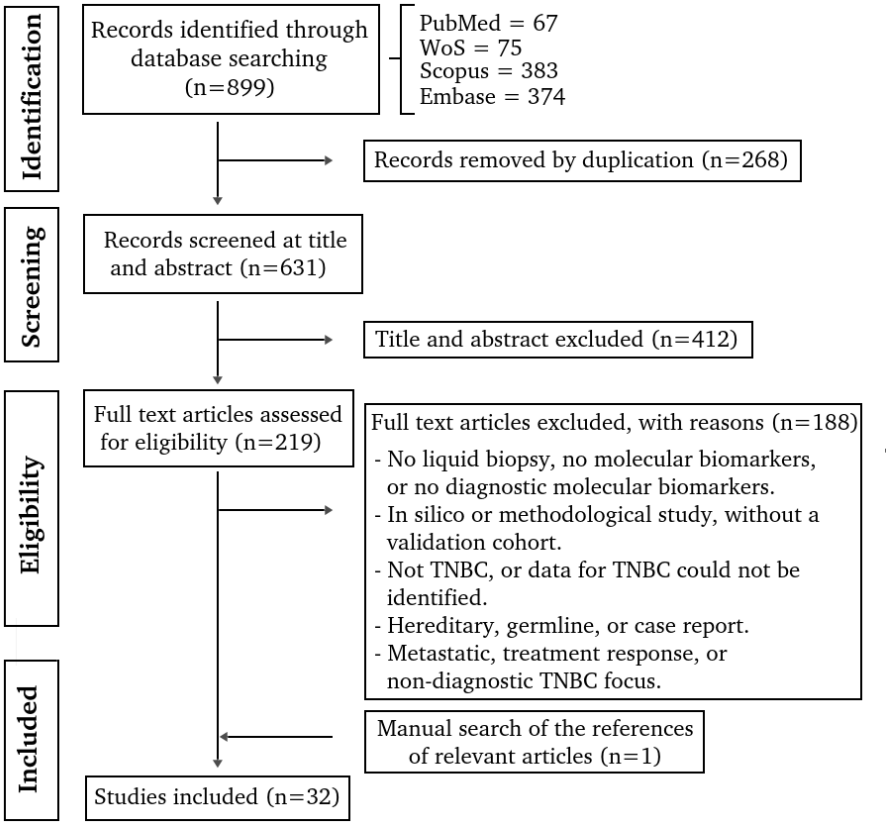

Figure 1. PRISMA flow diagram of the scoping review process.

The study design was defined in 28 studies, with 19 case–control (including variants such as nested or retrospective case–control), and the remaining were described as pilot (n = 3, including variants), experimental (n = 2), cohorts (n = 2), cross-sectional, and multi-phase. The studies were conducted primarily in Asia (n = 17), followed by Europe (n = 7), North America (n = 4), Africa (n = 2), and South America (n = 2). Among the 32 eligible studies, serum was the most commonly analyzed biological specimen (n = 17), followed by plasma (n = 11), whole or peripheral blood fractions (n = 3), buffy coat (n = 1), and a few mixed sample types. Finally, 15, 11, and 6 studies reported novel protein-based, RNA-based, and DNA-based molecular biomarkers, respectively. In one study, both protein-based and RNA-based biomarkers were reported. Of the 32 included studies, 17 reported the area under the receiver operating characteristic curve (AUC), while 14 and 13 studies provided data on sensitivity and specificity, respectively.

3.2. Patients Characteristics

Across the 32 studies, a total of 1532 TNBC cases and 3137 participants in the comparator group were analyzed. Comparator groups included healthy controls, benign breast disease, non-TNBC breast cancer, non–breast cancer participants, and, in one study, disease-free individuals. The approximate modal age was 52 years, with the majority of studies reporting mean or median ages within the 50–55-year range, with ages ranging from 21 to 93 years. Across the 32 studies, the median sample sizes were 29 TNBC cases and 48.5 controls per study. Regarding comparators, the most common study setups contrasted TNBC vs healthy controls and TNBC vs other breast cancer subtypes (e.g., luminal, HER2-positive); fewer studies included benign breast lesions or mixed comparator groups.

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3.3. Novel Protein-Based Molecular Biomarkers Associated with TNBC Diagnosis

The fifteen studies reporting novel protein-based molecular biomarkers associated with TNBC diagnosis (including one mixed Protein+RNA study, Table 1) were published between 2012 and 2024. The studies included 621 patients with TNBC and 1,664 compared group, for an overall cohort of 2,285 participants. Across the studies with available data, the reported age range spanned from 26 to 86 years, the mean and modal ages were approximately 52 years. Only one study provided separate age distributions for TNBC and control groups, reporting a mean age of 43.7 ± 7.8 years for TNBC patients and 46.1 ± 10.4 years for controls (Table 1, Study [15]).

Table 1. Proteins-based molecular biomarkers associated with diagnostic of TNBC.

AUC	Biofluid	Detection method	P-value	Alteration type	Biomarker	Comparator group	Case	Sample size	Study Ref.
Various	Plasma	Antibody microarray	p<0.05	Up-regulated	Panel: KIT, ITGB1, EFNA5, SRP54, FAS, BRCA1, XBP1, and others	28 not BC participants	28 TNBC patients	56	[12]
Not reported	Serum	2D-DIGE; MALDI-TOF-MS	p<0.05	Up-regulated Down-regulated	TTR, SERPINA1, HP	30 not BC participants	30 TNBC patients	60	[13]
0.853	Plasma	iTRAQ; WB; ELISA	A2M, C4BPA p<0.0001 FN1 p<0.018	Overexpression	FN1, A2M, C4BPA	20 not BC participants	8 TNBC patients	28	[14]
Not reported	Serum	2D-DIGE/MALDI, iTRAQ-LC-MS/MS, SWATH, WB, SRM	p<0.05	Up-regulated Down-regulated	Panel: CPN2, CO2, MYL6, HV101, APOA4, PI16, CXCL7, VTDB, IGJ, KNG1	20 not BC participants	19 TNBC patients	39	[15]

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Not reported	Serum	NP exposure, SDS-PAGE, LC–MS/MS, SWATH/SRM	p<0.05	Up-regulated Down-regulated	Panel: APOL1, CFH, VTNC, C3, C4A, C9, LGALS3BP, FCN3, RBP4, FN1, APOA4, ORM1, ZPI, TTR, APOC1, APOC3, IGHM, IG chains	8 not BC participants	8 TNBC patients	16	[16]
Not reported	Serum	IP, SDS-PAGE, HILIC, MALDI-TOF MS	p < 0.01	Glycosylation pattern change	MR	110 participants *	35 TNBC patients	55	[17]
Not reported	Serum	ELISA	p = 0.01 (size) p = 0.03 (stage)	Increased serum concentration	VEGF	35 not BC participants	30 TNBC patients	65	[18]
0.934	Serum	ELISA	p<1e-4	Increased serum concentration	RAI14	60 not BC participant**	46 TNBC patients	106	[19]
0.908	Serum	SELDI-TOF-MS, qRT-PCR, ELISA, WB	p<1e-4	Increased serum concentration	ApoC-I	215 participants ***	165TNBC patients	380	[20]
0.875	Serum	Serology	p<0.05	Lower concentration panel	KJ901215, FAM49B, HYI, GARS, CRLF3	776 participants ***	123 TNBC patients	389	[21]
1	Serum	Western blot, ELISA	p<1e-4	Higher circulating concentrations	ANXA2	179 participants *	58 TNBC patients	126	[22]

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TP53 = <0.63	Blood	ELISA	Non significant	Minimal diagnostic performance	GDF15, PKM, SPARC, CA125, WFDC2, COL1A1, FN1, CTGF, S100A7, SPP1, CCL5, hsa-miR-135b, Anti-TP53, HOXA5, SFRP1	87 not BC participant	28 TNBC patients	115	[23]
Various	Plasma	Ultracentrifugation; LC-MS/MS	Various	Panel changes	ApoA1, ApoA2, ApoC2, ApoC4 C3, CFB, IGLC2/3, GC, PLG, SERPINA3, IGHC1, C9, LRG1, C4B	204 participants *	20 TNBC patients	123	[24]
Not reported	Serum	ELISA	p = 0.010	Higher serum concentration	TRAF6	61 participants *	13 TNBC patients	39	[25]
Not reported	Serum	HuProt microarray	Not reported	Higher concentration	Anti-TXNL2	10 not BC participants	10 TNBC patients	20	[26]

* Includes non-TNBC and not BC. ** Includes benign breast disease and not BC. *** Includes non-TNBC, benign disease and not BC. TNBC, Triple negative breast cancer; BC, Breast cancer; KIT, Mast/stem cell growth factor receptor; ITGB1, Integrin beta-1; EFNA5, Ephrin-A5; SRP54, Signal recognition particle 54 kDa; FAS, TNFR superfamily member 6 (Ab1); BRCA1, Breast and ovarian cancer susceptibility protein 1; XBP1, X box-binding protein 1; TTR, Transthyretin; SERPINA1, Alpha-1-antitrypsin; HP, Haptoglobin; FN1, Fibronectin; A2M, Alpha-2-macroglobulin; C4BPA, Complement component-4-binding protein-alpha; CFB, Complement factor-B; CPN2, Carboxypeptidase N subunit 2; CO2, Carbon dioxide; MYL6, Myosin light chain 6; HV101, Voltage-gated hydrogen channel 1; APOA4, Apolipoprotein A4; PI16, Peptidase inhibitor 16; CXCL7, Chemokine C-X-C motif

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Ligand 7; VTDB, Vitamin D-binding protein; IGJ, Immunoglobulin J chain; KNG1, Kininogen-1; APOL1, Apolipoprotein 1; CFH, Complement factor H-related; VTNC, Vitronectin; C3, Complement C3; C4A, Complement C4-A; C9, Complement C9; LGALS3BP, Galectin-3-binding protein, FCN3, Ficolin-3; RBP4, Retinol binding protein 4; ORM1, Orosomucoid; ZPI, protein Z-dependent protease inhibitor; APOC1, apolipoprotein C-I; APOC3, apolipoprotein C-III; IGHM, Immunoglobulin heavy constant mu; IG chains, Immunoglobulin chains; MR, Mannose receptor; MRC1, Mannose receptor C-type 1; VEGF, Vascular endothelial growth factor; CA15-3, Cancer antigen 15.3; RAI14, Retinoic acid induced 14; FAM49B, Family with sequence similarity 49, member B; HYI**,** Hydroxypyruvate isomerase; GARS, Glycyl-tRNA synthetase 1; CRLF3**,** Cytokine receptor-like factor 3; ANXA2, Annexin A2; GDF15, *Growth differentiation factor 15; PKM, Pyruvate Kinase muscle; SPARC, Osteonectin; CA125, Cancer antigen 125; WFDC2, Human epididymis protein 4; COL1A1, Collagen type 1 alpha 1; CTGF, Connective tissue growth factor; S100A7, Psoriasin; SPP1, Osteoponin; CCL5, RANTES; HOXA5, Homeobox 5; SFRP1, Secreted frizzled-related protein 1;* IGLC2/3, Immunoglobulin lambda constant 2/3; GC, Vitamin D-binding protein; PLG, Plasminogen; SERPINA3, Serpin family A member 3; IGHC1, I*mmunoglobulin Heavy Constant Gamma 1*; C9, Complement Component 9; LRG1, *Leucine-rich alpha-2-glycoprotein 1*; C4B, Complement C4B; TWEAK, (TNF)-like weak inducer of apoptosis; TRAF6, TNF receptor-associated factor 6; Anti-TXNL2, Thioredoxin-like 2 autoantibody.

Most studies analyzed serum samples (n = 12), followed by plasma (n = 2), and whole blood fractions (n = 1). The predominant analytical techniques included ELISA-based assays, antibody microarrays, and proteomic mass spectrometry approaches (e.g., 2D-DIGE, MALDI-TOF-MS, LC–MS/MS, SWATH). These methodologies primarily aimed to identify circulating proteins differentially expressed in TNBC patients relative to healthy individuals or non-TNBC subtypes.

Among the 15 protein-based studies, al least 69 individual protein biomarkers were identify (Table 1), that could be grouped into 13 functional families, including apolipoproteins, complement components and regulators, immunoglobulins/autoantibodies, coagulation and protease inhibitors, acute-phase/transport proteins, extracellular matrix and adhesion molecules, growth factors/cytokines, and various signaling, enzymatic, and transcriptional regulators. Only APOA4 was reported in three independent studies, while TTR, FN1, APOC1, C3, and C9, were reported in two studies each. All other proteins were described in single studies.

3.4. Novel RNA-Based Molecular Biomarkers Associated with TNBC Diagnosis

Twelve studies investigated RNA-based molecular biomarkers (including one mixed Protein+RNA study). Across these investigations, the cumulative sample comprised 550 TNBC cases and 1,009 participants in the comparator group, with mean per-study sample sizes of ~48 TNBC cases and ~84 comparator participants. Publication years ranged from 2015 to 2023 (mean 2018). Diagnostic performance metrics were variably reported: AUC was provided in nine studies, while sensitivity and specificity were each reported in six studies. Serum and plasma were the most frequently analyzed biological specimens, whereas urine was used in only one study, and RT-qPCR/qRT-PCR approaches predominated, often combined with discovery arrays. Among the 12 RNA-based studies, five reported multiple RNAs or RNA panels, while seven focused on individual RNA biomarkers, resulting in a total of 40 unique RNAs identified as differentially expressed biomarkers in TNBC (Table 2). Among candidate RNAs, miR-21 was the most frequently investigated across studies (n=4), followed by miR-155 (n=2), whereas other microRNAs (e.g., miR-126-5p, miR-205, miR-199a-5p) and lncRNAs (e.g., ANRIL, SOX2OT, ANRASSF1, UCA1, HIF1A-AS2, NRIL) were each reported by single studies.

Table 2. RNA-based molecular biomarkers associated with diagnostic of TNBC.

AUC	Biofluid	Detection method	P-value	Alteration type	Biomarker	RNA type	Comparator group	Case	Sample size	Study Ref.

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Not reported	Peripheral blood	RT2 lncRNA PCR Array, qRT-PCR	p<1e-4	Up and down regulated	ZFAS1	lncRNA	40 not BC participants	40 TNBC patients	80	[27]
0.88	Plasma	miRNA arrays, RT-qPCR	p<0.0001	Down-regulated	miR-199a-5p, miR-16, miR-21	miRNA	255 participants*	72 TNBC patients	327	[28]
0.814	Plasma	Microarray, RT-qPCR	p = 1.4e-5	Concentration change	miR-126-5p	miRNA	21 not BC participants	21 TNBC patients	42	[29]
0.961	Serum	RT-qPCR	p<1e-4	Up and down regulated	miR-21, miR-155, miR-205	miRNA	51 not BC participants	139 TNBC patients	190	[30]
0.934	Serum	Microarray, RT-qPCR	p<0.01	Overexpressed	NRIL, HIF1A-AS2, UCA1	lncRNA	75 participants**	25 TNBC patients	100	[31]
0.74	Serum	NanoString nCounter	p≤0.05	Up-regulated	miR-25-3p	miRNA	69 participants**	12 TNBC patients	81	[32]
Not reported	Plasma	qRT-PCR	Not reported	Up-regulated	miR-21	miRNA	46 participants**	4 TNBC patients	50	[33]
Diagnostic panel = 0.929	Plasma	RT-qPCR	p =0.0008 –0.02	Serum differential concentration	Initial and diagnostic panel.	miRNA	91 participants***	36 TNBC patients	127	[34]
Not reported	Blood	ELISA; RT-qPCR	Non significant	Minimal diagnostic performance	miR-135b	miRNA	87 not BC participants	28 TNBC patients	115	[23]
0.785	Blood	RT-qPCR	p<0.0001	Up-regulated	miR-376c, miR-155, miR-17a, miR-10b	miRNA	34 not BC participants	37 TNBC patients	71	[35]

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0.959	Plasma	qRT-PCR	ANRIL = p<0.01 Others = p<0.05	Overexpressed	ANRIL, SOX2OT, ANRASSF1	lncRNA	220 participants**	120 TNBC patients	340	[36]
Not reported	Serum Urine	RT-qPCR	p<0.05	Increase and decrease in concentration	Serum: let-7a, let-7e, miR-21, miR-15a, miR-17, miR-18a, miR-19b miR-30b, GlyCCC2 Urine: miR-18a, miR-19b, miR-30b, miR-222, miR-320, GlyCCC2	miRNA	20 not BC participants	16 TNBC patients	36	[37]

* Includes non-TNBC, benign disease and breast cancer. ** Includes non-TNBC and healthy controls. *** Includes free disease and luminal breast cancer. TNBC, Triple negative breast cancer; BC, Breast cancer; lncRNA, long non-coding RNA; miRNA, micro RNA.

3.5. Novel DNA-Based Molecular Biomarkers Associated with TNBC Diagnosis

A total of six studies investigated DNA-based molecular biomarkers associated with TNBC diagnosis, encompassing 389 patients with TNBC and 432 participants in the comparator group. Publication years ranged from 2015 to 2023 (mean 2020). Most studies were based on plasma-derived DNA samples, with volumes ranging from 5 to 20 mL, although buffy coat and serum extracellular vesicle (EV) DNA were also analyzed. The most frequently used analytical approaches included Illumina 450K/EPIC methylation arrays, methylation-specific PCR (MSP), digital droplet PCR (ddPCR), and next-generation sequencing (NGS) platforms.

Collectively, 26 unique genes were reported across studies (Table 3). Methylation-based biomarkers, reported in four studies, included cfDNA differentially methylated regions in *SPAG6*, *IFFO1*, *SPHK2*, *TBCD/ZNF750*, *LINC10606* and *CPXM1*, as well as promoter methylation of *APC*, *RARB2*, *LINC00299*, and *ADAM12*. Mitochondrial DNA variants were identified in *MT-ND1*, *MT-ND2*, *MT-ND3*, *MT-ND4*, *MT-ND4L*, *MT-ND5*, *MT-ND6*, *MT-CYTB*, *MT-CO1*, *MT-CO2*, *MT-CO3*, *RNR2*, *MT-ATP6*, and *MT-ATP8* (1 study); and somatic mutations were examined in *PIK3CA* (1 study). Only one study reported AUC (0.74) along with sensitivity (86%) and specificity (90%).

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Table 3. DNA-based molecular biomarkers associated with diagnostic of TNBC.

AUC	Biofluid	Detection method	P-value	Alteration type	Biomarker	Variation	Comparator group	Case	Sample size	Study Ref
Test set = 0.78 Validation set = 0.74	Plasma	Illumina 450K/EPIC, XGBoost, ddPCR	P<0.0001	Hypermethylation and hypomethylation	*SPAG6*, *IFFO1*, *SPHK2*; *TBCD/ZNF750*, *LINC10606*, *CPXM1*	cfDNA methylation	84 not BC participants	139 TNBC patients	223	[38]
Not reported	Serum	MSP	p = 0.007 (RARB2)	Promoter methylation (RARB2 methylated in TNBC)	Promoter methylation: *APC*, *RARB2*	DNA methylation	145 participants*	71 TNBC patients	216	[39]
Not reported	Plasma	RT-PCR	---	No plasma mutations	*PIK3CA* hotspot mutations	Mutations	22 not BC participants	10 TNBC patients	32	[40]
Not reported	Buffy coat	ddPCR	P = 0.0025 p = 0.001 (tertile 1)	Hypermethylation (leukocyte DNA)	*LINC00299* (cg06588802)	Methylation	159 not BC participants	154 TNBC patients	313	[41]
Not reported	Serum	NGS (Illumina NovaSeq PE150)	p<0.0001 (EV concentration)	EV concentration Tumor-specific/shared EV mutations	mtDNA variants (*ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND5*, *ND6*, *CYTB*, *CO1*, *CO2*, *CO3*, *RNR2*, *ATP6*, *ATP8*)	mtDNA	9 not BC participants	9 TNBC patients	18	[42]
Not reported	Plasma	Illumina 450K,	Not reported	Hypomethylation	Promoter methylati	Methylation cfDNA	13 not BC	6 TNBC	19	[43]

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Pyrosequencing	on *ADAM12*	participants	patients

*Includes breast disease and healthy controls. TNBC, Triple negative breast cancer; BC, Breast cancer; cfDNA, Circulating cell-free DNA; *SPAG6*: *Sperm Associated Antigen 6; IFFO1, Intermediate Filament Family Orphan 1; SPHK2,* Sphingosine Kinase 2; TBCD, Tubulin-specific chaperone D; ZNF750, *Zinc Finger Protein 750; LINC10606,* Long Intergenic Non-Protein Coding RNA 606; CPXM1, Carboxypeptidase X, M14 Family Member 1; APC, Adenomatous polyposis coli; RARB2, *Retinoic Acid Receptor Beta; PIK3CA,* Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; LINC00299, Long Intergenic Non-Protein Coding RNA 299; ND1, Ubiquinone Oxidoreductase Core Subunit 1; ND2, Ubiquinone Oxidoreductase Subunit 2; ND3, Ubiquinone Oxidoreductase Subunit 3; ND4, Ubiquinone Oxidoreductase Subunit 4; ND4L, Ubiquinone Oxidoreductase Subunit 4L; ND5, Ubiquinone Oxidoreductase Subunit 5; ND6, Ubiquinone Oxidoreductase Subunit 6; CYTB, *Cytochrome b* (complejo III); CO1, Cytochrome c Oxidase Subunit 1; CO2, Cytochrome c Oxidase Subunit 2; CO3, Cytochrome c Oxidase Subunit 3; RNR2, 16S Ribosomal RNA; ATP6, ATP Synthase F0 Subunit 6; ATP8, ATP Synthase F0 Subunit 8; ADAM12, *ADAM Metallopeptidase Domain 12.*

4. Discussion

This scoping review synthesizes, for the first time to our knowledge, all molecular classes of liquid biopsy–derived biomarkers evaluated specifically for the diagnosis of TNBC. Across 32 primary studies published between 2012 and 2024, we identified proteins, RNAs, and DNA alterations as the three major biomarker categories investigated. Collectively, these findings underscore both the growing interest in minimally invasive diagnostics for TNBC and the considerable methodological and biological gaps that continue to limit their clinical translation.

A consistent observation across studies was the substantial heterogeneity in biomarker biology, study design, and analytical methods. This variability reflects not only the intrinsic molecular complexity of TNBC [44], but also differences in sample processing, controls groups, platforms, and reporting practices [45]. Protein-based biomarkers constituted the most frequently explored category (n = 15 studies), followed by RNA-based biomarkers (n = 11), while DNA-based biomarkers were less commonly evaluated (n = 6). Among RNA-derived candidates, miRNAs demonstrated comparatively stronger replication across independent studies. In contrast, somatic mutations were the least represented biomarker class (n = 1).

Despite being the predominant biomarker category, protein-based studies showed limited reproducibility. Only APOA4 was replicated in more than one independent study [15,16,24], however, the direction of change was inconsistent. In two studies, APOA4 was significantly up-regulated ($p < 0.05$) [15,16], whereas Santana et al. (2024) reported significant down-regulation ($p < 0.05$) [24]. These discrepancies highlight the sensitivity of protein measurements to pre-analytical workflows, comparator group selection, and population-level variability, reinforcing the need for standardized approaches to quantify spatial, temporal, and inter-individual heterogeneity [45,46].

RNA-based biomarkers included several promising candidates, most notably miR-21, which was significantly up-regulated in three of the four studies analyzed [28,30,33,37]. However, it has been validated in a limited number of samples, raising concerns about its reproducibility, generalizability, and suitability as a clinically reliable diagnostic biomarker [46]. Additional RNAs, including miR-199a-5p, miR-155, and miR-205 [28,30], as well as lncRNAs such as NRIL, HIF1A-AS2, and UCA1 [31], also demonstrated excellent diagnostic performance. Notably, miR-199a-5p achieved an individual AUC of 0.8838 [28], whereas the remaining markers, evaluated as part of multi-RNA panels, yielded AUC values exceeding 0.96 [30]. However, these values were reported in single studies only, underscoring the lack of external validation and the early-stage nature of this research field.

Among DNA-based biomarkers, only one study reported an AUC for a methylation panel [38] including *SPAG6, LINC10606, and TBCD/ZNF750, which* showed limited diagnostic performance (AUC: 0.74 in the validation set). The other three studies that analyzed methylation did not report

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AUC values. To date, none were replicated across independent populations, batches, or analytical platforms. The predominance of single-study biomarkers across all molecular classes reflects a fragmented evidence base where most discoveries remain unvalidated and therefore unsuitable for immediate clinical translation.

Beyond biomarker biology, study design limitations were pervasive. Most investigations employed retrospective case–control designs, which are vulnerable to spectrum bias and frequently overestimate diagnostic performance [47,48]. Diagnostic performance was inconsistently reported, with only 17 studies providing AUC values and even fewer reporting sensitivity or specificity. Validation cohorts were uncommon, and independent testing sets were rarely implemented. Clinical comparators were heterogeneous, often combining healthy individuals with patients with non-TNBC breast cancer, and demographic matching was generally inadequate, with only one study reporting age distributions separately for cases and controls. Sample sizes were typically small (median 29 TNBC cases and 35 controls), restricting subgroup analyses and limiting statistical robustness. Collectively, these issues hinder reproducibility, generalizability, and the translational potential of the biomarkers identified.

Strengths and limitations. Strengths of this scoping review include a comprehensive multi-database search strategy, adherence to PRISMA-ScR guidelines, independent screening and data extraction, and structured reporting by biomarker class. However, limitations inherent to scoping reviews also apply: no meta-analysis was conducted, and the high heterogeneity across studies prevented quantitative synthesis of diagnostic accuracy. Because only published studies were included, publication bias cannot be ruled out.

From a clinical perspective, the primary limitation is the lack of validation in independent or prospective cohorts. Most biomarkers were evaluated exclusively in discovery populations, making their real-world diagnostic utility uncertain. Until robust external replication is achieved, none of the identified circulating biomarkers (protein-, RNA-, or DNA-based), can be considered ready for clinical implementation in TNBC diagnosis.

5. Conclusions

This scoping review synthesizes the existing evidence on liquid biopsy–derived molecular biomarkers for the diagnosis of triple-negative breast cancer up to 2024. Although numerous protein-, RNA-, and DNA-based candidates have been proposed, the current evidence remains constrained by methodological heterogeneity, small cohorts, inconsistent reporting, and a lack of external and prospective validation. At present, no biomarker demonstrates sufficient reproducibility or robustness for clinical diagnostic implementation in TNBC. Advancing the field will require well-designed, adequately powered, and standardized multi-phase studies that incorporate independent validation cohorts and harmonized pre-analytical and analytical protocols.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Search strategy October 10, 2024. File S1: Supplementary file detailing the scoping review process, including (A) Identification, (B) Screening, (C) Eligibility, (D) Manual Search, and (E) Data Extraction.

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Abbreviations

The following abbreviations are used in this manuscript:

AUC	Area under the receiver operating characteristic curve
BC	Breast cancer
cfDNA	Cell-free DNA
CTC	Circulating tumor cell
ctDNA	Circulating tumor DNA
ddPCR	Digital droplet polymerase chain reaction
DOAJ	Directory of open access journals
ELISA	Enzyme-linked immunosorbent assay
EVs	Extracellular vesicles
HILIC	Hydrophilic interaction liquid chromatography
LD	Linear dichroism
lncRNA	Long non-coding RNA
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MDPI	Multidisciplinary Digital Publishing Institute
miRNA	MicroRNA
MSP	Methylation-specific polymerase chain reaction
NGS	Next-generation sequencing
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SRM	Selected reaction monitoring
SWATH	Sequential window acquisition of all theoretical fragment ion spectra
TLA	Three letter acronym
TNBC	Triple negative breast cancer
WB	Western blot

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