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ANNOTATION

Assessing the efficacy of cholesterol-lowering treatment

All things biologic vary, and cholesterol is no exception. The measurement of cholesterol in a patient usually represents an attempt to estimate not a momentary value but a long-term average, because it is the long-term exposure to high concentrations of LDL-cholesterol that damages the arterial wall. Within any individual, however, cholesterol levels fluctuate around this average. In addition, laboratory procedures and variation in blood sampling technique introduce random errors. Even a single homogenous serum pool will yield a range of values if analysed repeatedly. This variability has serious implications for our ability to monitor the effectiveness of cholesterol-lowering treatment in individual patients.

The extent of variability is usually reported in terms of the standard deviation (SD). The formula for calculating the SD is such that for a series of measurements, 68% of the values will differ from the mean of the series by no more than one SD, and 95% will differ from the mean by at most twice the SD. Standard deviations for serum cholesterol in normolipidaemic men typically range from 0.3 to 0.6 mmol/l [1]. These include laboratory error. Careful standardization can reduce the random fluctuations within one laboratory down to a relative SD (ie, the SD divided by the mean cholesterol concentration) of less than 0.7% [2]. However, clinical practice lags behind these developments: in the USA a typical relative SD is 3.5% [2]. Of even more serious concern are the systematic differences (bias) between different laboratories. In a recent survey, 5000 laboratories in the USA produced values for a single serum pool that ranged from 4.7 to 9.8 mmol/l, and this after arbitrary removal of the 107 most extreme values, which ran from 2.6 to 13.5 mmol/l. The true value of the pool was 6.79 mmol/l [2].

Variability inhibits rational treatment

What does all this imply for the physician who attempts to assess the effectiveness of cholesterol-lowering therapy in a particular patient? Suppose that in a hypothetical patient, cholesterol is measured just once and is found to be 7.0 mmol/l. The patient is also found to have a high intake of saturated fat and cholesterol, and is therefore put on a diet that should lower cholesterol by, on average, 1.0 mmol/l (a fairly typical result in outpatient studies, even though larger falls can be achieved). One month later, another single cholesterol measurement is made in this hypothetical patient by the same laboratory, and the concentration is now 7.2 mmol/l. Does this mean that the patient has been complying poorly with the diet, or that he is a 'non-responder'? Not necessarily. Because cholesterol levels vary, the differences between consecutive cholesterol measurements will also fluctuate. If a patient is on a constant diet, *differences* between sequential cholesterol measurements will fluctuate around zero, with an SD equal to $\sqrt{2}$ times the SD of the cholesterol level. If the SD of the cholesterol level is 0.5 mmol/l — caused by a combination of within-patient and laboratory fluctuations — then the SD of the *difference* between any two values will be 0.7 mmol/l and so will the SD of any change in level caused by therapy. One can calculate a 95% confidence interval for the diet-induced change in cholesterol. It represents the range where the true effect will lie in 95% of cases, and it equals the observed change plus or minus twice its SD. In the example described above, therefore, the observed

change of +0.2 has a 95% confidence interval that reaches from minus 1.2 to plus 1.6 mmol/l. For 5% of patients the true effect will be even farther out. In other words, the efficacy of the treatment is totally indeterminate; the patient showing a rise of 0.2 mmol/l may actually have experienced an undetected fall of 1.2 mmol/l, or a rise of 1.6 mmol/l, or anything in between. All this presupposes that all measurements were made in one laboratory; use of different laboratories or methods could easily add a shift of another 0.5 mmol/l, caused by method bias alone.

How can this situation be remedied? One way would be to increase the number of measurements. If the number of cholesterol observations is increased to four before and four after the onset of treatment then one can replace a difference between two casual samples by a difference between the means of two series of four, and get a more reliable impression of the effect of treatment. However, the SD decreases only with the square root of the number of observations. As a result, the SD of the difference will now be 0.35 instead of 0.7, and any change in cholesterol will carry an uncertainty of 2 x 0.35 = 0.7 mmol/l —clearly still unacceptable if one is trying to pick up a change of 1.0 mmol/l. It is much more useful to improve patient preparation and laboratory quality. Posture is important — if the patient has been standing before venepuncture at one occasion and been lying down at the next, his cholesterol will have fallen by an average 10% simply through haemodilution [3]. Acute infections can lead to large changes in cholesterol [4] and necessitate postponement of the measurement. Good laboratory practice and stringent quality control are, of course, of the utmost importance; laboratories should provide data on their performance in blind external proficiency tests. Measures such as these may halve the standard deviation; if it were halved again by routinely using the mean of four cholesterol determinations instead of a single value, then the problem of assessing the true course of cholesterol would become manageable. The SD of the change in our hypothetical patient is now reduced from 0.7 to 0.17, and the 95% confidence interval will stretch 0.35 mmol/l on either side of the observed change. Thus if the patient has conscientiously followed a regimen that can lower his cholesterol by 1.0 mmol/l then one may confidently expect to observe a change of not less than 0.65 mmol/l and no more than 1.35 mmol/l. Unlike the original situation, any increase now raises a definite suspicion of poor adherence to diet.

Making multiple measurements

Obtaining multiple measurments is common practice in the diagnosis and treatment of hypertension — no physician will put a patient on hypotensive medication because of a single high blood pressure reading. However, adopting the same practice for cholesterol appears prohibitive because of the cost and burden to the patient. In the long run, dry-chemistry desk top analysers utilizing fingerprick blood may offer a way out. The most convenient of these systems utilizes whole blood, with no need for preparation of plasma; however, reproducibility is not yet as good as that of systems using plasma, and early versions produced cholesterol values that ran 10% low [5,6]. In the absence of proper quality control materials, it was some time before this problem was recognised. Progress is being made, but control materials are still lacking; whole blood is too unstable, plasma often contains EDTA, which interferes with the analysis [7], and serum does not present the same matrix as whole blood. Special quality control programmes and well-trained personnel will be needed if desk top analysers are to reach the precision and accuracy now attainable in the better clinical chemistry laboratories [2].

If multiple determinations are needed to characterise a subject's serum cholesterol, at what intervals should the samples be obtained? Rotterdam *et al* [8] showed that in volunteers bled repeatedly under semimetabolic ward conditions, the intraindividual standard deviation increased as a function of the time between successive blood samples, and reached a plateau after about four days. Thus the range of cholesterol or HDL values that exists within an individual was covered most efficiently by spacing measurments at least four days apart. Values obtained within 24 hours of each other were usually fairly similar; if one value lay above the true average, the chances were the other would too, and the average of the two was not much closer to the true mean than either measurement separately. Incidentally, the statistical techniques employed were originally developed for oil prospectors who wanted to know how far from a previous hole they should drill the next one; the problem of obtaining independent measurements at a minimum distance is the same.

Conclusion

The practice of relying on a single cholesterol measurement will often yield misleading results. Changes in cholesterol, or the lack of them, in response to treatment will often be due either to random fluctuations within the patients or to laboratory error, rather than to good or poor compliance and/or responsiveness. Rational treatment of hypercholesterolaemia requires the averaging of multiple measurements — ideally four before and four after the start of treatment. In addition, good patient preparation, and painstaking control of laboratory performance are of cardinal importance.

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Forthcoming Meetings

May 8-10, 1989; Milan, Italy
Biotechnology of Dyslipoproteinemias: Clinical Applications in Diagnosis and Control (BIOTECH Ria 89, Fondazione Giovanni Lorenzini, Via Monte Napoleone 23, 20121 Milan, Italy)

May 8-10, 1989; Göteborg, Sweden
Workshop on Coffee and Coronary Heart Disease with Special Emphasis on the Coffee-Blood Lipids Relationship (Prof Dag S Thelle, The Nordic School of Public Health, POB 12133, S-402 42 Göteborg, Sweden, Tel 46 31/693900. Closing date Feb 28, 1989. Cost 3000 SEK)

June 18-21, 1989; Milan, Italy
IV European Meeting on Hypertension
(Prof A Zanchetti, Chairman, Organizing Committee, Centro di Fisiologia Clinica e Ipertensione, Universita di Milano, Ospedale Maggiore, Via F Sforza, 35, 20122 Milan, Italy.)

July 23-28, 1989; Rio de Janeiro, Brazil
XIII Inter-American Congress of Cardiology
(Igor Borges Abrantes, Chairman, Organizing Committee, AV Paula Souza, 364 — CEP 20271, Rio, Brazil)

Aug 17-18, 1989; Linköping, Sweden
European Atherosclerosis Society - 25th Anniversary Meeting (Anders G Olsson, Organizer, Attention: Kit Burmeister, Department of Internal Medicine, University Hospital, S-58185 Linkoping, Sweden)

September 10-14, 1989; Nice, France
XI Congress of the European Society of Cardiology (Wil FJ Neijmann, Executive Director, ECCO, 22 Rue Juste O vinde, CH-1260 Nyon, France)

September 17-22, 1989; Rome, Italy
XV World Congress of the International Union of Angiology (Prof Antonio Strano, President of the Congress, Via Di Vigna Stelluti, 40-00191, Rome, Italy)

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