Upsala J Med Sci 95: 279–285, 1990

Measuring Cholesterol A Handbook for Out-Patient Clinics and Laboratory Personnel

Peter Nilsson-Ehle

Department of Clinical Chemistry, University of Lund, Sweden

INTRODUCTION

Elevated serum cholesterol concentrations is a major risk factor for cardiovascular disease. If cholesterol levels are reduced, the relative risk decreases. With the introduction of new, potent hypolipidemic drugs there is now an increasing interest in cholesterol-lowering programs, with or without support of screening by measurements of S-Cholesterol in the population in general or in specific risk groups.

Guidelines for such intervention programs have been issues by international and national authorities. However, it has so far not been possible to determine the cost-effectiveness of such programs.

One important component in these considerations is the efficacy of the screening method used to distinguish subjects who should be treated from those who should not, and its ability to properly register alterations in cholesterol levels in individuals and populations as a result of intervention.

The quality requirements for biochemical measurements, when applied to health surveys or screening programs, seems - somewhat paradoxically - to be stricter than when such measurements are used in the traditional situation to confirm a diagnosis of hyperlipidemia or to monitor patients with overt lipid derangements during treatment. In screening programs, important medical decisions are based almost entirely on the result of a biochemical measurement, and the differences which occur after intervention in subjects with moderate hyperlipidemia are generally quite small and therefore difficult to detect and quantitate. In patients with hyperlipidemia, in contrast, the alterations and differences in lipid levels are much more pronounced and therefore easier to monitor. Also, medical decisions are

generally based on a more comprehensive evaluation of the multiple factors that may influence choice of therapy and prognosis in the individual patient. In an attempt to inform about the chatacteristics of the S-Cholesterol measurement and to facilitate its use in health surveys and screening, the Swedish Society for Clinical-Chemistry has produced a pamphlet (1) which has been distributed to out-patient clinics and health centers throughout Sweden. The primary target groups are nurses and technicians responsible for sampling and for cholesterol measurements with traditional methods or with decentralized techniques. The information covers various aspects on biological variation, the sampling procedure, and analytical performance. Finally, some calculations on the performance of the S-Cholesterol measurement in screening programs and in patient monitoring are presented. The major points are summarized below. Much or the information is based on recent investigations (2, 3).

ANALYTICAL VARIATION

Both standard methods and the dry chemistry techniques have low intra-assay variation for cholesterol (CV <1-2%). The inter-assay variation is clearly dependent on the setting but can be reduced to 2-3% (CV) provided that proper quality assurance programs are carried out.

ANALYTICAL BIAS

Samples drawn in EDTA tubes yield significantly lower cholesterol concentrations than serum samples, while there is no significant difference between heparin plasma and serum. On the average, cholesterol concentrations in EDTA samples are 4% lower than in serum. In EDTA tubes which have not been properly filled the difference can amount to 12-15%. The lower concentrations measured in EDTA samples can be attributed to the osmotic effect of the salt, which extracts fluid from the red blood cells, thereby diluting the plasma.

ERRORS ASSOCIATED WITH THE SAMPLING PROCEDURE

The random error associated with blood sampling, as assessed by comparing samples obtained by simultaneous venous punctures in the left and right antecubital vein, is 2-3% (CV). However, the sampling procedure is critical because of the heavy impact of posture on the concentration of cholesterol in the circulation. In sitting and in the supine position, S-Cholesterol levels are significantly lower than in standing; the average differences are 7 and 11%, respectively, but in extreme cases the difference may amount to 22%.

INTRAINDIVIDUAL BIOLOGICAL VARIATION

The variations in S-Cholesterol over the day in healthy subjects is <5% (CV). The variation in fasting samples obtained on consecutive mornings is of similar magnitude. In women, there is a small increase in S-Cholesterol around the time of ovulation, but the biological variation over a month is still <6% (CV).

DISCUSSION AND RECOMMENDATIONS

The technical performance of modern S-Cholesterol measurements seems sufficient to meet the high standards of screening activities. The inter-assay variation, which is relevant for patient monitoring, can be kept below 3% provided that proper quality assurance programs are enforced. This holds true also for the dry chemistry equipment primarily designed for decentralized laboratory work. However, it should be emphasized that proper training and instruction is critical for this setting.

Because of the marked and potentially unpredictable reduction of S-Cholesterol which may occur in EDTA blood it is strongly recommend that analyses of lipid and lipoprotein concentrations be performed in serum. If an anticoagulant is needed heparin is the substance or choice.

The errors associated with the sampling procedure can also be kept within acceptable limits. However, information on the marked effects of posture is essential to warrant an informative result.

We strongly advocate the recommendations of the International Federation for Clinical Chemistry, which state that all venous blood sampling should be performed after the subject has been resting in recumbent position for 15 min.

The biological variation in young healthy volunteers is about 5% (CV) under various conditions. Also including the trends for serum lipid alterations during the menstrual cycle, the biological variation of S-Cholesterol is not greater than 6% (CV). Since the concentration of cholesterol in the circulation is not affected by food intake, samples can be taken at any time during the day to be representative. Instructions given to the subjects before sampling, in order to minimize biological variation, include abstinence from alcohol the day before sampling, since alcohol intake may markedly increase lipid levels. Also, strenuous physical activities should be avoided the day before sampling, since this may decrease S-Cholesterol concentrations.

Besides these short-term influences on serum lipoprotein levels, it is well known that transient alterations in lipid and lipoprotein levels occur in a variety of situations, which may invalidate the result of serum lipid measurements. Of special interest in this context is the marked reduction in S-Cholesterol which occurs as a result of the acute phase reaction after infections and after myocardial infarction. To ensure a representative result, sampling for serum lipids should be postponed till 3 months after the acute phase.

The differentiation between analytical error, sampling error and biological variation is necessary to accurately evaluate the contribution of these sources of variation to the total variation of the cholesterol measurement. Fig 1 illustrates that the sampling error and the analytical error, when procedures are carried out competently, is minor compared to the biological variation, which sets the limits for the performance of the S-cholesterol measurement.

Under optimal conditions, the total variation for the S-Cholesterol measurement is around 6,5%. Consequently, the average, typical value for an individual, with 95% probability, is in the range ±13% (±2 SD) from the reported value (e g, reported value 6,5 mmol/l corresponds to 6,65 - 7,35 mmol/l). A change in S-Cholesterol must amount to 18% (2,8 x 6,5%) to be

detected with 95% probability. To detect an expected decrease in S-Cholesterol e g during cholesterol-lowering treatment (one-tailed test) the reduction must be at least 15% (2,3 x 6,5%) to be detected with 95% probability. If smaller reductions are to be detected, several samples have to be obtained on different occasions to obtain a more accurate assessment of the typical cholesterol level. For example, by using the mean of 2 (3) determinations it is possible to detect changes of 11 (8,5) % ($15/\sqrt{2}$ and $15/\sqrt{3}$, respectively).

It should be pointed out that the performance of the method, especially in the screening situation, is also profoundly influenced also by a possible systematic analytical error (bias, inaccuracy) which will primarily affect the number of individuals correctly assigned to intervention or non-intervention. The interaction between random and systematic errors in the screening situation is discussed in another contribution this volume (Hyltoft-Pedersen et al.).

COMMENTS

About 10 000 copies of the pamphlet have been distributed through the Central Clinical Chemistry Laboratories in Sweden. Judging from spontaneous comments from the target groups, the information is well received and contributes substantially to the improvement of laboratory procedures. Also, there has been considerable interest from physicians, indicating that it is important to present this type of information to all levels of medical personnel. Furthermore, some drug companies and manufacturers of analytical instruments have incorporated the pamphlet into their information with positive response from their customers.

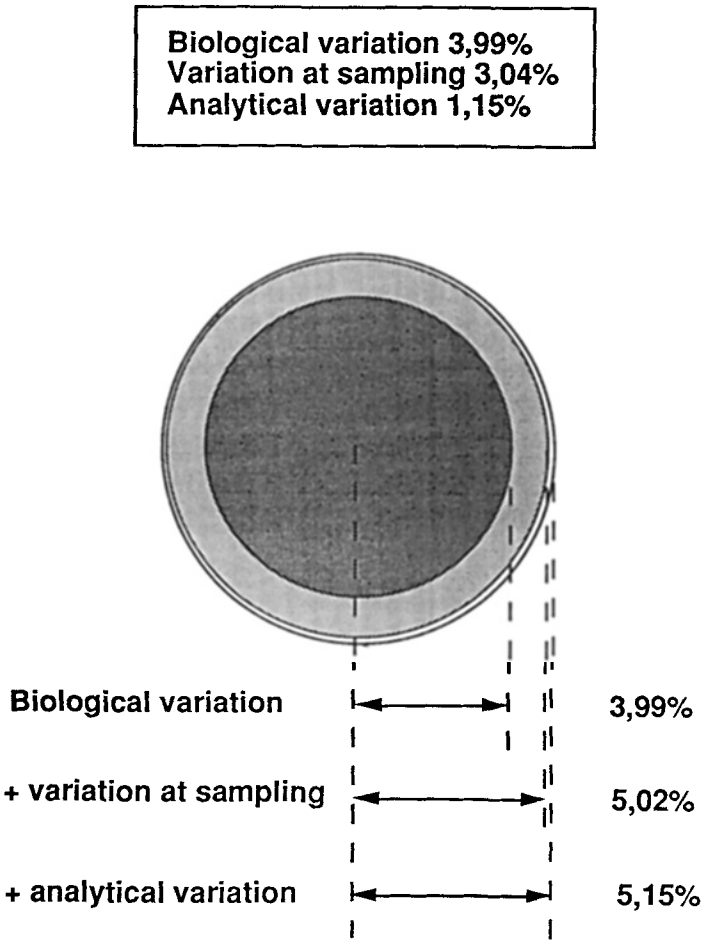

Figure 1. The contribution of biological variation, sampling error and analytical error to the total variation of a S-cholerol measurement carried out by trained personnel. The numbers have been calculated by addition of random variances, i e

$$(\text{total variation})^2 = (\text{biological variation})^2 + (\text{sampling error})^2 + (\text{analytical error})^2$$

REFERENCES:

1. Att mäta kolesterol. En handbok för mottagningar och laboratoriepersonal. Svensk Förening för klinisk kemi. Almqvist och Wiksell, 1990.

2. Nilsson-Ehle, P, Nordin G, Nilsson, J E Tryding N. 1989. Kolesterol - svårare att mäta än att sänka? Läkartidningen 14, 1263 - 1269.

3. Nilsson-Ehle P. 1990. Biological and analytical variation of the determination of serum cholesterol levels: implications for screening and patient monitoring. Submitted for publication.

Correspondence:
Peter Nilsson-Ehle, Associated professor,
Department of Clinical Chemistry
University Hospital
S-221 85 Lund, Sweden