

Sampling Time Is Important but May Be Overlooked in Establishment and Use of Thyroid-Stimulating Hormone Reference Intervals

To the Editor:

In the current debate on reference interval(s) for serum thyroid-stimulating hormone (TSH) concentrations, a lowering of the upper reference limit from ~ 4.0 to 2.5, or even 2.0, mU/L has been proposed by the National Academy of Clinical Biochemistry (NACB) (1). This proposal is based in part on the observation that populations with the lowest prevalence of antithyroid antibodies have the lowest TSH upper limits. Other arguments for the lowering of the upper limit of the reference interval are related to the question of whether mild TSH increases have any clinical consequences. This question, however, illustrates the problem of mixing the concepts of (a) decision limits (e.g., discrimination values, cutoffs, action limits), which are based on the clinical consequences and treatment strategies and (b) reference intervals, which are based solely on biology and mathematics applied in an appropriate reference population. This confusion is also addressed in a recent paper on TSH reference interval(s) (2).

The debate for lowering the upper TSH reference limit also in-

cludes the argument that the reference distribution for serum TSH should be gaussian in nature, but the upper tail of the distribution is currently skewed by: (a) euthyroid outliers such as may occur in patients recovering from nonthyroidal illness, (b) measurement of bioinactive TSH isoforms, (c) TSH receptor gene polymorphisms, and (d) occult autoimmune thyroid dysfunction. As a consequence, some authorities suggest the distribution tail to be deleted (1). In our opinion, however, this upper tail is an essential part of the distribution. In fact, when all values from individuals at risk are removed, log-gaussian distributions are common for most serum components (3), as we demonstrated for serum TSH, which is unimodal and log-gaussian (4).

We now focus on the newer documentation regarding serum TSH reference intervals and methods. Despite the fact that several publications suggest an upper limit of ~ 4 mU/L, NACB proposes an upper limit of 2.5 mU/L, although only one of several population-based studies supports this (Table 1).

As evidenced in Table 1, studies vary widely in time of sampling and analytical methods used, as well as inclusion and exclusion criteria. The study with the highest relative median serum TSH in the Deutschen Gesellschaft für Klinische

Chemie und Laboratoriumsmedizin hormone survey could have been expected to demonstrate the highest upper reference limit, but clearly it does not (Table 1), suggesting that factors other than method standardization play a role. Repeated data from external quality assessment performed from 2000 to 2005 disclose that between-method variation is only a minor source of the variation in serum TSH. The exclusion of individuals at risk, however, has been based on nonstandardized criteria, and the importance of time of sampling has been ignored. In fact, in the majority of publications the time of sampling has not been specified.

There is evidence of a considerable diurnal variation in serum TSH concentration, with a maximum around midnight (6). A decrease of up to 50% occurs from 8:00 to 9:30 AM; thereafter the concentration remains relatively constant until evening, with a smaller nadir in the late afternoon. Because serum TSH concentration decreases markedly during the morning and time of sampling is unknown in most studies, sampling time differences between studies may be a primary reason for the discrepancies in published reference intervals. Individuals working night shifts have displaced or reduced diurnal rhythms, a phenomenon that

Table 1. Data from recently published studies on TSH reference intervals.

Publication	Time of blood sampling	Reference interval	Sample size		Exclusion due to:			DGKL Quality Assessment ^a		
			n	Excluded, %	Antibodies, %	US	TSH methods	Relative median	CV, %	n
Canaris et al. (7)	?	0.30–5.10	25 862	0	0	No	London diagnostics			
Hollowell et al. (8)	?	0.45–4.12	17 353	23.1	14.0	No	Nichols			
Jensen et al. (4)	0800–0900	0.58–4.07	1441	31.6	17.3	No	AutoDELFLIA	0.97	4.4	22
Kratzsch et al. (9)	0800–1800	0.40–3.77	870	47.9	22.6	Yes	Elecsys	1.00	4.6	22
Völzke et al. (10)	?	0.25–2.12	4298	65.4	3.5	Yes	Byk Sangtec	1.12	3.5	16
Eskelinen et al. (11)	0800–1000	0.47–5.60	1252	35.9	22.0	No	AutoDELFLIA	0.97	4.4	22
d'Herbomez et al. (12)	?	0.35–3.48	763	6.9	6.9	No	Access	0.92	4.3	14
Surks et al. (13)	?	0.45–4.17	17 353	17.4	14.0	No	Nichols			
Hoogedorn et al. (14)	0800–2000	0.34–4.66	6434	19.7	13.9	No	Architect	0.87	4.7	22

^a In the Deutschen Gesellschaft für Klinische Chemie und Laboratoriumsmedizin (DGKL) hormone survey (5) 2 samples (concentrations 1–20 mU/L) were dispatched 1 to 4 times per year during 2000 to 2005. The Nichols test is not used in Europe. Byk Sangtec is used by very few. Exclusion US relates to morphological alterations by ultrasound. Eskelinen et al. (12), studied only in individuals >65 years. US, ultrasound.

should also be acknowledged (or such individuals excluded) when establishing reference intervals. Consequently, our proposal is to establish reference intervals as a function of time of sampling to reveal the influence of time on reference limits for serum TSH. The outcome of sampling time investigations will indicate whether such data will lead to recommendations for time of sampling or to time-dependent reference intervals.

Studies to establish decision limits for serum TSH should be based on standardized measurements performed in longitudinal follow-up of cohorts with various concentrations of serum TSH. Such studies may well support intervention below a serum TSH concentration of 4.0 mU/L. At present, however, such a decision is not based on unequivocal evidence (2).

References

- Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Spencer CA, et al. Laboratory Medicine Practice Guidelines: Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid* 2003; 13:3–126.
- Brabant G, Beck-Peccoz P, Jarzab B, Laurberg P, Orgiazzi J, Szabolcs, et al. Is there a need to redefine the upper normal limit of TSH? *Eur J Endocrinol* 2006;154:633–7.
- Hyltoft Petersen P, Blaabjerg O, Andersen M, Jørgensen LGM, Schousboe K, Jensen E. Graphical interpretation of confidence curves in rankit plots. *Clin Chem Lab Med* 2004;42:715–24.
- Jensen E, Hyltoft Petersen P, Blaabjerg O, Hansen PS, Brix TH, Hegedüs L. Establishment of a serum thyroid stimulating hormone (TSH) reference interval in healthy adults. The importance of environmental factors, including thyroid antibodies. *Clin Chem Lab Med* 2004;42: 824–32.
- Deutsche Gesellschaft für Klinische Chemie (DGKL). Ringversuch Hormone survey. *HM* 2/00 to *HM* 2/05.
- Weeke J, Gundersen HJG. Circadian and 30 minutes variations in serum TSH and thyroid hormones in normal subjects. *Acta Endocrinol*. (Copenh.) 1978;89:659–72.
- Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. *Arch Intern Med* 2000;160:526–34.
- Hollowell JG, Staehling NW, Flanders WD, Hanon WH, Gunter EW, Spencer CA, et al. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* 2002; 87:489–99.
- Kratzsch J, Fiedler GM, Leichtle A, Brügel M, Buchbinder S, Otto L, et al. New reference intervals for thyrotropin and thyroid hormones based on National Academy of Clinical Biochemistry criteria and regular ultrasonography of the thyroid. *Clin Chem* 2005;51:1480–6.
- Völzke H, Alte D, Kohlmann T, Lüdemann J, Nauck M, John U, et al. Reference intervals of serum thyroid function tests in a previously iodine-deficient area. *Thyroid* 2005;15:279–85.
- Eskelinen S, Suominen P, Vahlberg T, Löppönen M, Isoaho R, Kiveliä SL, et al. The effect of thyroid antibody positivity on reference intervals for thyroid stimulating hormones (TSH) and free thyroxine (fT₄) in an aged population. *Clin Chem Lab Med* 2005;43:1380–5.
- d'Herbomez M, Jarrige V, Darte C. Reference intervals for serum thyrotropin (TSH) and free thyroxine (fT₄) in adults using the Acces[®] Immunoassay System. *Clin Chem Lab Med* 2005;43:102–5.
- Surks MI, Goswami G, Daniels GH. Controversy in clinical endocrinology: the thyrotropin reference range should remain unchanged. *J Clin Endocrinol Metab* 2005;90:5489–96.
- Hoogendoorn EH, Hermus AR, de Vegt F, Ross HA, Verbeek ALM, Kiemeneij LALM, et al. Thyroid function and prevalence of anti-thyroperoxidase antibodies in a population with borderline sufficient iodine intake: influences of age and sex. *Clin Chem* 2006;52:104–11.

Esther Jensen^{1*}
Ole Blaabjerg¹
Per Hyltoft Petersen²
Laszlo Hegedüs³

¹ Department of Clinical Biochemistry
Odense University Hospital
Odense, Denmark

² NOKLUS, Norwegian quality
improvement of primary care
laboratories
Division for General Practice
University of Bergen
Bergen, Norway

³ Department of
Endocrinology and Metabolism
Odense University Hospital
Odense, Denmark

* Address correspondence to this author at: Department of Clinical Biochemistry, Odense University Hospital, DK-5000 Odense C, Denmark. Fax 45-65-41-19-11; e-mail Esther.Jensen@ouh.fynsamt.dk.

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A Combinatorial Haplotype of the UDP-Glucuronosyltransferase 1A1 Gene (*60-#IB) Increases Total Bilirubin Concentrations in Japanese Volunteers

To the Editor:

UDP-glucuronosyltransferases (UGTs) are a family of enzymes that glucuronidate many endogenous and exogenous substrates (1). Of the *UGT1A* gene isoforms, *UGT1A1* is primarily responsible for glucuronidation of bilirubin (1). In east Asians, 2 well-known genetic variants, A(TA)₆TAA>A(TA)₇TAA (allele *28, reduced transcription) and G71R (211G>A, allele *6, reduced activity), are causative factors for increased plasma bilirubin concentrations in Gilbert syndrome (1). The *28 allele is almost always linked to the *60 allele (-3279T>G), with reduced in vitro transcription (2).

In a previous study (2) in which we divided *UGT1A1* into 2 haplotype blocks (the 5'-flanking region and exon 1 in block 1 and common exons 2 to 5 in block 2), *60 and *IB (perfectly linked 1813C>T, 1941C>G, and 2042C>G in the 3'-untranslated region in Japanese persons) showed increased total bilirubin concentrations in non-*28 patients. Because of the small number of patients, however, it was not clear whether bilirubin concentrations were affected by *60 and *IB acting independently or cooperatively when they were on the same chromosome. To clarify this point, we reinvestigated the associations between the *UGT1A1* haplotypes and total bilirubin concentrations in 554 healthy Japanese volunteers. The ethical review boards of the participating institutions approved this study, and informed consent was obtained from all participants.

For genotyping of *60, *28, *6, and *IB marker variations, DNA was extracted from Epstein-Barr-virus-transformed lymphoblastoid cells. The genotyping methods for the *60, *6, and *IB alleles were described previously (3, 4). For *IB, 1941C>G was genotyped (3). For *28, -364C>T, which is perfectly linked with the *28 allele in Japanese persons (2), was used as a surrogate polymorphism, as described in Fig. 1 in the Data Supplement that accompanies the online version of this Letter at <http://www.clinchem.org/content/vol53/issue2>, which also shows the allele frequencies of the variations. The diploidy configuration (combination of haplotypes) for each volunteer was inferred by an expectation-maximization-based program, LDSUPPORT, as