Biological Variability of Thyroid Autoantibodies (Anti-TPO and Anti-Tg) in Clinically and Biochemically Stable Patients With Autoimmune Thyroid Disease

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The biological variation of anti-TPO and anti-Tg autoantibodies was studied in 17 clinically and biochemically stable female patients with autoimmune thyroid disease (AITD), at regular monthly intervals over a period of 6 consecutive months. The mean and standard deviation (SD), within-subject coefficient of variation (CV), between-subject CV, index of individuality, reliability coefficient, and critical differences were as follows: for anti-TPO 238 (197) U/ml, 9.2%, 81.4%, 0.11, 0.96, and 27.6%; and for anti-Tg 1,785 (3,170) U/ml, 6.9%, 174%, 0.04, 0.99, and 22.3%. The data indicate a low within-subject CV, and a high between-sub-

ject CV that is particularly pronounced for anti-Tg. The high individuality of both autoantibodies indicates that an isolated result compared to conventional population-based reference intervals is of very little value for diagnosis. Furthermore, the near to 1 reliability coefficient for both autoantibodies correctly classifies the patient with respect to his or her homeostatic mean antibody concentration in a 6-month period of clinical and biochemical stability of thyroid disease. Imprecision goals for anti-TPO and anti-Tg antibodies are attainable with current methodology. *J. Clin. Lab. Anal.* 16:37–39, 2002. © 2002 Wiley-Liss, Inc.

Key words: anti-Tg antibodies; anti-TPO antibodies; autoimmune thyroid disease; biological variability

INTRODUCTION

Thyroid peroxidase autoantibodies (TPO-Ab) and thyroglobulin autoantibodies (Tg-Ab) are hallmarks of autoimmune thyroid disease (AITD) (1,2). On the other hand, all variables measured in the clinical laboratory change with time. The components of biological variation permit us to assess the usefulness of traditional reference values, delineate objective analytical goals for precision, and assess what degree of change is required in a series of results for statistical significance (3). The objective of this study was to investigate the biological variation of Tg-Ab and TPO-Ab in a cohort of 17 patients with clinically and biochemically stable AITD.

MATERIALS AND METHODS

Subjects

Seventeen Caucasian female patients with AITD, mean age 44.2 (18.6) years, were examined monthly over a period of 6 consecutive months to define their clinical and biochemi-

cal stability according to asymptomatic health status and normal TSH levels. We analyzed only women because AITD is much more prevalent in females, and we wanted a homogeneous group.

Collection of Blood Samples

A standardized protocol according to the hospital ethics committee was used for blood collection, and handling and storage of specimens. To minimize preanalytical variance, the same phlebotomist drew the specimens at the same time each day (between 08:00 and 9:30 hr). Serum was separated and stored in frozen aliquots at -70°C until assayed.

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METHODS

Anti-TPO and Anti-Tg antibodies were measured by enzyme immunoassay with fluorimetric detection using the UniCAP System (Pharmacia Diagnostics, Freiburg, Germany) according to the manufacturer's recommendations. When all the specimens had been collected, the study period was deemed complete. On the day of analysis all the specimens were thawed at room temperature and mixed by gentle swirling or inversion. To minimize analytical variation, the samples were all randomly sequenced and assayed in a single analytical run using the same lot of reagents, calibrators, and quality control materials.

Statistical Analysis

From the entire set of data on all subjects, and after excluding outliers and assuming that both within-subject and analytical variances are constant over the entire range of analyte concentrations, we used a simple nested analysis of variance (ANOVA) to obtain coefficients of variation (CVs) (within-subject CV (CV_i) and between-subject CV (CV_g)). We also obtained the index of individuality (II), reliability coefficient (R), critical differences (also called reference change values (RCVs)), and the amount of variability added to the true test results through analytical variation (E_a) (4,5). According to biological variation data, we obtained desirable performance standards for the measurement of thyroid antibodies (6).

RESULTS

The descriptive statistics are reported in Table 1. The overall mean (and SD) for anti-TPO and anti-Tg were 238 (197) U/ml and 1,785(3,170) U/ml, respectively. The median was quite different from the mean, suggesting a non-Gaussian distribution of anti-TPO and anti-Tg values in the population. Twenty-five and 75 percentiles are also presented. Data show that dispersion around the mean and the median are high; hence, we can expect a relatively wide reference interval for these autoantibodies.

Components of variance, between-subject, within-subject, and analytical variation expressed as CVs for anti-TPO and anti-Tg are shown in Table 2. As regards biological variation, both anti-TPO and anti-Tg between-subject variation was high (CV_g 81.4% and 174%, respectively)—much higher than within-subject biological variation (CV_i 9.2% and 6.9%,

TABLE 1. Descriptive statistics for the study

Analyte	Overall mean	Standard deviation	Median	P25	P75
Anti-TPO (U/mL)	238	197	138	86	376
Anti-Tg (U/mL)	1785	3170	291	95	1593

P, percentile.

TABLE 2. Components of variance

Analyte	CV_i (%)	CV_g (%)	CV_a (%)
Anti-TPO	9.2	81.4	3.8
Anti-Tg	6.9	174.0	4.2

CV_i , within-subject CV; CV_g , between-subject CV; CV_a , analytical CV.

respectively). The anti-TPO and anti-Tg analytical variations (CV_a) of the methods used were 3.8% and 4.2%, respectively.

Indexes derived from biological variation (index of individuality, reliability coefficient, critical differences, heterogeneity index, and amount of variability added to true test results through analytical variation) are shown in Table 3. Both autoantibodies exhibit a low index of individuality, a reliability coefficient near 1.0, and critical differences of about 25%. Desirable analytical goals for imprecision and inaccuracy are also reported in Table 3.

DISCUSSION

This study demonstrates a low within-subject biological variation and a high between-subject biological variation for thyroid autoantibodies. The low within-subject variation of anti-TPO and anti-Tg is probably due to the fact that the patients studied were all clinically and biochemically stable.

Anti-Tg and anti-TPO antibodies show a high individuality with an II lower than 0.6. Thus, we can obtain unusual results from a particular individual within the conventional population-based reference values. Differences lower than 27.6% and 22.3% for anti-TPO and anti-Tg, respectively, could be explained only by biological and analytical variability.

A recent study (7) reported anti-TPO intra- and interassay CVs between 2–8% and 3.2–19%, respectively, and anti-Tg intra- and interassay CVs between 3–9% and 5–12%, respectively. In our study, the imprecision goal expressed as a desirable coefficient of analytical variation was 4.6% for anti-TPO and 3.4% for anti-Tg. These quality goals for imprecision are very stringent for autoantibody measurements; however, our method attains the goal for anti-TPO, and almost attains it for anti-Tg. Quality goals for imprecision have been expanded to overcome some of the philosophical and practical difficulties with the concept (8). A new quality specification could be used for those quantities for which desirable performance standards were not attainable with current technology and

TABLE 3. Indices derived from biological variation and desirable performance standards

Analyte	II	R	RCV (%)	E_a (%)	Imprecision (CV_a %)	Inaccuracy (Bias %)
Anti-TPO	0.11	0.96	27.6	8.2	4.6	20.7
Anti-Tg	0.04	0.99	22.3	17.2	3.4	44.4

II, individuality index; R, reliability coefficient; RCV, reference change values; E_a , amount of variability added to true test results through analytical variation; CV_a , analytical CV.

methodology; this is the minimum performance defined by $CV_a < 0.75 CV_i$. In our study, this corresponds to 5.2% for anti-Tg, which is greater than the method's CV_a . The amount of variability added to the true test through analytical variation is 8.2% for anti-TPO and 17.2% for anti-Tg. Our study shows that the inaccuracy goals for anti-TPO and anti-Tg tests were 20.7% and 44.4%, respectively. These figures are easily obtained with the method used.

In conclusion, we obtained components of variance of thyroid autoantibodies in a group of 17 female patients with clinically and biochemically stable AITD. A high between-subject variation of both anti-TPO and anti-Tg autoantibodies was observed. Indexes derived from biological variation show that conventional reference intervals based on population are of limited value in anti-TPO and anti-Tg antibody tests. However, a single measurement correctly characterizes an individual's average autoantibody concentration. Critical differences must be taken into account when interpreting two autoantibody measurements. Imprecision goals for anti-TPO and anti-Tg antibodies are attainable with current methodology.

REFERENCES

1. Rapoport B, McLachlan SM. Thyroid peroxidase autoantibodies. In: Peter JB, Shoenfeld Y, editors. *Autoantibodies*. Amsterdam: Elsevier Science; 1996. p 816–821.
2. Burek CL, Rose NR. Thyroglobulin autoantibodies. In: Peter JB, Shoenfeld Y, editors. *Autoantibodies*. Amsterdam: Elsevier Science; 1996. p 810–815.
3. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 1989;27:409–437.
4. Harris EK. Statistical principles underlying analytic goal-setting in clinical chemistry. *Am J Clin Pathol* 1979;72:374–382.
5. Harris EK, Boyd JC. *Statistical bases of reference values in laboratory medicine*. New York: Marcel Dekker; 1995.
6. Stockl D, Baadenhuisjen H, Fraser CG, et al. Desirable routine analytical goals for quantities assayed in serum. *Eur J Clin Chem Clin Biochem* 1995;33:157–169.
7. Feldt Rasmussen U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. *Clin Chem* 1996;42:160–163.
8. Fraser CG, Hyltoft Petersen P, Lieber JC, Ricos C. Proposals for setting generally applicable quality goals solely based on biology. *Ann Clin Biochem* 1997;34:8–12.