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Guiding methimazole therapy of autoimmune thyroid disease with thyroid antibody profiles: A predictive and causal inference study

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Abstract: The predictiveness and diagnostic utility of thyroid antibodies-thyroid-stimulating hormone receptor antibody, anti-thyroglobulin antibody and anti-thyroid peroxidase antibody-were investigated in autoimmune thyroid disease. The charts of 85 patients with Hashimoto's thyroiditis or Graves' disease were retrospectively analyzed with causal inference techniques and machine learning algorithms to estimate adjusted associations and interactions among antibodies. The most robust estimated disease diagnosis association was with anti-thyroid peroxidase antibody (mean treatment effect = 0.731) which also gave the best predictive accuracy (area under the receiver operating characteristic curve = 0.875) especially in the middle-aged and older population. Predictive accuracy was enhanced by using several antibodies (area under the curve = 0.913) whereas, interaction effects were trivial. Thyroid-stimulating hormone receptor antibody positivity was highly predictive of the clinical decision to start Methimazole treatment in Graves' disease. The findings suggest that anti-thyroid peroxidase antibody preference for screening and thyroid antibody profile use in treatment planning can guide clinical decision-making, within the constraints of causal inference assumption employed.

Keywords: Autoimmune thyroid disease; Graves' disease; Hashimoto's thyroiditis; Methimazole; Personalized therapy; Precision diagnostics; Thyroid antibodies; TPOAb; TRAb

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INTRODUCTION

Autoimmune thyroid disease, such as Hashimoto's thyroiditis and Graves' disease, is one of the most prevalent endocrine conditions globally. The disease has a considerable impact on long-term health, metabolic homeostasis and quality of life (Trzos *et al.,* 2022; Silva *et al.,* 2024; Gallo *et al.,* 2023). In spite of universal acknowledgment of its clinical significance, precision-based diagnosis and treatment lacunae persist, for instance, the application of thyroid antibody profiles in individualizing streamlined management. Diagnosis at an early stage and early intervention are crucial, especially in Graves' disease, when antithyroid drugs like methimazole are conventional therapies for the normalization of thyroid function and the prevention of disease advancement or complications (Czarnywojtek *et al.,* 2023). Thyroid antibody tests, such as anti-thyroid peroxidase antibody (TPOAb), anti-thyroglobulin antibody (TGAb) and thyroid-stimulating hormone receptor antibody (TRAb), are routine to confirm diagnosis and guide the management of autoimmune thyroid disease (Han *et al.,* 2022; Ludgate *et al.,* 2024; Kravchenko and Zakharchenko, 2023).

While such antibodies are routinely measured in clinical practice, the relationship among antibody titers, patient heterogeneity and disease outcomes and their value in predicting the need for treatment, is poorly characterized. Methimazole therapy is generally started in patients who are TRAb positive or have high titers of antibodies seen in active Graves' hyperthyroidism, but patient population differences, duration of disease, age, sex and antibody expression confound traditional decision-making (Xie *et al.,* 2025). For instance, TPOAb is strongly predictive of hypothyroidism in Hashimoto's thyroiditis and the titers of TRAb correlate well with hyperthyroidism in Graves' disease, influencing both the timing and dose of methimazole treatment (Bolakale-Rufai *et al.,* 2023).

Expanding on this, quantifying antibody profiles and coupling it with patient-specific clinical characteristics may augment personalization of treatment regimens. Sophisticated statistical and machine learning methods enable modeling intricate interactions between numerous antibodies, demographic variables and disease subtypes and offer a more refined prediction of treatment need and outcome. These models may be able to detect patients at higher risk of speeding disease or unfavorable response to treatment, thus facilitating earlier and more targeted interventions and optimal dosing of methimazole to maintain efficacy while reducing the risk (Ngan *et al.,* 2025; Chan *et al.,* 2025; Ebadi and Selamoglu, 2025).

In addition, previous research has principally investigated each of these antibodies separately, with minimal exploration of composite predictive value or antibody interactions. Filling these gaps is needed to move precision medicine forward in autoimmune thyroid disease and reduce risks of over-treatment, under-treatment and medication adverse effects (Ludgate *et al.,* 2024; Stasiak *et al.,* 2023). Insight into how patient heterogeneity and antibody interactions determine treatment outcomes can be directly applied to personalized treatment planning.

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To fill these gaps, we used causal inference and machine learning techniques to estimate adjusted associations and interaction effects of TPOAb, TGAb and TRAb with the possibility of predicting methimazole initiation need. These sophisticated analytical techniques provide rigorous examination of direct and interaction effects that are unachievable by standard analyses. By bridging the gap between antibody profiles and clinical pharmacology, this research creates an evidence-based precision-guided methimazole treatment paradigm, combining laboratory diagnostics and real-world treatment guidelines for Hashimoto's thyroiditis and Graves' disease. In the process, this research moves precision medicine in AITD forward by synthesizing previous research and clinical guidelines critically, by pointing out limitations of traditional antibody interpretation and by illustrating the promise of combined causal modeling and machine learning for individualized treatment optimization.

MATERIALS AND METHODS

Study subjects

Ninety-five patients who attended a specialty thyroid disease clinic between April 2021 and December 2024 were recruited to the present study. All patients were screened for thyroid antibodies and thyroid function prior to initiation of any antithyroid treatment, including methimazole. Exclusion criteria required participants to be over 18 years of age, should have complete imaging and lab data and should have a confirmed clinical diagnosis of Graves' disease or Hashimoto's thyroiditis. Participants with an unclear final diagnosis were excluded from test and training sets in order not to affect the accuracy of the model and misclassify. Severe renal or hepatic disease, pregnancy or lactation, previous radioactive iodine treatment, concomitant autoimmune disorders (except autoimmune thyroid disease) and malignancies were also applied as exclusion criteria.

Clinical data collection

A fasting venous blood sample of 5 mL was given by each patient to evaluate thyroid antibodies and thyroid function tests by electrochemiluminescence immunoassay on a Roche Cobas e601 analyzer. Antibodies that were assayed were thyroid-stimulating hormone receptor antibody, anti-thyroglobulin antibody and anti-thyroid peroxidase antibody. Anti-thyroid peroxidase antibody and anti-thyroglobulin antibodies titers were deemed negative, low (1-3× above upper limit of normal), medium (3-10× above upper limit of normal), or high (>10× above upper limit of normal), while thyroid-stimulating hormone receptor antibody titers were deemed negative, low (1-3× above upper limit of normal), or medium (>3× above upper limit of normal).

Thyroid function studies like thyroid-stimulating hormone, free triiodothyronine and free thyroxine were performed to assess the disease severity and to determine the initiation of therapy. Inflammatory markers like erythrocyte sedimentation rate and C-reactive protein were quantified to account for potential confounders influencing antibody titers. Thyroid ultrasonography was also performed in all patients on a GE LOGIQ E9 unit with a 9 MHz linear transducer. Thyroid morphology was graded as normal, diffuse lesion, or nodular lesion. These clinical and laboratory data were used as the rationale for methimazole treatment.

Cohort composition and limitations

The cohort included 50 patients with Hashimoto's thyroiditis and 35 patients with Graves' disease and the gender distribution was 70.6% female (60 patients) and 29.4% male (25 patients). Disease severity ranged from mild to severe based on clinical assessment and thyroid function tests. We acknowledge that the typically small sample size might compromise model stability, especially for complex machine learning models like Random Forest and XGBoost. Five-fold cross-validation was used to prevent overfitting, but replication in larger populations is required to confirm model performance. Other potential confounders, such as disease duration, adherence with medication, co-morbid conditions and lifestyle factors, were not controlled in the analysis and this limitation is discussed to point out directions for future studies.

Clinical diagnosis and methimazole decision

Two experienced endocrinologists made the clinical diagnosis of autoimmune thyroid disease using the 2019 Guidelines for the Diagnosis and Treatment of Autoimmune Thyroid Disease. Laboratory results, ultrasound information and presentation were considered. Patients were classified as Hashimoto's thyroiditis or Graves' disease, with agreement between hyperthyroid status and Graves' disease diagnosis. Undiagnosed patients were excluded from all analyses. In case of disagreement, a third senior endocrinologist was consulted. In Graves' disease patients, thyroid-stimulating hormone receptor antibody positivity and biochemical hyperthyroidism both were employed to make a decision on the initiation of methimazole. Treatment with methimazole was titrated and those with Hashimoto's thyroiditis or subclinical disease were not treated.

Directed acyclic graph and causal inference

The directed acyclic graph was employed to explore adjusted associations among thyroid antibodies, disease status and initiation of methimazole with the assumption of no unmeasured confounding, proper temporal ordering and no collider bias. Confounders that mattered, such as age, sex and inflammatory markers, were adjusted to block backdoor paths.

Double machine learning with double robust estimation was used to estimate causal effects. Nuisance parameters

like propensity scores and outcome regressions were cross-fitted to random forests in order to minimize overfitting as well as bias. Average treatment effects of each of the antibodies on autoimmune thyroid disease diagnosis and initiation of methimazole were calculated. Conditional average treatment effects within age-stratified subgroups were estimated and sample sizes within groups were examined to ensure estimates stability. Sensitivity analyses were conducted by altering confounder specification, changing antibody positivity criteria and excluding outliers. Collinearity among antibodies was examined and no robust collinearity was found.

Machine learning modeling and evaluation

Two independent clinical prediction tasks were trained that were predicting diagnosis of autoimmune thyroid disease and predicting initiation of methimazole treatment. Independent models and datasets were used for each task to avoid contamination. Only patients with confirmed diagnosis were included; undiagnosed patients were not included. Clinical labels were aligned with hyperthyroid status in Graves' disease.

Three machine learning models-logistic regression, random forest and extreme gradient boosting-were trained to each task. Logistic regression learned linear relations, while random forest and extreme gradient boosting learned nonlinear relations between thyroid function, antibodies and patient features.

Double-counting was prevented by normalizing antibody values. Only one representation per antibody was used across all tasks-raw values or categorical flags-such that feature importance and interaction term estimates were still interpretable and unbiased. Hyperparameter tuning was conducted with five-fold cross-validation:

• *Random forest*: no. of trees (100-500), max depth (3-20), min samples per leaf (1-10), max features ('sqrt', 'log2')

• *Extreme gradient boosting*: learning rate (0.01-0.3), max depth (3-10), no. of estimators (100-500), subsample ratio (0.5-1.0), column sample by tree (0.5-1.0)

Model precision was quantified by receiver operating characteristic plot and area under the curve value by cross-validation fold. The other metrics were sensitivity, specificity, positive predictive value, negative predictive value and confusion matrices. Feature importance and antibody interaction term were computed to predict disease prediction and treatment contribution. Logistic regression interaction term estimates and 95% confidence intervals were calculated and DeLong tests to examine statistical significance of area under the curve gains. To avoid circularity, thyroid-stimulating hormone receptor antibody was not used as a predictor for the start of methimazole. Modeling to test predictive value of clinical features and thyroid antibodies rather than reinventing clinical decision rules. Collinearity between antibodies was tested prior to modeling and there was no collinearity found significant.

Statistical analysis

Continuous data were expressed as mean ± standard deviation and categorical data in number and percentages. Between-group differences were compared by t-test or analysis of variance for continuous variables and chi-square or Fisher's exact test for categorical variables. The correlation was quantified by Pearson or Spearman coefficients appropriate. Heatmaps were generated to display correlations between antibodies, thyroid function and clinical parameters. The analyses were conducted in Python 3.8 with the following libraries: pandas, numpy, scikit-learn, xgboost, matplotlib, seaborn and statsmodels. Two-tailed p-values of < 0.05 were considered statistically significant.

RESULTS

Demographic and clinical characteristics

85 patients of thyroid disease were recruited (Table 1), of whom 60 (70.6%) were women and 25 (29.4%) were men, which is consistent with the higher prevalence of autoimmune thyroid disease (AITD) in women. The age varied from 18 to 72 years (mean 43.05 ± 13.73 years). Clinical diagnoses were established in 38 (undiagnosed, 44.7%), 32 (Hashimoto's thyroiditis, 37.6%) and 15 (Graves' disease, 17.6%); in the diagnosed subjects, 68.1% had Hashimoto's and 31.9% had Graves', which replicated normal patterns of prevalence. Thyroid antibody positivity was as follows: TPOAb 33 patients (38.8%), TGAb 26 (30.6%), TRAb 26 (30.6%), high-titer TPOAb in 2.4% and high-titer TGAb in 5.9%.

TRAb positivity was strongly associated with the initiation of Methimazole in Graves' disease. Inflammatory markers (ESR, CRP) and thyroid function (TSH, FT4, FT3) were evaluated for confounding factors and severity of the disease. Ultrasound thyroid morphology identified 32 patients (37.6%) with normal morphology, 31 (36.5%) with diffuse lesions and 22 (25.9%) with nodular lesions.

Association of thyroid antibodies with clinical markers

Correlation analysis (Fig. 1) revealed the following key findings. There was a moderate positive correlation between TPOAb and TGAb ($r = 0.45$) and both antibodies also correlating positively with TSH (TPOAb: $r = 0.40$; TGAb: $r = 0.31$). TRAb showed positive strong correlations with FT4 ($r = 0.40$) and FT3 ($r = 0.48$) and its importance was thus implied in hyperthyroid status and utility in guiding methimazole therapy. Predicted correlations were observed between markers of thyroid function, for example, between TSH and FT4 ($r = -0.58$), TSH and FT3 ($r = -0.63$) and FT4 and FT3 ($r = 0.64$). Age, CRP and ESR were less correlated with thyroid function markers and, therefore, had limited impact on the antibody-based diagnosis.

Causal pathway analysis

A causal pathway diagram (Fig. 2) depicted direct effects of TPOAb, TGAb and TRAb on AITD diagnosis with indirect effects through inflammation and thyroid function markers. Confounders of significance were found to be age and sex that influence antibody levels and disease risk. Interestingly enough, TRAb had a direct causal effect on the initiation of methimazole, which confirmed its clinical application in the treatment of Graves' disease.

Average treatment effect (ATE)

The ATE values of all the antibodies for diagnosis of AITD and treatment with methimazole are presented in Fig. 3. TPOAb contributed the highest causal effect (ATE = 0.731), followed by TGAb (ATE = 0.533) and TRAb (ATE = 0.478). The findings indicate that TPOAb is the strongest antibody to increase disease likelihood, whereas TRAb is critical to guide methimazole treatment.

Conditional average treatment effect (CATE)

CATE analysis by age group (Fig. 4) showed greater causal effects of TPOAb in middle age and old age, confirming its utility as the first-line screening test for AITD. TGAb and TRAb showed less variability by age group, confirming their ancillary diagnostic and therapeutic uses.

Predictive model evaluation

Single-antibody model performance

Single-antibody predictive performance was evaluated, with TPOAb being the optimal individual-antibody predictor (AUC = 0.875), followed by TGAb (AUC = 0.813) and TRAb (AUC = 0.700). The results confirm TPOAb's great diagnostic value and emphasize TRAb's specific suitability for guiding methimazole treatment (Table 2).

Multi-antibody model performance

Insertion of interaction terms into multi-antibody models had little impact on predictive ability (Figs. 5 & 6), suggesting that each antibody makes an independent contribution to prediction. This suggests that simpler biomarker panels may be able to match the performance of more advanced models.

Feature importance analysis

Random Forest and XGBoost feature importance analysis concluded that TPOAb titer, TPOAb positivity and TSH were the most significant predictors for AITD diagnosis and TRAb was the most significant predictor of methimazole treatment options (Fig. 7).

Antibody interaction effects (Logistic Regression)

Logistic model with interaction terms revealed main effects of TPOAb, TGAb and TRAb to be significant, while interaction terms were not (Table 3), suggesting absence of synergistic or antagonistic effects among antibodies.

Random forest and XGBoost interaction analyses

Addition of antibody interaction terms to Random Forest and XGBoost models yielded only modest increments in AUC (Random Forest: 0.912 → 0.917; XGBoost: 0.915 → 0.920) (Figs. 8 & 9). This also supports the argument that main effects are adequate for clinical judgments, e.g., treatment with methimazole.

In general, the results determine TPOAb to be the most critical predictive marker for the diagnosis of AITD, yet TRAb remains relevant in the management of methimazole therapy of Graves' disease. Single-antibody models are as efficient as multi-antibody models, confirming the use of reduced antibody testing in the clinic.

DISCUSSION

This study integrated causal inference and machine learning methods to evaluate the diagnostic and predictive roles of thyroid autoantibodies (TPOAb, TGAb, TRAb) in autoimmune thyroid disease (AITD) for the purpose of guiding methimazole treatment in Graves' disease (Vargas-Uricoechea *et al.,* 2023). Of the three antibodies, TPOAb demonstrated the strongest causal association with AITD diagnosis (ATE = 0.731), confirming its use as a first-line screening biomarker (Yang *et al.,* 2025). Age-stratified analysis proved TPOAb positivity to be most useful in middle-aged and older adults and suggested age-stratified testing strategies to optimize clinical usefulness (Othman, 2023; Kalra *et al.,* 2024). Multiparameter prediction models that use several antibodies improved diagnostic performance (AUC = 0.913); however, interaction terms did not contribute significantly to model performance, as though each antibody provides predominantly independent predictive information (Rufino-Moya *et al.,* 2024; Ebadi, 2025; Ebadi *et al.,* 2025b).

TRAb positivity was strongly associated with hyperthyroid activity and directly associated with the initiation of methimazole treatment in Graves' disease patients, suggesting its use as a therapeutic biomarker (Takizawa *et al.,* 2025; Pourzardosht *et al.,* 2022). Clinical use of antibody profiles, such as TRAb status, may complement existing treatment recommendations to allow stratification of patients by antibody positivity, individualized treatment and potentially improved outcomes for Graves' disease. These findings warrant the employment of stratified antibody screening, enhanced diagnostic specificity and tailored treatment recommendations (Zhang *et al.,* 2023; Ke *et al.,* 2025). Pathophysiologically, the strong correlation of TPOAb with AITD accords with earlier studies (Yao *et al.,* 2024).

TPOAb targets thyroid peroxidase (TPO), an enzyme that is essential in the production of thyroid hormones. Persistent TPOAb positivity very likely represents ongoing autoimmune loss of thyroid follicular cells, possibly with hypothyroidism or fluctuating thyroid function (Gu *et al.,* 2023). The causal inference estimate (ATE = 0.731).

Table 1: Demographic and clinical profile of study participants

Variable	Category	Count	Mean	Std	Percent (%)
Age	–	85	43.05	13.73	–
TPOAb	–	85	57.84	110.19	–
TGAb	–	85	71.82	151.08	–
TRAb	–	85	1.8	2.1	–
ESR	–	85	14.71	5.91	–
CRP	–	85	2.78	1.57	–
TSH	–	85	3.14	1.57	–
FT4	–	85	14.67	2.95	–
FT3	–	85	4.46	1.36	–
Sex	Female	60	–	–	70.6
	Male	25	–	–	29.4
Clinical diagnosis	Undiagnosed	38	–	–	44.7
	Hashimoto's thyroiditis	32	–	–	37.6
	Graves' disease	15	–	–	17.6
Thyroid function	Normal	78	–	–	91.8
	Hypothyroidism	5	–	–	5.9
	Hyperthyroidism	2	–	–	2.4
Ultrasound examination	Normal	32	–	–	37.6
	Diffuse lesion	31	–	–	36.5
	Nodule	22	–	–	25.9
TRAb status	Negative	59	–	–	69.4
	Positive	26	–	–	30.6
TPOAb status	Negative	52	–	–	61.2
	Positive	33	–	–	38.8
TGAb status	Negative	59	–	–	69.4

Table 2: Performance of single-antibody models

Antibody	Effect size	AUC	Sensitivity	Specificity
TPOAb	2.637	0.875	0.75	1.0
TGAb	1.537	0.813	0.625	1.0
TRAb	1.628	0.700	0.50	0.90

Table 3: Logistic regression including antibody interaction terms

Variable	β	95% CI	p-value
Intercept	-3.12	(-4.21, -1.89)	<0.01
Age	0.02	(-0.01, 0.04)	0.17
Sex (Female=1)	0.64	(-0.31, 1.49)	0.11
ESR	0.06	(-0.02, 0.14)	0.09
CRP	0.03	(-0.07, 0.13)	0.54
TSH	0.55	(0.31, 0.79)	<0.01
FT3	-0.38	(-0.91, 0.15)	0.15
FT4	-0.41	(-0.88, 0.06)	0.09
TPOAb	2.18	(1.27, 3.09)	<0.01
TGAb	1.35	(0.67, 2.03)	<0.01
TRAb	0.92	(0.19, 1.65)	0.01
TPOAb×TGAb	0.46	(-0.32, 1.24)	0.21
TPOAb×TRAb	0.29	(-0.41, 0.99)	0.42
TGAb×TRAb	0.34	(-0.31, 0.99)	0.32

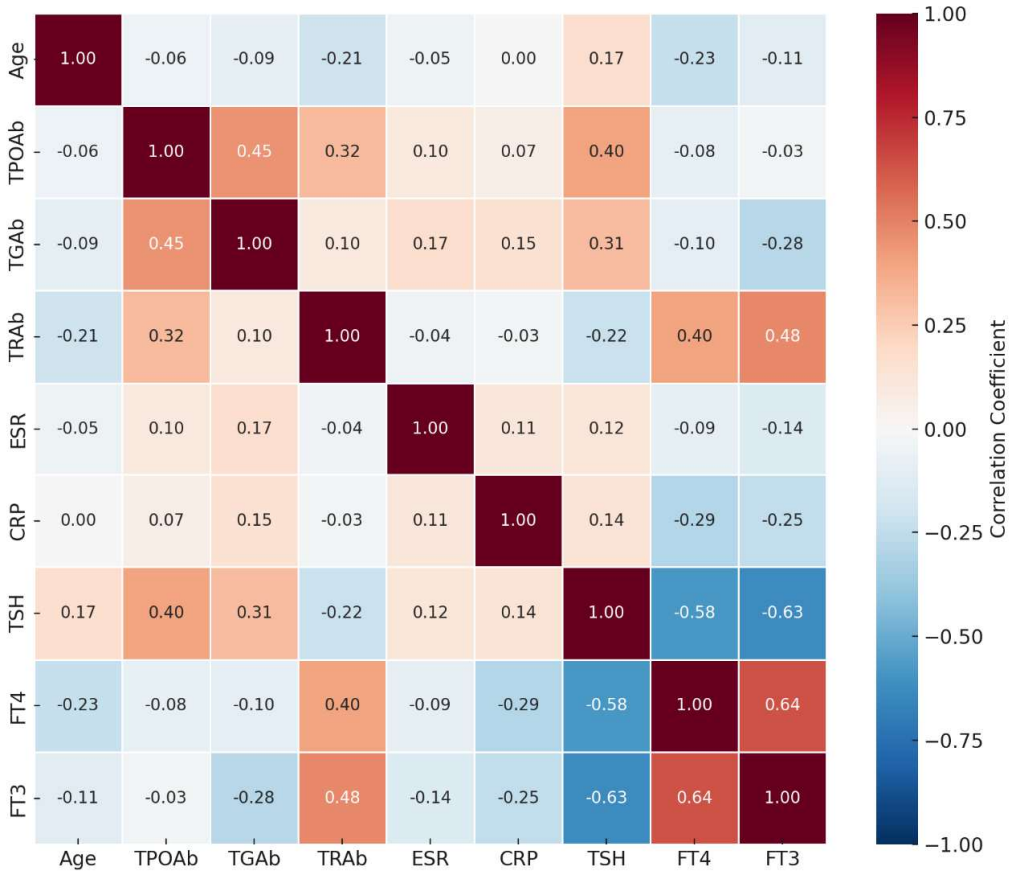

Fig. 1: Thyroid antibodies and clinical parameters correlation matrix; Spearman correlation coefficients among thyroid antibodies (TPOAb, TGAb, TRAb), thyroid function tests (TSH, FT3, FT4), inflammatory markers (ESR, CRP), and patient age. Positive correlations in blue, negative in red. TRAb has positive correlations with FT3 and FT4, confirming its utility in hyperthyroidism and Methimazole therapy decision-making.

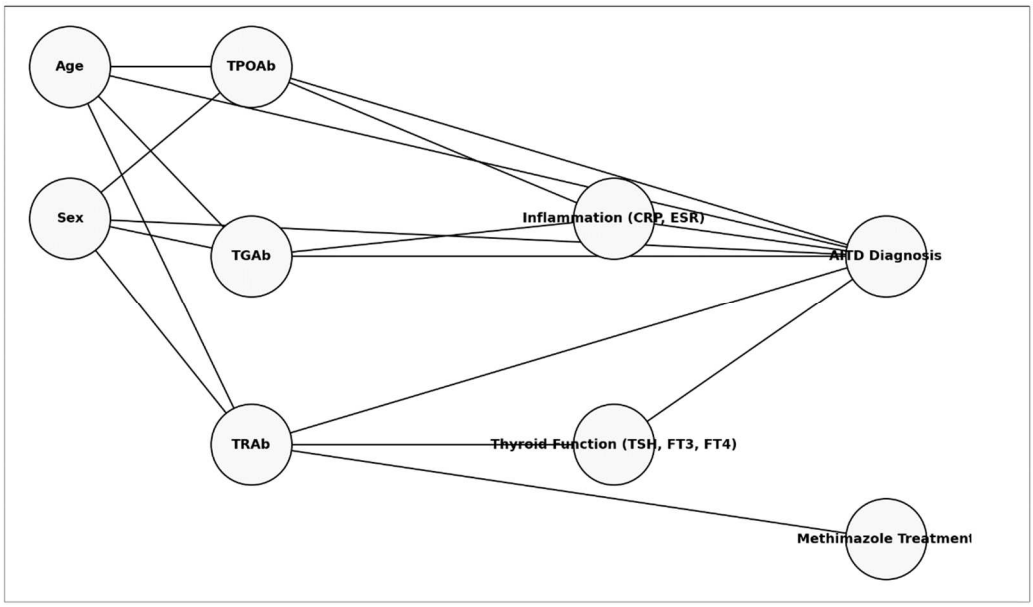

Fig. 2: Directed acyclic graph of causal pathway diagram for diagnosis of AITD and thyroid antibodies; Directed acyclic graph of causal pathways among thyroid antibodies, inflammatory markers, thyroid function, and diagnosis of AITD. Age and sex are taken as confounding variables. TRAb shows a direct causal effect for starting Methimazole treatment.

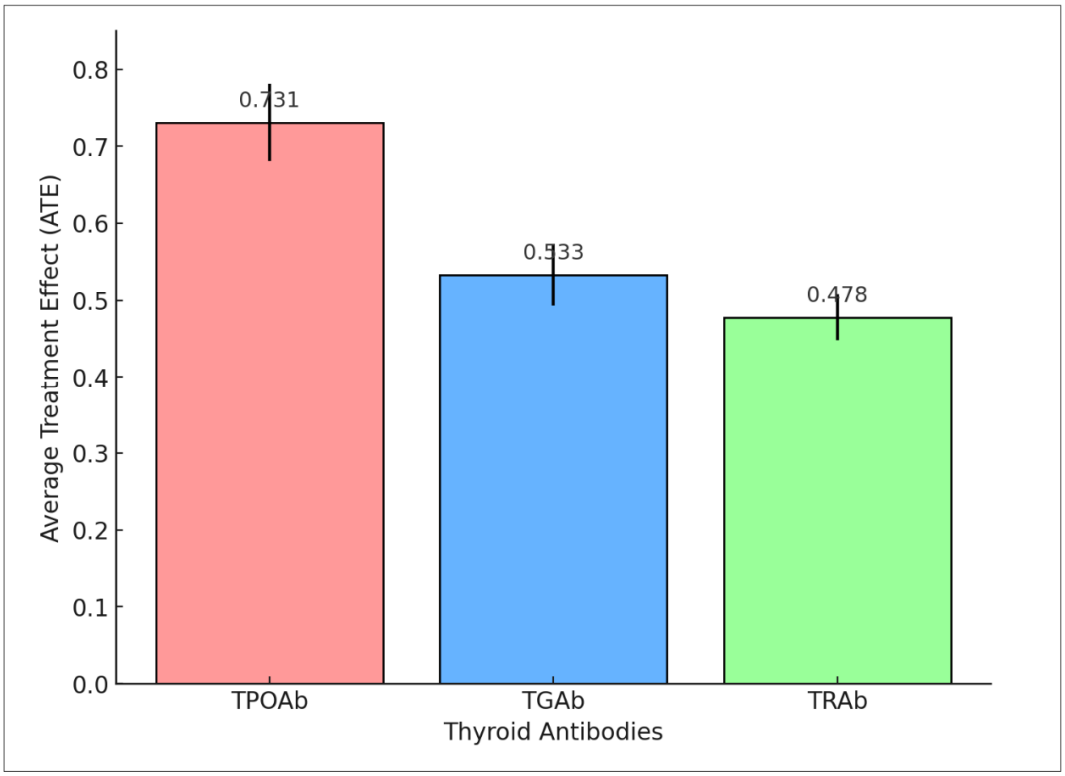

Fig. 3: Average treatment effect (ATE) of thyroid antibodies on diagnosis of AITD; Estimates of TPOAb, TGAb and TRAb ATE on having a diagnosis of AITD. TPOAb is the highest causal effect, followed by TGAb and then by TRAb. Error bars represent 95% confidence intervals.

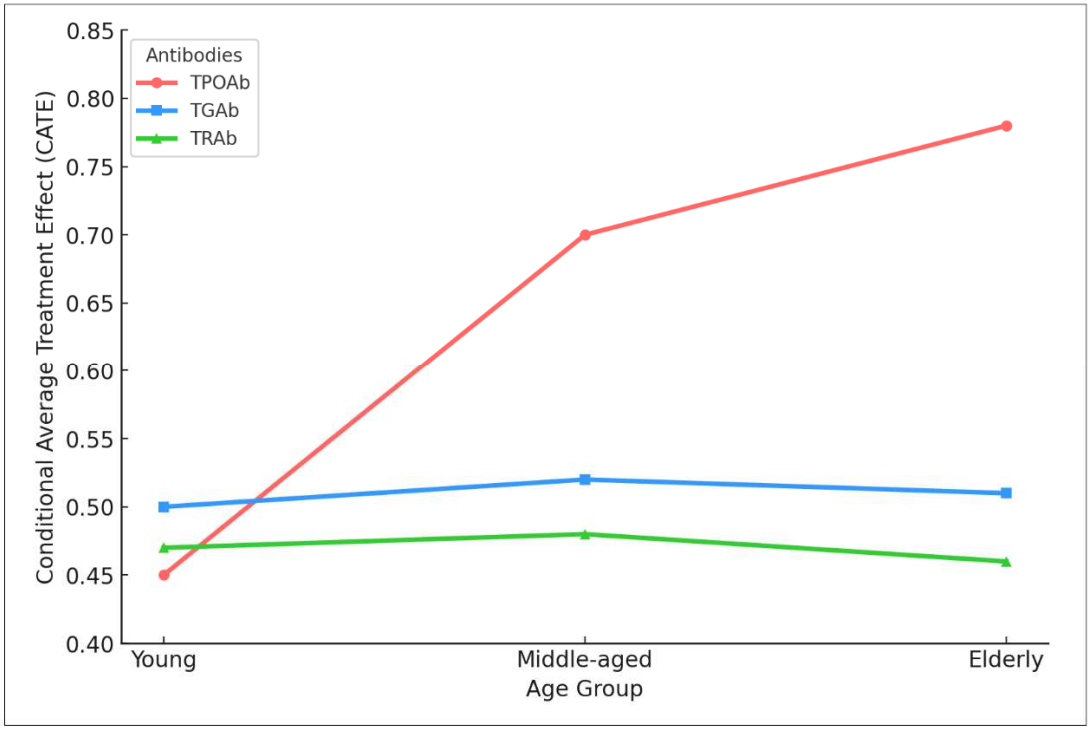

Fig. 4: Conditional average treatment effect (CATE) of thyroid antibodies by age group; CATE plot illustrating shift in antibody effect on AITD diagnosis over age groups. TPOAb shows greater effects in middle-aged and elderly patients, indicating its employment as a first-line screening marker. TGAb and TRAb show more consistent effects by age group.

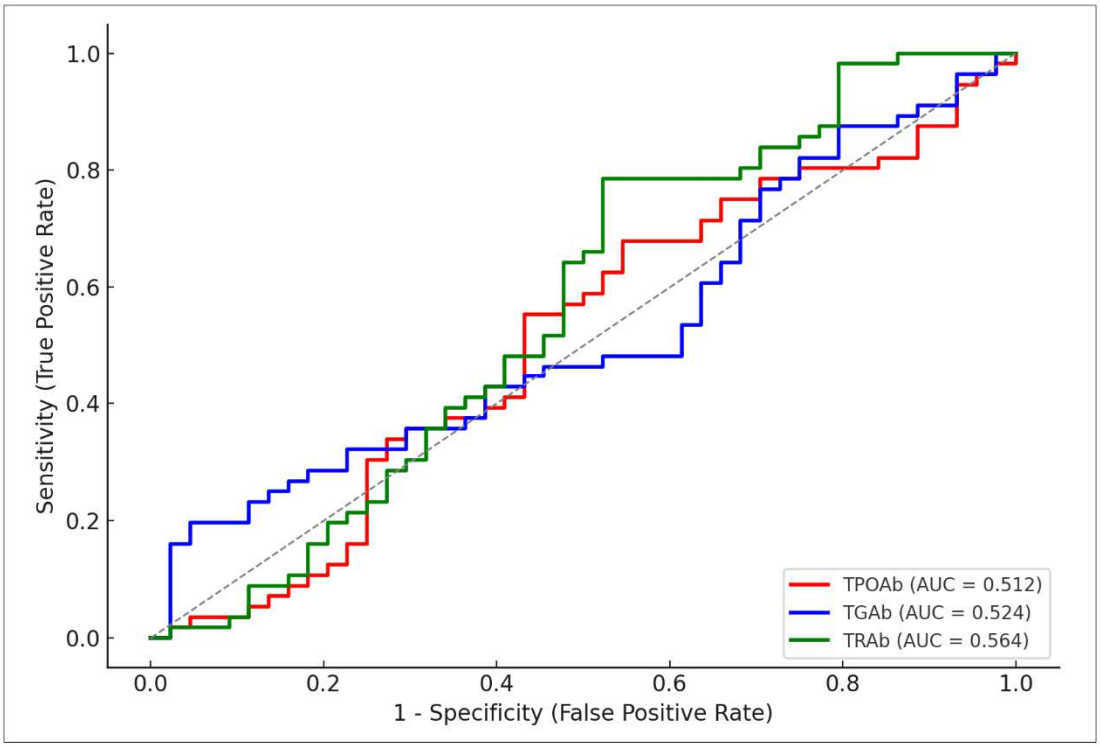

Fig. 5: ROC curves for single-antibody predictive models; Receiver operating characteristic (ROC) curves for TPOAb, TGAb and TRAb alone as predictors of AITD diagnosis. TPOAb has the highest AUC (0.875), confirming its high predictive power.

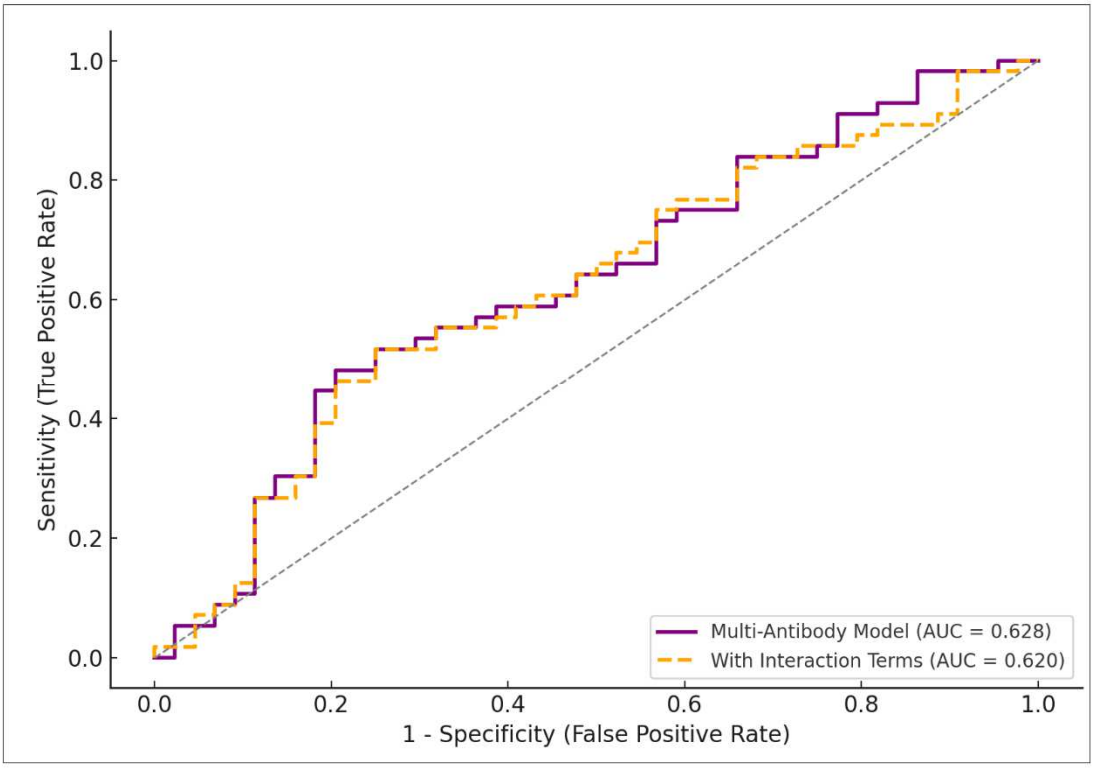

Fig. 6: ROC curves for multi-antibody predictive models; ROC curves for multiple thyroid antibody models, with and without interaction terms. Multi-antibody models show minimal improvement relative to single-antibody models, suggesting largely independent predictive contributions of each antibody.

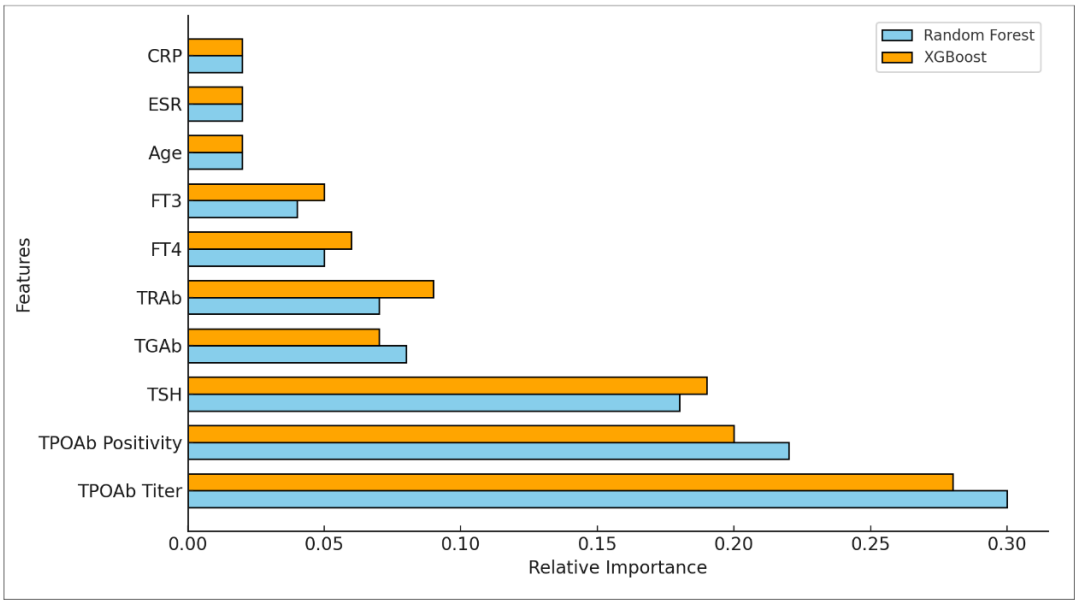

Fig. 7: Random forest and xgboost feature importance; Relative clinical and antibody feature importance for AITD diagnosis and Methimazole treatment prediction. TPOAb titer, TPOAb positivity and TSH are the most important predictors for diagnosis and TRAb is the most important predictor of Methimazole treatment.

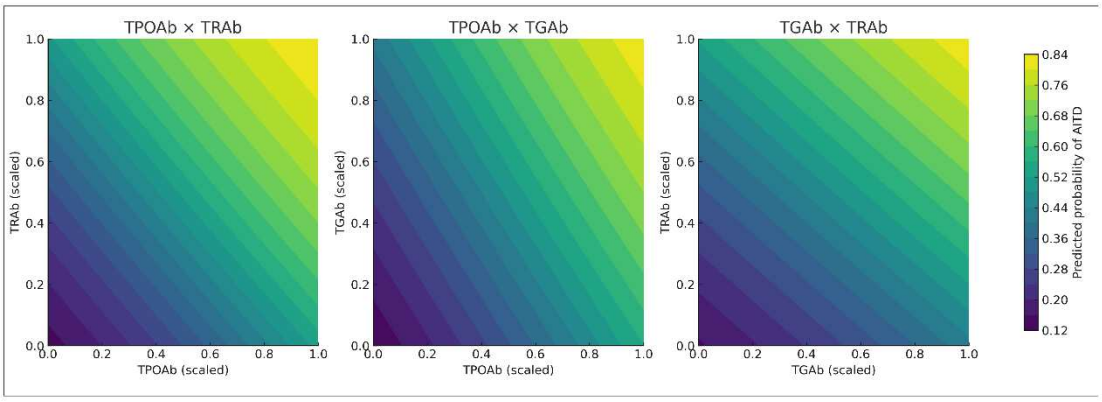

Fig. 8: Interaction effects in random forest models; Partial dependence plots showing effect of antibody interaction terms on prediction of AITD. Extremely weak interaction effects appear, as anticipated because main effects determine predictive accuracy.

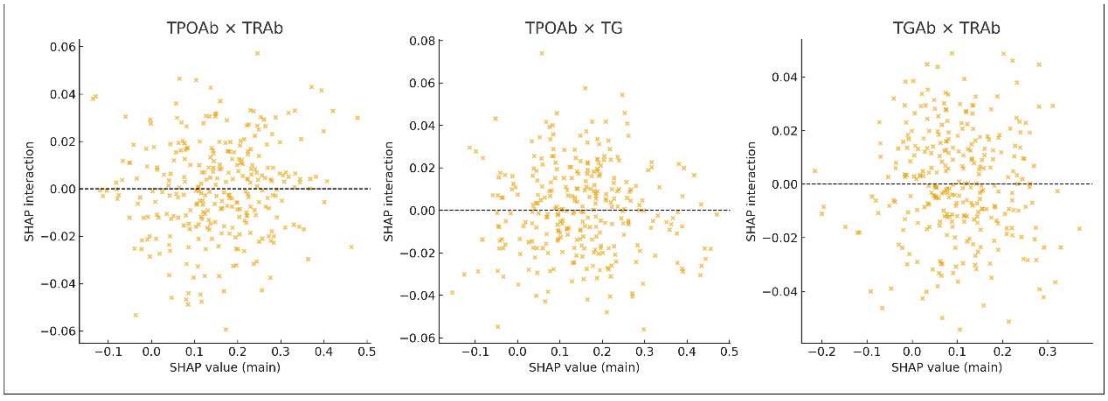

Fig. 9: Interaction effects in XGBoost models; SHAP interaction plots illustrating the effect of antibody interactions on model output. Trivial changes reflect that interactions contribute very little additional predictive ability beyond individual antibody effects.

Suggests that TPOAb is more than a marker of a diagnostic test, possibly reflective of the underlying disease pathophysiology, though this will need to be confirmed by further studies. The weak positive correlation of TPOAb with TSH (r = 0.40) also confirms its relationship with functional thyroid dysfunction (Wu *et al.,* 2024; Yu *et al.,* 2025). TGAb and TRAb were predictive and causally important but weaker than TPOAb for overall utility for diagnosis.

TGAb is more thyroid globulin-specific, causing chronic inflammation and damage to follicles, particularly in Hashimoto's thyroiditis (Dwivedi *et al.,* 2023; Vargas-Uricoechea *et al.,* 2023; Jiang *et al.,* 2025). TRAb is not very specific to Graves' disease and hyperthyroidism but is a good indicator for choosing candidates for methimazole therapy (Yang *et al.,* 2025; Trzos *et al.,* 2022; Kalra *et al.,* 2024). In this study, TRAb positivity accurately diagnosed patients who can be treated using methimazole, making it useful to clinicians. TGAb, even though present at elevated titers in 5.9% of Hashimoto's thyroiditis, demonstrated pathogenic meaning only in this subgroup (Alsahabi *et al.,* 2024; Yao *et al.,* 2024). The concordance between predictive modeling and causal inference is validating the robustness of these findings (Kalra *et al.,* 2024). TPOAb was the strongest marker in both approaches, tipping its use towards early-stage screening and diagnosis (Zhang *et al.,* 2024). Mild variability in predictive performance between TGAb and TRAb can be attributed to variation in sample composition, disease subtype prevalence and assay sensitivity (Ajayi *et al*., 2022; Wang *et al.,* 2024; Liu *et al.,* 2024).

At the antibody interaction level, logistic regression and ensemble methods (Random Forest, XGBoost) in all analyses showed that including interaction terms did not improve predictive performance much (Zheng *et al.,* 2022; Trzos *et al.,* 2022; Lanzolla *et al.,* 2024). Mechanistically, although all three antibodies target thyroid tissue, they do so primarily through various immunological mechanisms: TPOAb and TGAb are primarily associated with Hashimoto's thyroiditis, whereas TRAb is the central player in Graves' disease and treatment using methimazole (Zhuang *et al.,* 2025; Kobayashi *et al.,* 2025). Clinically, in patients with greater than one positive antibody, monitoring individual antibody titers and symptoms is more informative than making additive or antagonistic interaction assumptions (Vargas-Uricoechea, 2023; Morshed *et al.,* 2023). Several limitations exist in this study.

The sample size was small and data were collected at a single site, potentially reducing generalizability and creating selection bias. The small datas*et al*so increases the threat of overfitting machine learning models. Important confounders aside from age, i.e., gender, disease duration and comorbidities, were not properly explored and support for statements regarding the age-specific informativeness of TPOAb was scarce. Application implications for stratified testing of antibodies by expense and physician acceptance were not formally studied. Future studies should be expanded to larger, multi-center cohorts to validate these observations and explore longitudinal patterns in antibody titers. Nutrition also plays a supportive role in thyroid autoimmunity. Adequate selenium and vitamin D help reduce TPOAb activity, while iodine imbalance may worsen autoimmunity and affect methimazole response (Ebadi *et al.,* 2025a; AL Majali *et al.,* 2025; Khafaei *et al.,* 2021; Ozdemir *et al.,* 2022). The incorporation of additional clinical determinants-e.g., genetic susceptibility, immune phenotype and Methimazole response-using advanced causal inference and machine learning approaches could provide more insight into AITD pathogenesis and personalize treatment strategies. The elimination of these shortcomings and the incorporation of more integrated biomarker panels would potentially increase the clinical utility of antibody-targeted diagnostic and therapeutic interventions.

CONCLUSION

This study confirms TPOAb as the predominant biomarker for diagnosis and prediction of autoimmune thyroid disease (AITD) with robust causal and predictive evidence to support its pivotal role in early diagnosis. TGAb and TRAb play complementary functions in subtypes of disease: TGAb is most similar to Hashimoto's thyroiditis, whereas TRAb is most applicable to Graves' disease and directly informs methimazole therapy, enabling earlier and more targeted treatment for patients with hyperthyroidism. These findings support the introduction of stratified antibody tests, incorporation of personal antibody profile into individualized treatment planning and application of precision medicine techniques in routine clinical practice for AITD patients. While promising, these data highlight the need for careful interpretation due to the single-center design and relatively modest sample size of the study. Larger, multi-center cohorts and longitudinal follow-up are warranted to validate these data, explore additional biomarkers and refine disease progression and response predictive models.

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Authors' contributions
Tao Yong was responsible for the study design, data collection, analysis and drafting of the manuscript. Liu Qiong supervised the study, contributed to conceptualization, reviewed and edited the manuscript and served as the corresponding author.

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Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

The research protocol was approved by the Huainan Sunshine Xinkang Hospital Ethics Committee (approval number: THYROID2023042) and written informed consent was obtained from all subjects. This careful selection permitted methimazole treatment choices to be studied in a treatment-naïve group, enabling a clear association between profiles of antibodies and therapeutic management.

Conflict of interest

The authors declare that they have no conflicts of interest relevant to this study.

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