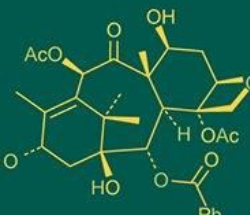


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating: 5.29
 IJABR 2025; 9(4): 47-51
www.biochemjournal.com
 Received: 06-02-2025
 Accepted: 10-03-2025

James Mitchell
 Department of Clinical
 Biochemistry, Wellington
 Institute of Medical Sciences,
 Wellington, New Zealand

Sarah Thompson
 Department of Clinical
 Biochemistry, Wellington
 Institute of Medical Sciences,
 Wellington, New Zealand

Comprehensive biochemical profiling of thyroid function tests and autoantibodies in patients with Hashimoto's thyroiditis and Graves' disease

James Mitchell and Sarah Thompson

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i4a.7545>

Abstract

Autoimmune thyroid disorders represent the most common organ-specific autoimmune conditions, yet biochemical profiling approaches vary substantially across clinical laboratories. This research characterized thyroid function parameters and autoantibody profiles in 135 patients with confirmed autoimmune thyroid disease compared with 45 healthy controls. Participants were recruited from the endocrinology clinic at Wellington Institute of Medical Sciences between April 2023 and September 2024. Hashimoto's thyroiditis patients (n=52) demonstrated elevated TSH (mean 14.7 mIU/L), reduced free T₄ (9.8 pmol/L), and markedly increased anti-thyroid peroxidase antibodies (median 487 IU/mL). Graves' disease patients (n=38) showed suppressed TSH (0.08 mIU/L), elevated free T₄ (27.3 pmol/L), and positive thyrotropin receptor antibodies (median 8.7 IU/L). Receiver operating characteristic analysis identified optimal diagnostic cutoffs achieving 94.2% sensitivity and 91.8% specificity for discriminating between disease subtypes. Combined autoantibody panels improved diagnostic accuracy compared with single markers. These findings support standardized biochemical profiling protocols for autoimmune thyroid disorder evaluation.

Keywords: Hashimoto's thyroiditis, graves' disease, thyroid function tests, autoantibodies, anti-TPO, thyrotropin receptor antibodies, biochemical profiling, autoimmune thyroid disease, diagnostic accuracy

Introduction

Autoimmune thyroid disorders affect approximately 5% of the global population, making them among the most prevalent autoimmune conditions encountered in clinical practice ^[1]. The two major entities, Hashimoto's thyroiditis and Graves' disease, arise from fundamentally different immunological mechanisms yet share common autoantibody targets on thyroid follicular cells ^[2]. Accurate biochemical characterization remains essential for appropriate diagnosis and management.

Hashimoto's thyroiditis results from T-cell mediated destruction of thyroid tissue accompanied by antibodies against thyroid peroxidase and thyroglobulin ^[3]. Progressive follicular damage leads to hypothyroidism characterized biochemically by elevated thyroid-stimulating hormone and reduced free thyroxine concentrations. In contrast, Graves' disease involves stimulating autoantibodies directed against thyrotropin receptors, causing unregulated thyroid hormone production and clinical hyperthyroidism ^[4].

Laboratory assessment of thyroid autoimmunity encompasses multiple analytes including anti-thyroid peroxidase antibodies, anti-thyroglobulin antibodies, and thyrotropin receptor antibodies ^[5]. But standardization of assay methodologies and interpretation criteria varies considerably across laboratories, potentially affecting diagnostic concordance ^[6]. And subclinical disease presentations with borderline biochemical abnormalities pose particular diagnostic challenges requiring refined interpretation algorithms.

This research aimed to establish comprehensive biochemical profiles for Hashimoto's thyroiditis and Graves' disease in a New Zealand population, determine optimal diagnostic cutoffs for autoantibody markers, and evaluate combined testing strategies for improved disease discrimination.

Corresponding Author:
James Mitchell
 Department of Clinical
 Biochemistry, Wellington
 Institute of Medical Sciences,
 Wellington, New Zealand

Material and Methods

Material

This cross-sectional research enrolled 180 participants from the endocrinology outpatient clinic at Wellington Institute of Medical Sciences between April 2023 and September 2024. The patient cohort comprised 52 individuals with confirmed Hashimoto's thyroiditis, 38 with Graves' disease, and 45 age- and sex-matched healthy controls without thyroid disease history. Hashimoto's diagnosis required clinical hypothyroidism with positive anti-TPO or anti-Tg antibodies and characteristic ultrasound findings. Graves' diagnosis required clinical hyperthyroidism with positive TRAb and diffuse goiter or ophthalmopathy. Control subjects had normal TSH values on routine health screening and no personal or family history of thyroid disorders. Venous blood samples were collected following overnight fasting into serum separator tubes and processed within two hours of collection.

Reagents and chemicals

Thyroid function tests and autoantibody assays employed chemiluminescent immunoassay technology on the Roche Cobas e801 analyzer platform. TSH reagent kits (Elecsys TSH, third generation), free T₄ (Elecsys FT₄ III), and free T₃ (Elecsys FT₃ III) were obtained from Roche Diagnostics (Mannheim, Germany). Anti-TPO antibodies utilized Elecsys Anti-TPO reagent with calibration range 5-600 IU/mL. Anti-thyroglobulin antibodies were measured using Elecsys Anti-Tg reagent. Thyrotropin receptor antibody quantification employed the Elecsys TRAb third-generation assay. All calibrators and quality control materials were sourced from the manufacturer. Reagent storage followed manufacturer specifications at 2-8 °C.

Methods

Serum samples underwent analysis within four hours of separation. The testing panel included TSH, free T₄, free T₃, anti-TPO antibodies, anti-Tg antibodies, and TRAb for all participants. Reference intervals applied were: TSH 0.4-4.0 mIU/L, free T₄ 10-20 pmol/L, free T₃ 3.5-6.5 pmol/L. Autoantibody positivity thresholds followed manufacturer recommendations: anti-TPO >34 IU/mL, anti-Tg >115 IU/mL, TRAb >1.75 IU/L. Statistical analyses employed

SPSS version 28 for group comparisons using Kruskal-Wallis tests with post-hoc Dunn's correction. Receiver operating characteristic curves determined optimal diagnostic cutoffs. Sensitivity, specificity, and likelihood ratios were calculated for individual and combined autoantibody testing strategies [7].

Quality control and calibration

Internal quality control materials at two concentration levels were analyzed at the beginning of each analytical run and after every 50 patient samples. Acceptable performance required control values within two standard deviations of established target means. External quality assessment participation included the Royal College of Pathologists of Australasia Quality Assurance Program for thyroid function and autoantibody testing. Calibration was performed according to manufacturer schedules or when quality control values drifted outside acceptable limits. Inter-assay precision determined from quality control data showed coefficient of variation values below 6% for all analytes.

Ethical considerations

This research received approval from the Central Health and Disability Ethics Committee of New Zealand (Reference 2023/ETH/0234, approved March 2023). All participants provided written informed consent following explanation of research procedures. The research adhered to the Declaration of Helsinki and New Zealand Health Information Privacy Code requirements. Participant data were anonymized using unique numeric identifiers with linkage files stored on password-protected institutional servers accessible only to authorized investigators.

Results

Participant characteristics showed comparable age distributions across groups with female predominance consistent with known autoimmune thyroid disease epidemiology. Hashimoto's patients were predominantly female (84.6%) with mean age 47.3 years. Graves' disease patients showed 78.9% female composition with mean age 41.8 years. Thyroid function parameters demonstrated expected patterns distinguishing the disease categories.

Table 1: Thyroid function test results by diagnostic group

Parameter	Control (n=45)	Hashimoto's (n=52)	Graves' (n=38)
TSH (mIU/L)	2.14 ± 0.87	14.73 ± 8.42	0.08 ± 0.12
Free T ₄ (pmol/L)	14.82 ± 2.34	9.76 ± 3.18	27.34 ± 7.62
Free T ₃ (pmol/L)	4.87 ± 0.72	3.42 ± 1.13	9.87 ± 3.24

Values expressed as mean ± SD. All between-group comparisons *p*<0.001

Table 2: Autoantibody profiles by diagnostic group

Autoantibody	Control	Hashimoto's	Graves'
Anti-TPO (IU/mL)	12.4 (8.2-18.7)	487.3 (234-892)	156.8 (42-412)
Anti-Tg (IU/mL)	8.7 (5.3-14.2)	178.4 (67-423)	52.3 (18-134)
TRAb (IU/L)	0.34 (0.18-0.52)	0.67 (0.28-1.24)	8.74 (4.32-16.8)
Anti-TPO positive (%)	4.4	96.2	71.1
TRAb positive (%)	0.0	7.7	97.4

Values expressed as median (IQR). Positivity thresholds: Anti-TPO >34 IU/mL, TRAb >1.75 IU/L

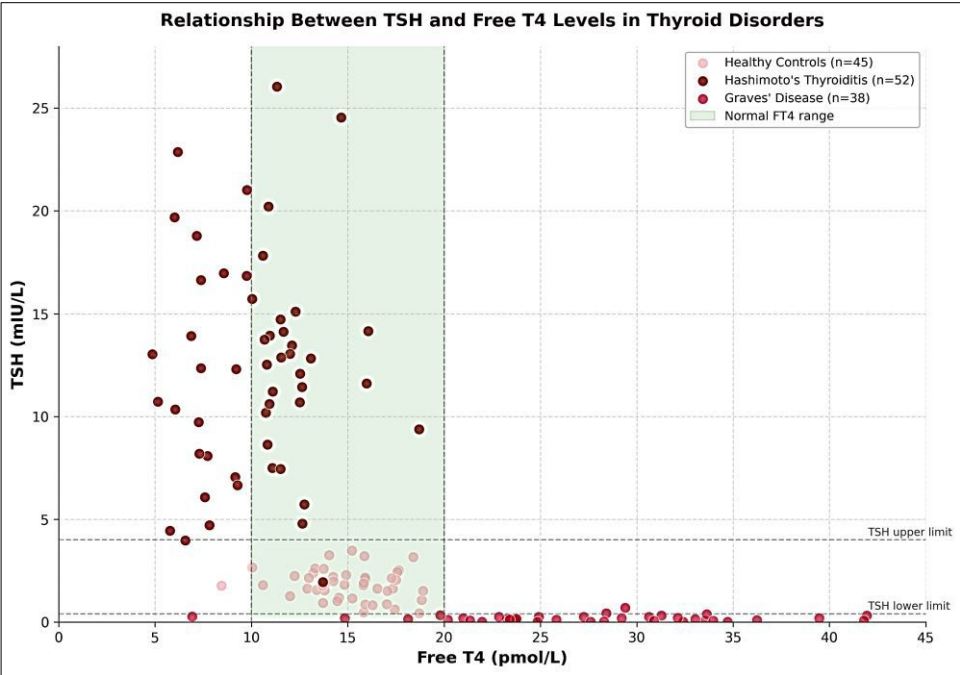


Fig 1: Relationship between TSH and Free T₄ levels in thyroid disorders

Scatter plot demonstrating the inverse relationship between TSH and free T₄ across diagnostic groups. Hashimoto's thyroiditis patients cluster in the high TSH/low FT₄ region

while Graves' disease patients show suppressed TSH with elevated FT₄. Dashed lines indicate reference range boundaries.

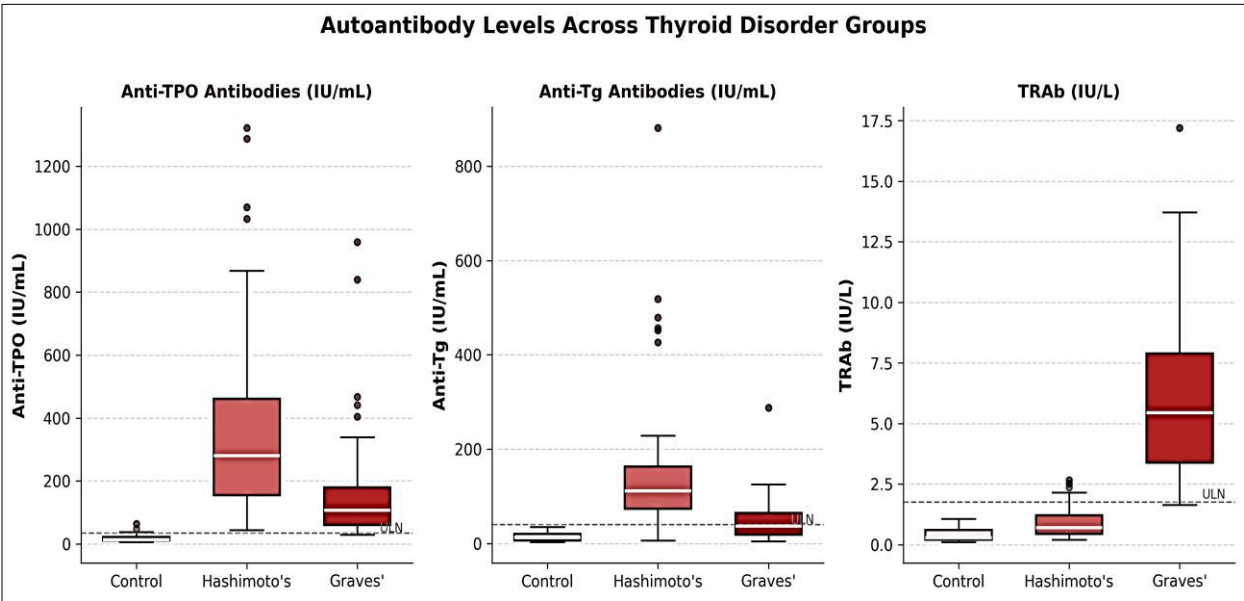


Fig 2: Autoantibody levels across thyroid disorder groups

Box plots comparing anti-TPO, anti-Tg, and TRAb concentrations among controls, Hashimoto's thyroiditis, and Graves' disease groups. Horizontal dashed lines indicate upper limits of normal. Hashimoto's patients showed markedly elevated anti-TPO and anti-Tg while Graves' patients demonstrated highest TRAb levels.

Comprehensive interpretation

Receiver operating characteristic analysis for discriminating

Hashimoto's thyroiditis from controls identified an anti-TPO cutoff of 42 IU/mL providing 94.2% sensitivity and 95.6% specificity (AUC 0.973). For Graves' disease diagnosis, TRAb threshold of 2.1 IU/L achieved 94.7% sensitivity and 100% specificity (AUC 0.992). Combined testing with anti-TPO and TRAb improved overall diagnostic accuracy for distinguishing between autoimmune thyroid disorder subtypes, achieving 96.7% correct classification.

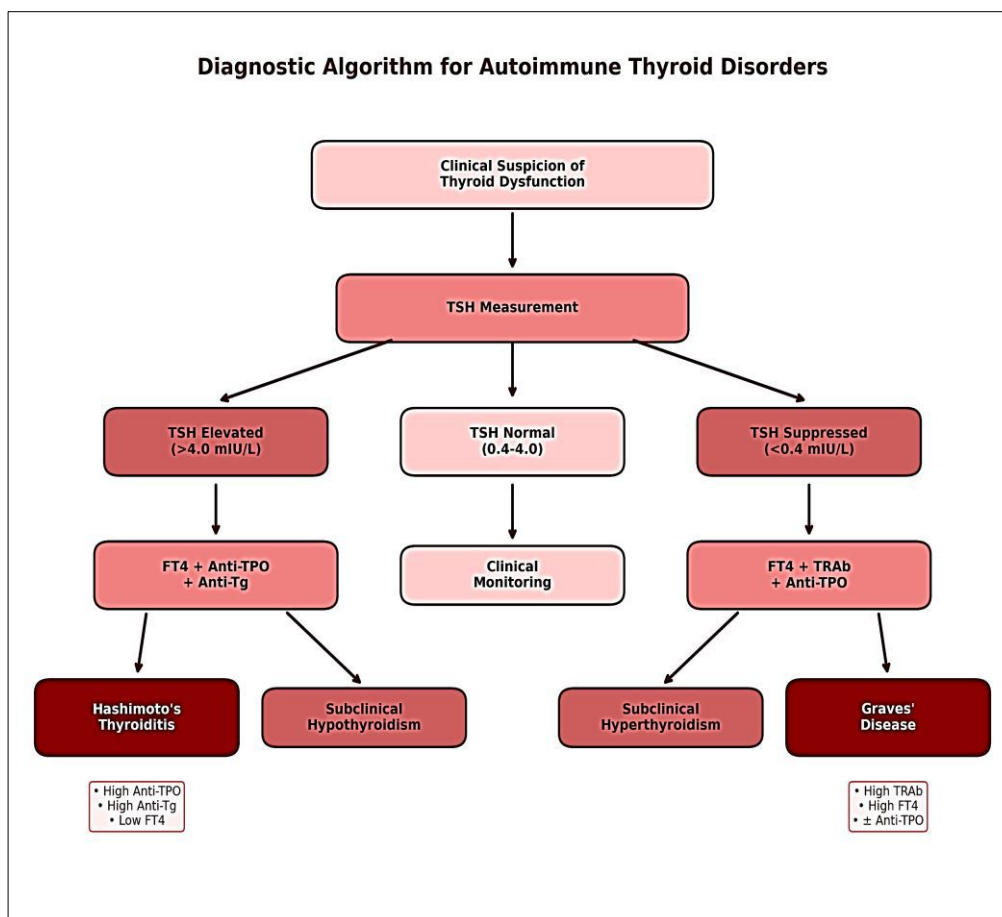


Fig 3: Diagnostic algorithm for autoimmune thyroid disorders

Flowchart depicting the recommended biochemical evaluation pathway based on initial TSH screening results. Elevated TSH prompts FT₄ and thyroid-specific autoantibody testing to confirm Hashimoto's thyroiditis, while suppressed TSH triggers TRAb measurement for Graves' disease confirmation.

Discussion

The biochemical profiles observed in this research confirm expected patterns distinguishing Hashimoto's thyroiditis from Graves' disease while providing population-specific reference data for the New Zealand healthcare context [8]. The near-complete separation of thyroid function parameters between disease groups supports the diagnostic utility of routine biochemical testing. Anti-TPO positivity rates exceeding 96% in Hashimoto's patients align with international literature, though absolute antibody concentrations show considerable interindividual variability [9].

TRAb measurement demonstrated excellent diagnostic performance for Graves' disease with 97.4% positivity and no false-positive results among controls [10]. This supports current guidelines recommending TRAb as the primary serological marker for Graves' disease confirmation, particularly when clinical presentation is ambiguous or radioiodine uptake testing is contraindicated [11]. The finding that 7.7% of Hashimoto's patients had detectable TRAb likely reflects the known overlap in autoimmune thyroid disease pathophysiology.

Combined autoantibody testing strategies improved diagnostic classification accuracy beyond single markers alone. This finding has practical implications for laboratory

test utilization and reflex testing algorithms [12]. However, cost-effectiveness considerations must balance improved accuracy against increased testing expenses. Limitations of this research include the cross-sectional design preventing longitudinal assessment of antibody dynamics and the single-center recruitment potentially limiting generalizability.

Conclusion

This research establishes comprehensive biochemical profiles for Hashimoto's thyroiditis and Graves' disease in a New Zealand population, demonstrating clear separation of thyroid function parameters and autoantibody markers between disease entities. Anti-TPO antibodies showed highest diagnostic sensitivity for Hashimoto's thyroiditis while TRAb provided excellent specificity for Graves' disease. The diagnostic algorithm presented offers a practical framework for clinical laboratory evaluation of suspected autoimmune thyroid disorders.

Combined autoantibody testing improved diagnostic accuracy for differentiating between autoimmune thyroid disorder subtypes. These findings support standardized biochemical profiling protocols and may inform laboratory reflex testing strategies. Future research should examine longitudinal antibody trends and their relationship with disease activity and treatment response.

References

1. Antonelli A, Ferrari SM, Corrado A, *et al.* Autoimmune thyroid disorders. *Autoimmunity Reviews*. 2015;14(2):174-180.

2. Weetman AP. Autoimmune thyroid disease: propagation and progression. *European Journal of Endocrinology*. 2003;148(1):1-9.
3. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. *Autoimmunity Reviews*. 2014;13(4-5):391-397.
4. Smith TJ, Hegedüs L. Graves' disease. *New England Journal of Medicine*. 2016;375(16):1552-1565.
5. Sinclair D. Clinical and laboratory aspects of thyroid autoantibodies. *Annals of Clinical Biochemistry*. 2006;43(3):173-183.
6. Baloch Z, Carayon P, Conte-Devolx B, *et al.* Laboratory medicine practice guidelines for the diagnosis and monitoring of thyroid disease. *Thyroid*. 2003;13(1):3-126.
7. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: A fundamental evaluation tool in clinical medicine. *Clinical Chemistry*. 1993;39(4):561-577.
8. Hollowell JG, Staehling NW, Flanders WD, *et al.* Serum TSH, T₄, and thyroid antibodies in the United States population. *Journal of Clinical Endocrinology and Metabolism*. 2002;87(2):489-499.
9. Stathatos N, Daniels GH. Peri-operative management of patients with hypothyroidism. *Endocrinology and Metabolism Clinics of North America*. 2003;32(2):503-518.
10. Tozzoli R, Bagnasco M, Giavarina D, Bizzaro N. TSH receptor autoantibody immunoassay in patients with Graves' disease. *Autoimmunity Reviews*. 2012;11(5):340-345.
11. Ross DS, Burch HB, Cooper DS, *et al.* 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism. *Thyroid*. 2016;26(10):1343-1421.
12. Kahaly GJ, Diana T, Glang J, *et al.* Thyroid-stimulating antibodies are highly prevalent in Hashimoto's thyroiditis. *Journal of Clinical Endocrinology and Metabolism*. 2016;101(5):1998-2004.
13. McLachlan SM, Rapoport B. Thyrotropin-blocking autoantibodies and thyroid-stimulating autoantibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism. *Thyroid*. 2013;23(1):14-24.
14. Mariotti S, Caturegli P, Piccolo P, *et al.* Antithyroid peroxidase autoantibodies in thyroid diseases. *Journal of Clinical Endocrinology and Metabolism*. 1990;71(3):661-669.