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Estimation of individual reference intervals in small sample sizes

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Abstract

In occupational health studies, the study groups most often comprise healthy subjects performing their work. Sampling is often planned in the most practical way, e.g., sampling of blood in the morning at the work site just after the work starts. Optimal use of reference intervals requires that the population, on which the reference interval is based, is representative for the study group in question. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommends estimating reference interval on at least 120 subjects. It may be costly and difficult to gain group sizes of that order of magnitude for all topics in question. Therefore, new methods to estimate reference intervals for small sample sizes are needed. We present an alternative method based on variance component models. The models are based on data from 37 men and 84 women taking into account biological variation from various variables such as gender, age, BMI, alcohol, smoking, and menopause. The reference intervals were compared to reference intervals calculated using IFCC recommendations. Where comparable, the IFCC calculated reference intervals had a wider range compared to the variance component models presented in this study. The presented method enables occupational health researchers to calculate reference intervals for specific groups, i.e. smokers versus non-smokers, etc. In conclusion, the variance component models provide an appropriate tool to estimate reference intervals based on small sample sizes.

Keywords: Reference interval; Biological variation; Blood; Healthy subjects

Introduction

In occupational health studies, the study groups most often comprise healthy subjects performing their work. Sampling is often planned in the most practical way, e.g., sampling of blood in the morning at the work site just after the work starts. Optimal use of reference intervals requires that the population, on which the reference interval is based, is representative for the study group in question. From that point of view, the best reference intervals for use in preventive and/or occupational medicine are reference intervals based on biological samples from healthy subjects performing their routine work. Reference intervals for clinical purposes are often established for hospitalised reference subjects living under the same conditions, but not suffering from the same disease as the patient (Kouri et al., 1994). Hence, the published reference intervals are not suitable for use in occupational medicine. The International Federation of Clinical Chemistry and laboratory medicine (IFCC) recommends that the parametric confidence intervals of fractiles of reference intervals should be based on an approximation to the normal distribution after transformation of reference intervals (Solberg, 1986). For estimating reference interval as

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recommended by IFCC samples from more than 120 subject are recommended (Poulsen et al., 1997). If the component in question is influenced by, e.g., age, body mass index (BMI), gender, or smoking it can be costly and difficult to gain group sizes of that order of magnitude. Therefore new methods to estimate reference intervals for small sample sizes are needed. Variance component models used to determinate biological variation may serve this purpose.

Hormones have been used as effect measures for a variety of different exposures including perceived job strain in human health service organisations (Ohlson et al., 2001), job decision latitude in shift workers (Tenkanen et al., 1997) and working with information technology (Arnetz, 1997), and in relation to other outcome parameters: cardiovascular disease (Arnetz, 1997), acute onset of low back or neck/shoulder complaints (Hasselhorn et al., 2001), and physiological changes such as increased catabolism (Kawakami et al., 1995; Netterstrøm et al., 1988). Only few studies have published reference intervals established for biochemical compounds in blood from healthy subjects performing their routine work, i.e. two studies concerning IgA in blood from healthy subjects (Irjala et al., 1990; Negri et al., 1995) and two studies deal with DHEA-S in blood from healthy men (Denti et al., 1997; Thomas et al., 1999). Further, it has recently been stressed that knowledge of the underlying biological variation is crucial to use and understand the population-based reference intervals in clinical settings (Fraser, 2004).

The purposes of this study was to present variance component models as an alternative method to estimate reference intervals in small sample sizes for use in preventive and/or occupational medicine among healthy subjects, and to use it to calculate specific, where appropriate, reference intervals for gender, different age groups, smoking, or BMI for serum dehydroepiandrosterone-sulphate (S-DHEA-S), serum free-testosterone (S-free-testosterone), blood glycated haemoglobin A_{1c} (B-HbA_{1c}), serum immunoglobin (S-IgA), serum prolactin (S-prolactin) and serum total-cholesterol (S-total cholesterol) in healthy men and women performing their routine work. Finally, the established reference intervals were compared to reference intervals calculated as recommended by IFCC, where possible.

Subjects and methods

Study group

A total of 42 healthy men and 88 healthy women responded to public advertising. Two weeks prior to a health examination a questionnaire concerning the health including conditions regarding demographic data and known diseases, such as hypertension, was completed. Five men and four women were excluded due to known hypertension. Hence 37 men (88%) and 84 women (94%) comprised the study group. In the female group 65 women were pre-menopause and 19 were postmenopausal. Mean and range for demographic data of the study group are given in Table 1. Six men were smokers, 16 were former smokers, and 15 had never smoked. Twenty-three women were smokers, 32 were former smokers, and 29 had never smoked. All subjects were wage earners at the time of sampling. All subjects gave informed consent and the local ethics committee approved the study to comply with the Helsinki declaration.

Table 1. Demographic data of 37 healthy men and 84 healthy women

	Men ($n = 37$)		Women ($n = 84$)	
	Mean	Range	Mean	Range
Age (year)	45	31–60	44	30–60
BMI (kg/m^2)	25.4	21.2–33.6	23.6	18.4–29.8
Alcohol (gram per week)	96	0–420	84	0–504

Sample collection

The study was carried out on a working day in October, November, or December 1998. Blood was collected from the antecubital vein by venepuncture in 10 ml Vacutainer® tubes (Becton Dickinson, Rutherford, NJ). Samples for HbA_{1c} analysis were collected in tubes containing ethylene-diamine-tetra-acetic-acid (EDTA). Samples for S-total cholesterol, S-DHEA-S, S-IgA, S-prolactin, and S-free-testosterone were collected in plain tubes with no additives, and after two hours at room temperature centrifuged for 10 min at 3000 rev/min (928 relative centrifugal force (RCF)). Serum was separated from the sediment and stored frozen (−20 °C) for 2–12 months until assayed.

Chemical analysis

High performance liquid chromatography (HPLC) was used for determination of HbA_{1c}. The HPLC consisted of a Waters 625 LC system together with a Waters photo-diode-array detector model 996 and a WISP 717 auto sampler for automatic injection of the samples. Millennium chromatography software was used for calculation of concentrations (Waters Associates Inc., Milford, United States). A cation exchange column Mono S HR 5/5 from Pharmacia Biotech AB, Uppsala, Sweden was used to separate B-HbA_{1c} from