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Circulating epithelial cells as potential biomarkers for detection of recurrence in patients of papillary thyroid carcinoma with positive serum anti-thyroglobulin antibody

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ABSTRACT

Background: Serum thyroglobulin (Tg) is not a reliable tumor marker for monitoring disease status after treatment in patients with papillary thyroid carcinoma (PTC) with positive anti-thyroglobulin antibody (TgAb). The aim of this study was to evaluate the clinical role of circulating epithelial cells (CECs) in PTC patients with positive serum TgAb and undetectable serum Tg.

Methods: A pilot study was performed to evaluate CECs in 25 PTC patients with positive serum TgAb and undetectable serum Tg. CECs were isolated and enriched from peripheral blood with a negative selection system PowerMag. Immunofluorescence staining with anti-epithelial cell adhesion molecule (anti-EpCAM) and anti-thyroid stimulating hormone receptor (anti-TSHR) antibodies were used to define $EpCAM^+$-CECs and $TSHR^+$-CECs. After CECs testing, 25 patients were classified into two groups: recurrence group (n = 7) and remission group (n = 18) based on biopsy or imaging studies. The diagnostic accuracy and cutoff points of $EpCAM^+$-CECs and $TSHR^+$-CECs were evaluated using receiver operating characteristic (ROC) curves. The optimal cut-off values of CECs were determined by the Youden index (sensitivity + specificity − 1).

Results: The median numbers of $EpCAM^+$-CECs (72.5 vs. 10.75) and $TSHR^+$-CECs (54 vs. 5.25) were significantly increased in recurrence group compared to remission group. The area under the curve (AUC) showed good performance of $EpCAM^+$-CECs (0.937) and $TSHR^+$-CECs (0.825) to discriminate between recurrence and remission. The cut-off value for $EpCAM^+$-CECs and $TSHR^+$-CECs were set at 48 cells/ml and 10 cells/ml, respectively and showed a sensitivity ($EpCAM^+$-CECs: 85.7%; $TSHR^+$-CECs: 85.7%) and a specificity ($EpCAM^+$-CECs: 100%; $TSHR^+$-CECs: 77.8%) in predicting the recurrence.

Conclusions: Our study suggests CECs testing could be a potential biomarker to identify recurrence in PTC patients with positive serum TgAb and undetectable serum Tg.

1. Introduction

Thyroid follicular epithelial-derived carcinomas include papillary, follicular, and anaplastic cancers. Among them, papillary thyroid carcinoma (PTC) is the most common type of all thyroid cancers, and accounts for > 80% cases [1]. Surgery is the main therapy for patients with PTC, followed by radioactive iodine (RAI) therapy (if indicated) and then treated with thyroid hormone suppression. After surgery, serum thyroid-stimulating hormone (TSH), levels of serum thyroglobulin (Tg) and neck ultrasonography should be performed in order

Abbreviations: anti-EpCAM, anti-epithelial cell adhesion molecule; anti-TSHR, anti-thyroid stimulating hormone receptor; AUC, area under the curve; CECs, circulating epithelial cells; CT, computed tomography; FT4, free thyroxine; LN, lymph node; MRI, magnetic resonance imaging; PET, positron emission tomography; PTC, papillary thyroid carcinoma; RAI, radioactive iodine; ROC, receiver operating characteristic; Tg, thyroglobulin; TgAb, anti-thyroglobulin antibody; TSH, thyroid-stimulating hormone

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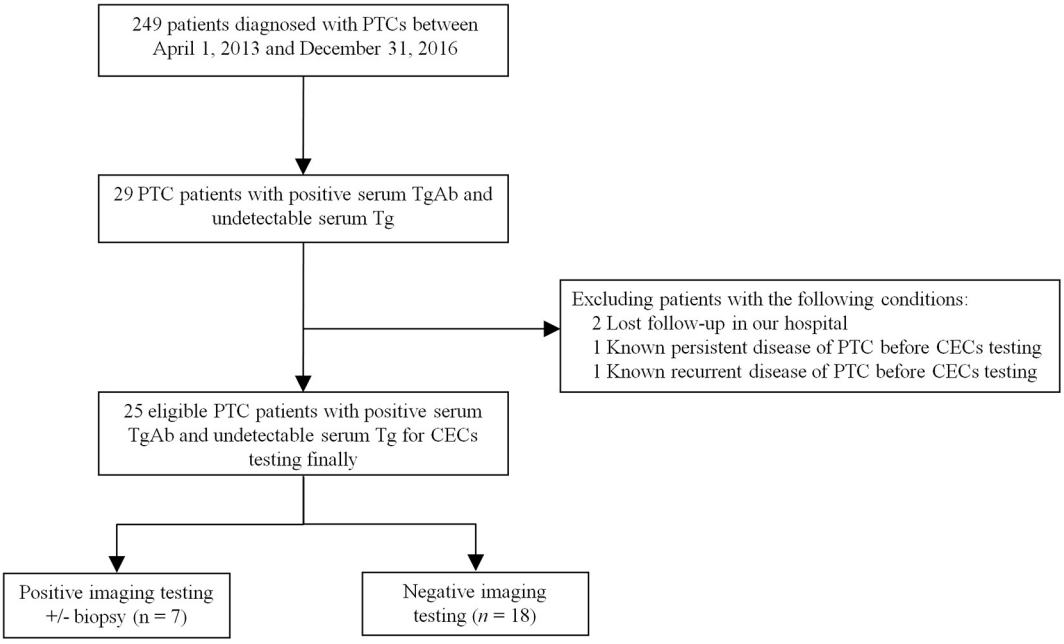

Fig. 1. Flow chart of study subjects selection.

to detect the possible persistent or recurrent disease during the first year and to determine the need for additional treatments, especially for RAI therapy.

Serum Tg is a routine and useful test to monitor disease status in PTC patients after total or near-total thyroidectomy with or without RAI therapy [2]. However, for patients with positive serum anti-thyroglobulin antibody (TgAb), serum Tg cannot be a reliable tumor marker for follow-up of recurrent or persistent disease because of highly false negative rate [3,4]. Positive serum TgAb are present in around 15% of thyroid cancer patients [5,6] and an elevated titer of TgAb with or without an increased serum Tg suggests a recurrent disease; on the contrary, a significantly decreased titer of serum TgAb suggests no recurrence [7]. Nevertheless, optimal levels of serum TgAb needed to define recurrent or persistent PTC is still conflicting currently [8,9]. Although longitudinal changes in titers of serum TgAb can help physicians in detection of recurrent or persistent disease in progression [10], considerable time is still needed to observe this trend and this may cause a delayed diagnosis. Therefore, for PTC patients with positive serum TgAb, there remains a critical need to develop new biomarkers to monitor the disease status.

Evaluation of circulating epithelial cells (CECs) or circulating tumor cells (CTCs) could be considered as a “liquid biopsy” that can monitor treatment responses and disease status in several types of cancers, such as breast, lung, colorectal and prostate cancers [11–13]. Because PTC is a tumor of epithelial origin, CECs could be considered as CTCs in a defined population with known PTC. However, only a few studies have evaluated the clinical applications of CECs or CTCs in patients with PTC [14–17], especially for PTC patients who have positive serum TgAb. An explore study including 162 persons by Winkens et al. [14] indicated that $EpCAM^+$-CECs could be detected in differentiated thyroid carcinoma (DTC) consisted of papillary or follicular thyroid carcinoma and showed a moderately positive correlation with Tg which suggested increased $EpCAM^+$-CECs may correlate with thyroid cancers in progression but $EpCAM^+$-CECs were not specific to DTC [14]. Our previous study including 65 persons by Lin et al. [15] conducted the research not only with $EpCAM^+$-CECs but also $TSHR^+$-CECs, a new biomarker, and suggested both of them could identify patients of PTCs with distant metastases who defined by elevated Tg with positive imaging studies from those in disease-free status [15]. Another study including 42 patients (18 medullary thyroid carcinoma (MTC), 14 DTC and 10 thyroid carcinoma with disease-free status) by Xu et al. [16] pointed out that the count of $EpCAM^+$-CTCs ≥ 5 in patients with metastatic MTC is associated with worse overall survival [16]. One recent study by Tseng et al. [17] analyzed the treatment response of 129 patients with PTC and revealed that CECs counts are adequate for monitoring the therapeutic outcome of these patients. Patients with structural incomplete response usually had higher CECs counts when compared to other patients with excellent, biochemical incomplete, and indeterminate responses [17]. Although these studies [14–17] have evaluated the clinical applications of CECs or CTCs in patients with thyroid carcinoma, none of them are designed and specific to verify the use of CECs or CTCs in PTC patients who have positive serum TgAb and undetectable serum Tg. Whether testing for CECs is able to identify disease status in patients of PTC with positive serum TgAb is still lack of data and not clear. As a result, the aim of this pilot study is to analyze the clinical role of CECs testing to identify the recurrence in PTC patients with positive serum TgAb and undetectable serum Tg.

2. Materials and methods

2.1. Ethics statement

This prospective study was approved by the Ethics Committee and the Institutional Review Board of the Chang Gung Memorial Hospital (approval ID: 102-3433B and 104-3901B). Written informed consents of this study were obtained from all patients or their guardians.

2.2. Study population and patients treatment before CECs testing

This is a prospective study at a tertiary medical center, the Linkou Chang Gung Memorial Hospital between April 1st, 2013 and December 31st, 2016. The enrollment criteria for patients included being of age 18 years or older, able to understand and sign the informed consent, having positive serum TgAb and undetectable serum Tg and no evidence of recurrent or persistent PTC by image studies before CECs testing. After applying inclusion and exclusion criteria, 25 PTC patients with positive serum TgAb and undetectable serum Tg were included for CECs testing finally (Fig. 1). The peripheral blood (4 ml/test) was drawn from these 25 PTC patients for CECs testing. After CECs testing, all of these 25 patients received image studies to evaluate any evidence