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Calcitonin as a Clinical Biomarker: Unmet Diagnostic needs and Biosensor-based Solutions

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Abstract

A key biomarker for medullary thyroid cancer (MTC), calcitonin (CTN), is a peptide hormone released by thyroid parafollicular C-cells. It facilitates early identification, prognostic evaluation, and post-treatment recurrence monitoring. In nodular thyroid disease, elevated serum CT levels of 60–100 pg/ml are more suggestive of MTC or C-cell hyperplasia (CCH) than imaging or fine-needle aspiration alone. Nevertheless, there are still unmet diagnostic needs, such as false positives from non-thyroidal conditions, difficulties in pediatric populations, and the rise of calcitonin-negative MTC (CNMTC), in which up to 5–10% of cases have normal CTN levels that confuse detection, requiring the use of alternative markers such as procalcitonin (PCT), or progastrin-releasing peptide (proGRP). Despite their dependability, traditional immunoassays have drawbacks such as costly, time-consuming procedures and matrix interferences in complicated biofluids. By using nanomaterials like graphene, carbon nanotubes, gold nanoparticles, and quantum dots for ultrasensitive, real-time CTN detection at picogram levels, biosensor-based systems provide viable substitutes. By improving specificity by molecular imprinting or antibody immobilization, electrochemical, optical, and piezoelectric biosensors allow point-of-care applications in serum or saliva with little sample preparation. This mini review outlines diagnostic gaps, summarizes the most recent data on CTN's therapeutic efficacy, and investigates biosensor advances as alternative instruments for individualized MTC therapy. With the ultimate goal of reducing MTC mortality by proactive screening, next avenues include wearable sensors for ongoing monitoring and clinical validation to bridge bench-to-bedside translation.

Introduction

Mostly produced by the thyroid glands' C-cells, calcitonin (CTN) is a polypeptide hormone made up of 32 amino acids with a disulphide bridge connecting positions 1 and 7 [1]. In the 1960s, perfusion tests on dogs revealed that CTN is a second hormone, after parathyroid hormone, that regulates calcium levels [2]. The thyroid glands' C-cells, also known as parafollicular cells, are primarily responsible for producing calcitonin [3]. CTN is a biosynthesised component of procalcitonin (ProCT), a bigger prohormone. It results from posttranslational changes of preprocalcitonin, a precursor protein consisting of 136 amino acids [1]. Glycine is the last amino acid in the nonaminated, immature 33-amino acid version of CTN that is seen in ProCT. Several other free peptides and immature CTN are produced as a result of ProCT's posttranslational processing. The result of further altering immature versions of CTN is a protein molecule with a disulfide bridge connecting its different chains and an aminated C-terminus [4,5].

The calcitonin gene, Calcitonin I (CALC-I) gene (Figure 1), has 6 exons and is found on chromosome 11's short arm. In addition to calcitonin, alternative splicing produces calcitonin gene-related peptide (CGRP), a second gene product that is tissue-specific. The calcitonin family includes CGRP, amylin, adrenomedullin, and calcitonin receptor-stimulating peptide 1. The first four exons of the transcribed mRNA are spliced and combined to create calcitonin itself. Calcitonin makes up almost 95% of all mature transcripts in C-cells [1]. The calcitonin receptor, a serpentine protein that is highly expressed in the kidney and hypothalamus, has been demonstrated to be the mechanism by which calcitonin works [6].

Figure 1. Tissue specific calcitonin transcript expression: Tissue-specific alternative splicing processes the CALC-I gene into three mRNAs: CGRP-I (all exons except 4) in neural tissues, CT-II (exons from 1 to 3, partial 4, 5, and 6) in the liver, and CT-I (exons from 1 to 4) in thyroid parafollicular cells [3].
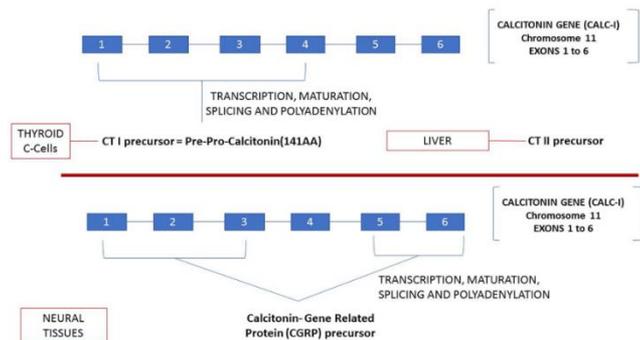
The diagnostic significance of calcitonin as a tumor marker for medullary thyroid carcinoma (MTC) is undeniable, despite the fact that its function as a regulator of calcium and bone metabolism is still up for debate. MTC is a rare thyroid tumor secreting calcitonin [7]. About 75% of MTC cases are sporadic, whereas 25% of cases are inherited tumors [8]. CT has shown itself to be a highly effective tumor marker for both the follow-up therapy of MTC [9] and the preoperative identification of nodular thyroid disorders [10]. Patients with MTC are reported to exhibit higher baseline and pentagastrin or calcium-stimulated CTN concentrations than either healthy persons or patients with C-cell hyperplasia (CCH) [6] (Raue and Frank-Raue, 2015). Serum mature calcitonin values were the sole method used to identify afflicted relatives in cases of familiar MTC prior to the era of RET mutation diagnosis [11]. In addition to the mature monomeric CTN (1–32 amino acids), the blood contains a number of additional isoforms, precursors, and metabolites [12, 13]. The calcitonin immunoreactivity of MTC patients varies greatly [14]. Patients with MTC have both polymeric and monomeric forms of calcitonin in comparison to healthy persons. Disulfide bridges to unrelated proteins or between molecules may cause polymeric versions of CTN in MTC patients [15]. Preoperative diagnosis of MTC greatly benefits from CTN concentration measures [11]. Additionally, when less than 50 pg/ml, preoperative mature CTN levels in MTC may potentially be predictive of ultimate cure and suggest tumoral volume [16].

Current diagnostic methods

Immunoassay techniques are characterized as being used generally to measure CTN. Around the end of the 1960s, the first radioimmunoassays (RIA) for CTN were developed [17]. Later, two-sided immunoassays with noticeably higher specificity (immunoradiometric assays, or IRMA) gained popularity. Two distinct antibodies were employed in these studies to target distinct, spatially separated CTN molecule epitopes [18]. In 2000 assay producers switched from radiolabeled systems to fluorescent and chemiluminescent assays [19, 20]. In order to increase analytical sensitivity, labels with a somewhat greater signal efficiency were used in place of the uncomfortable radioactivity [6]. The chemiluminescence test from Nichols Institute Diagnostics (NID) was regarded as the "gold standard" for CTN readings in the years that followed since several studies have assessed its efficacy [21, 7, 22].

However, issues related to the kind of antibody (polyclonal or monoclonal) utilized in the test may have an impact on the CTN measuring techniques [21]. Different types of immunoreactive CTN can be produced by MTC, and patient-to-patient variability exists [21]. The antibody used in the immunoassays, whether RIA or IRMA, detects various forms to varying degrees, indicating that in order to guarantee MTC detection, much attention must be taken in both assay selection and result interpretation [22].

The diagnosis of MTC has been a significant difficulty in clinical practice ever since it was originally described. Actually, cytologic examination has a number of drawbacks for diagnosing MTC, even if fluids of thyroid nodule aspirate (FNA) cytology is a trustworthy diagnostic method for evaluating thyroid nodules produced from follicular cells [23]. Although serum calcitonin (CT) measurement is acknowledged as the most sensitive diagnostic for identifying MTC, there is ongoing discussion over its regular application in patients with nodular thyroid disease [24, 25]. Furthermore, ultrasonography is not very specific in this situation. Due to these restrictions, a sizable portion of MTC are undetected prior to surgery, increasing the possibility of an insufficient treatment strategy [23].

Additionally, a false-positive diagnosis, which might result in a needless thyroidectomy, or a false-negative diagnosis, which could result in a bad prognosis for MTC, can be caused by variables that influence the measurement of calcitonin concentration. Therefore, while evaluating calcitonin values, it is important to take into account potential affecting factors too [6].

Biosensor platforms for calcitonin detection

Early identification of thyroid cancer is crucial as it is highly treatable. The techniques described above do have certain drawbacks, though, such as the fact that radioactive material contamination in radioimmunoassay can be detrimental to human health cells. Analysis with other immunoassay methods takes a lengthy time [26].

Serum CTN concentration measurements are crucial for the diagnosis and monitoring of medullary thyroid cancer patients. Development of detection techniques with adequate sensitivity was essential to the use of CTN as a tumor marker. There is a need for very sensitive techniques appropriate for calcitonin testing. Simple sample processing and a limited number of tests make the assay user-friendly, and its excellent sensitivity and accuracy enable the identification of analyte concentrations at low levels.

A good alternative and solution may be the development of biosensors for the detection of this important marker. With the help of nanomaterials, researchers are concentrating on blood-based biomarkers in addition to conventional diagnostic techniques.

The following will describe recently described and published biosensors for detecting calcitonin. Sha et al. in their study described a surface molecular imprinting technique used to

create a CTN-imprinted polymer on the surface of TiO2 NPs, which allowed for the sensitive and selective detection of calcitonin. The sensor made from the polymer might pick up on minute changes in the CTN concentration in the human body. This type of sensor may offer a dependable, disposable, renewable, and economical method for clinical examination that circumvents the matrix effect and cross-reactivity of actual specimens [27]. In the same year researchers Alarfaj and El-Tohamy developed an ultrasensitive electrochemical immunosensor without a label to detect calcitonin. The active surface of a glassy carbon electrode was coated with graphene oxide and gold nanoparticles to create the immunosensor. The addition of the nanocomposite increases the immunosensor's surface area, which results in a higher sensitivity. The updated immunosensor surface enhances anti-calcitonin capture antibody loading. Ferrocene-graphene oxide molecules were used to modify the sensor surface, which immobilizes the anti-calcitonin detection antibodies in order to detect the amount of calcitonin. The shift in peak current, which results from the alteration in surface charge brought on by the antigen–antibody sandwich-type immunoreaction, is used to calculate the signal of the modified sensor. The output peak current shift, with a dynamic linearity range of 0.001 to 1.0 ng/ml and a detection limit as low as 0.7 pg/ml, was precisely proportional to the calcitonin concentration under ideal circumstances. At low concentrations, the developed immunosensor demonstrated a high sensitivity and adequate selectivity for the detection of human calcitonin [28]. Recently another biosensor for CTN detection was described by Omer et al. They created a brand-new, easy-to-use, sensitive, accurate, and exact technique to measure calcitonin in various blood samples from patients with medullar thyroid cancer. For the effective measurement of calcitonin, sol-gel/polyethylene glycol-doped flower-shaped thin-film gold nanoparticles are employed as an optical biosensor. Transmission electron microscopy, scanning electron microscopy, energy-dispersive X-ray microanalysis, X-ray diffraction, and Fourier-transform infrared spectroscopy were used to characterize the sensor. With a correlation value of 0.99 and a detection limit of 0.707 pg/ml, the calibration plot was created for the calcitonin concentration range of 0.01–1000 pg/ml. The proposed technique increases calcitonin's sensitivity as a valuable biomarker for medullar thyroid cancer early detection. This approach is thought to be a starting point for developing a novel prototype for monitoring thyroid cancer in the spinal cord both before and after therapy [26].

To improve the detection, a variety of sensing systems have been created utilizing nanomaterial-mediated techniques. Xu and Wu discussed a review on nanomaterials conjugated with biomolecules to enhance the interaction with the target analyte and are successfully used in biosensors for surface functionalization [29].

Future perspectives

Biosensors for measuring calcitonin levels in human blood have enormous potential to transform the early detection of thyroid cancer, especially MTC, by enabling quick, non-invasive, point-of-care (POC) diagnostics that are more accessible and sensitive than conventional immunoassays. Developments in nanomaterial-based technologies, such as electrochemical immunosensors that use graphene oxide or molecularly imprinted polymers or gold nanoparticle-enhanced optical sensors, are opening the door to ultrasensitive detection at picogram levels, enabling real-time monitoring in complex biological samples like serum with little interference. By providing photostable, multiplexed assays that combine with AI-driven analytics for predictive trend analysis, quantum dots further improve this environment. They may also change paradigms toward personalized medicine and ongoing endocrine surveillance via wearable technology or microfluidic platforms.

Continuous advancements, such as expanding piezoelectric biosensors from procalcitonin (a calcitonin precursor) to direct calcitonin assays, could enable widespread adoption, lowering diagnostic delays and improving outcomes in high-risk populations by combining calcitonin with complementary biomarkers like carcinoembryonic antigen, even though issues like sensor stability, biocompatibility, and clinical standardization still exist.

Conclusion

Summarizing everything, in individuals with thyroid nodules or hereditary disorders, calcitonin continues to be the most precise and clinically validated biomarker for MTC, enabling early identification, risk assessment, and long-term monitoring. Significant unmet needs in current immunoassays, which are frequently constrained by lengthy processing, cost, and susceptibility to matrix interferences, are highlighted by persistent diagnostic challenges despite their demonstrated utility. These challenges include the confounding phenomenon of calcitonin-negative MTC, difficulties in interpreting moderate elevations without stimulation tests, and false positives from non-malignant conditions.

In complex biofluids, such as serum, biosensor-based technologies that use nanomaterials like gold nanoparticles, graphene, quantum dots provide compelling solutions by enabling ultrasensitive, quick, and point-of-care detection of calcitonin at picogram levels. Through improved specificity, reduced sample volumes, and integration with microfluidics for multiplexed biomarker analysis (procalcitonin, or proGRP), these innovations hold the potential to overcome the limitations of traditional assays. This will help to address calcitonin-negative cases and support more precise diagnostics.

Finally, by facilitating extensive, proactive screening, cutting down on diagnostic delays, and enhancing outcomes in high-risk patients, the conversion of these biosensor platforms from

lab prototypes to proven clinical tools has the potential to revolutionize MTC care. To reach their full potential in customized endocrine oncology and move toward preventative, patient-centered therapy, more research on sensor stability, biocompatibility, standardization, and large-scale clinical trials will be necessary.

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Declarations:

Authors' Contribution:

- [a-b-c]Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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