Advances in Laboratory Diagnosis of Lymph Node Metastasis in Papillary Thyroid Carcinoma: From Diagnostic Biomarkers to Multimodal Combined Applications

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Abstract: Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the head and neck, with a generally slow disease progression and favorable prognosis in most patients. However, lymph node metastasis in a small subset of patients can reduce the five-year disease-free survival rate from 94.8% to 73.1%. Studies have confirmed that lymph node metastasis is a risk factor for poor prognosis. Therefore, this article analyzes and compares the diagnostic value of biomarkers, liquid biopsy, and combined detection techniques to evaluate their diagnostic efficacy in lymph node metastasis and prognosis, aiming to provide evidence-based support for optimizing clinical diagnostic pathways.

Keywords: papillary thyroid carcinoma; lymph node metastasis; laboratory diagnosis; serological markers; molecular biological testing

1. Introduction

Thyroid carcinoma (TC) can be classified into four pathological types. Among these, papillary thyroid carcinoma (PTC) has the highest incidence rate, accounting for 85% of all types and representing the most common form of TC [1]. Furthermore, 25% to 30% of PTC patients experience disease recurrence, significantly reducing survival rates. Reports indicate that 20% to 50% of PTC patients develop lymph node metastasis (LNM), primarily concentrated in the neck region. Lymph node metastasis in papillary thyroid carcinoma serves as a valid prognostic factor for disease recurrence [2]. Furthermore, some metastatic foci remain occult in the early disease stage, making their prediction crucial for formulating appropriate clinical management strategies [3]. This article reviews the current advances in laboratory detection techniques for lymph node metastasis in papillary thyroid carcinoma as follows.

2. Serological Markers

2.1 Exosomal MicroRNAs

Containing both RNA and proteins, exosomes serve as carriers of signaling molecules, participating in intercellular communication and the regulation of diverse biological processes. MicroRNAs (miRNAs) are a class of non-coding RNAs approximately 22 nucleotides in length that regulate physiological functions by modulating target genes [4]. The miRNAs contained within exosomes are intrinsically linked to tumorigenesis and progression. They transfer genetic information to other cells within the tumor microenvironment, altering it into a favorable setting for tumor growth, thereby promoting tumor cell proliferation and migration [5]. Exosomal miRNAs also play a significant role in the development of papillary thyroid carcinoma (PTC). Elevated expression of exosomal miR-485-3p correlates with tumor size and lymph node metastasis (LNM) in PTC patients, making it a potential biomarker for diagnosing LNM in PTC patients [6].

2.2 MicroRNAs

MicroRNAs (miRNAs) are a class of short non-coding RNA molecules that participate in cellular biological processes by regulating gene expression. Research indicates that miRNAs play a crucial regulatory role in the initiation and progression of papillary thyroid carcinoma (PTC) [7-10].In PTC, miR-221 and miR-222 expression correlates with tumor size and invasive metastasis [11,12,13]. miR-181b expression is associated with recurrence and lymph node metastasis [14].The diagnostic utility of miRNAs continues to grow, establishing them as important biomarkers for PTC diagnosis and prognosis [15].

2.3 Circulating Tumor Cells

Circulating tumor cells (CTCs) are tumor-derived cells present in the peripheral blood of PTC patients. They are significantly associated with tumor staging, metastasis, and prognosis, serving as novel biomarkers for PTC diagnosis and prognostic assessment. Studies indicate that preoperative CTC positivity rates are high in PTC patients (up to 87%), and

CTC counts correlate positively with tumor aggressiveness (e.g., lymphatic invasion, lymph node metastasis, BRAF V600E mutation) and metastatic risk [16]. Furthermore, CTC levels correlate with tumor size and staging. Particularly in patients with tumors ≤1.0 cm in diameter, elevated CTC levels (≥8.7 FU/3mL) have been identified as an independent risk factor for CLNM [17].

2.4 hsa_circRNA_0073762

hsa_circRNA_0073762 is a circular RNA formed by reverse splicing of its host gene. Unlike linear RNA, its closed circular structure confers enhanced stability and resistance to nuclease degradation, making it a highly promising biomarker for early cancer diagnosis and prognosis assessment. By regulating the miR-548c-3p/ENAH pathway, it plays a pivotal role in the proliferation, migration, and invasion of PTC cancer cells. Experimental results indicate that knocking down this circRNA significantly inhibits the proliferation, migration, and colony-forming capacity of PTC cancer cells [18]. Given its well-defined pro-cancer mechanism, hsa_circ_0073762 demonstrates significant application value in diagnosing lymph node metastasis in PTC.

3. Molecular Markers

3.1 BRAF Gene

The BRAF gene is a key gene in the mitogen-activated protein kinase (MAPK) signaling pathway. The BRAF V600E mutation is a critical driver gene in PTC, with a mutation rate as high as 73% [19]. This mutation is significantly associated with prognosis, cervical lymph node metastasis (CLNM), and recurrence [20]. BRAF mutations play a crucial role in enhancing PTC invasiveness and LNM-related mortality [21]. Studies confirm that detecting PTC BRAF V600E mutations in fine-needle aspiration washings improves CLNM accuracy [22].

3.2 RAS Genes

In PTC, mutations within the RAS gene family (HRAS, KRAS, NRAS) also account for a significant proportion. RAS gene mutations typically represent early molecular events in PTC development. Compared to BRAF V600E mutations, RAS mutations are associated with lower aggressiveness but remain linked to lymph node metastasis. Studies indicate that the incidence of lymph node metastasis in RAS-mutated cases shows no significant difference compared to other mutation types [23].

3.3 RET/PTC Rearrangement

As a key molecular marker of PTC, the RET/PTC rearrangement is a common genetic abnormality, particularly prevalent in younger patients and radiation-exposed populations. Studies indicate that RET/PTC rearrangement is significantly associated with lymph node metastasis and also correlates significantly with factors such as gender and TSH levels [24]. Among PTC patients with RET/PTC rearrangement, 80% of cases present with lymph node metastasis, and some cases also exhibit extrathyroidal invasion [25].

4. Combined Testing

Combining FNAB with Tg detection in aspirate fluid and BRAFV600E/RET gene testing can compensate for the limitations of single-method approaches, reduce patient discomfort, and lessen reliance on operator expertise [26]. This combined strategy demonstrates significantly higher sensitivity, specificity, and accuracy than single methods in identifying cervical lymph node metastasis in PTC, thereby enhancing preoperative diagnostic efficacy [27]. The combination of ultrasound parameters, C-TIRADS scores, BRAF gene status, Tg levels, and patient clinical indicators holds significant value for predicting cervical CLNM and LLNM in PTC. A nomogram prediction model constructed based on these indicators can effectively forecast the risk of LNM occurrence in PTC [28].

5. Novel Diagnostic Technologies

Liquid biopsy enables molecular profiling of cancers by detecting tumor biomarkers (e.g., cfDNA, ctDNA, CTCs, exosomes) in bodily fluids such as blood and urine, thereby advancing precision oncology [29]. In PTC, circulating tumor DNA (ctDNA) detection in peripheral blood can reveal mutations such as BRAF V600E, TP53, and PIK3CA, demonstrating high concordance with tumor tissue pathology (92.9%, 82.1%, and 92.9%, respectively) [30,31]. Furthermore, a diagnostic miRNA panel comprising miR-146b, miR-221, and miR-222 enhances the diagnostic efficacy of fine needle aspiration biopsy (FNAB) [32]. Although liquid biopsy offers non-invasive and repeatable advantages, positioning it as a promising tool for PTC diagnosis and monitoring, its detection sensitivity, specificity, and standardized protocols require further optimization.

6. Discussion

Papillary thyroid carcinoma (PTC) is a common endocrine malignancy characterized by favorable biological behavior, slow growth, and good prognosis. However, it often presents with early-stage lymph node metastasis (LNM). LNM is considered an independent risk factor for PTC recurrence. Currently, ultrasound, CT, and MRI exhibit insufficient diagnostic accuracy for lymph node metastasis. Therefore, predicting the occurrence of cervical lateral lymph node metastasis (CLNM) and central lymph node metastasis (LLNM) in PTC is crucial for preoperative planning of optimal surgical strategies, which constitutes the ultimate significance of this study [28].

Without pathological confirmation, it remains unclear whether lymph node metastasis has occurred in the lateral neck region [33]. Therefore, improving the preoperative assessment of lateral neck lymph node metastasis in PTC holds significant clinical value for predicting recurrence and prognosis. Laboratory diagnosis of papillary thyroid carcinoma has made remarkable progress in recent years. Multi-marker combined detection has become a research hotspot.

In the future, PTC diagnosis will evolve toward multi-omics integration and minimally invasive approaches. By combining liquid biopsy, serum biomarkers, and imaging examinations, it is expected to enable more precise personalized diagnosis and treatment, optimize surgical strategies, and improve patient outcomes. This paper reviewed databases such as PubMed and CNKI to compile PTC diagnostic models constructed using joint laboratory indicators from 2020 to 2025. See Table 1 for details.

Table 1. Statistical Analysis of Diagnostic Models for Papillary Thyroid Carcinoma from 2020 to 2025

Author	Diagnostic Model	Advantages
Shen Zhiqiu	Combining TG and TGAb with CT Enhancement to Construct a Predictive Model for PTC Lymph Node Metastasis	The combination of Tg and TgAb testing with CT demonstrates significant predictive value for lateral neck lymph node metastasis in PTC. This approach substantially improves accuracy, providing clinicians with a more precise preoperative assessment tool [34].
Wang Yan	Building a Predictive Model for PTC Lymph Node Metastasis Based on Radiomics	Five predictive models for forecasting lateral neck micrometastasis in PTC were constructed based on clinical-pathological and omics features. Among them, the clinical-omics Catboost model demonstrated the highest performance, providing valuable guidance for managing suspicious lymph nodes with a short axis <8 mm [35].
Guan Pan	Establishing a Predictive Model for Papillary Thyroid Carcinoma Lymph Node Metastasis Using Conventional Ultrasound Combined with Ultrasonic Microvascular Imaging	A nomogram model based on clinical and ultrasound SMI characteristics can help identify PTC patients at high risk for CLNM, provide individualized risk assessment, and serve as a reference for clinical treatment decisions [36].
Jin Huayu	A Radiomics-Based Predictive Model for Lymph Node Metastasis in Papillary Thyroid Carcinoma Using Transfer Learning	A transfer learning radiomics (TLR) model based on thyroid ultrasound images was established to predict lymph node metastasis (LNM) risk in papillary thyroid carcinoma (PTC) patients. Results demonstrated that the TLR model consistently predicted LNM risk [37].

Acknowledgments

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