Accuracy of Cardiovascular Risk Estimation

To the Editor:

Middleton (1) describes the effect of analytical variation in high-sensitivity C-reactive protein and lipid assay on cardiovascular risk calculation, using a Monte Carlo simulation technique and the Ridker-Rifai quintile model, and demonstrates that relative risk is over- or underestimated in a substantial proportion of cases. From this he concludes that multiple HDL-cholesterol estimations may reduce misclassification that occurs because of assay imprecision. This is true, but this approach underestimates the imprecision of risk calculation.

The factor that is neglected in this investigation of the precision of risk estimation is the important role played by biological variation. We have recently published a similar analysis scenario in which we mathematically modeled a hypothetical “true” population derived from data from the National Health Survey for England. We investigated the effect of combined biological and analytical variation in total cholesterol and HDL-cholesterol, as well as blood pressure, on calculated risk and likely treatment decisions (2). Using the Framingham (1991) risk model (3) at the various internationally recommended 10-year coronary-heart-disease-risk treatment threshold concentrations of 15%, 20%, and 30%, the 95% confidence limits at these points were ±5.1%, ±6.0%, and ±6.9% for singlicate estimates; ±3.6%, ±4.2%, and ±4.9% for duplicate estimates; and ±2.8%, ±3.3%, ±3.9% for triplicate estimates, respectively (i.e., for singlicate 15% risk, 95% confidence interval is 9.9–20.1%). Consequently, using the UK 30% risk threshold and singlicate estimation, 30% of patients who should receive treatment would be denied it and 20% would receive treatment unnecessarily.

As implied by Middleton (1), the greatest problem arises in those closer to risk thresholds. This suggests that higher-risk thresholds [e.g., the 3%/year used in the UK vs the 2%/year used elsewhere (4, 5)] allow for greater precision by placing more individuals in the clearly lower-risk group, but the potential for misdiagnosis of patients close to higher thresholds is, in fact, greater because the confidence intervals are wider. In the group around the threshold value, multiple measurements improved precision, but the nature of the risk equation means that one cannot absolutely define risk in any individual. The risk equation is asymptotic with respect to the number of determinations, so that an infinite number of measurements are required to achieve perfect accuracy. It is clearly impossible, however, to test every patient 30 or more times before deciding on treatment, and a pragmatic screening policy must, therefore, be devised.

The usual statistical limit of confidence is 5%; therefore, it was decided that the optimum number of repetitions would be the point at which the decrease in false-positive and false-negative results at each step was <5%. This was achieved at nine repetitions, but again, testing each patient on nine separate occasions would be excessive for obviously low-risk cases (2). Because the detection limit and specificity of less than three repetitions were poor and because there is always the possibility of laboratory error, it follows that the minimum standard in very low- or very high-risk cases should be three repetitions. Logically, all patients whose risk estimates then lie within the 95% confidence interval of the risk threshold should continue to be tested until nine repetitions have been carried out. Because the 95% confidence interval is not a round number, however, it is more convenient in routine practice to use the risk cutoff ±5% (e.g., for a 15% cutoff, all results from 10% to 20%), which would effectively serve the same function, and then apply clinical judgement on whom to treat.

It has been demonstrated that 46% of first cardiovascular events in women occur in patients with LDL-cholesterol below the National Cholesterol Education Program decision limit of 1300 mg/L (3.36 mmol/L) (6). The baseline data on which risk calculators are based suffer the same problems of variation that affect the interpretation of patient data. The failure of decision thresholds to correctly identify patients and the implications of both of these studies (1, 2) demonstrate that it is essential that mathematical properties of cardiovascular risk calculators are investigated before public policy decisions are made to introduce such models for the identification of high-risk individuals requiring treatment. Secondly, any results from such calculators need to cite the confidence interval for the risk estimate to allow for informed decision-making.

References


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Detection of a Novel 1905C→T Mutation within the Dihydropyrimidine Dehydrogenase Gene and Potential for Misclassification with the Exon 14-Skipping Mutation

To the Editor:

Dihydropyrimidine dehydrogenase (DPYD) is the initial and rate-limiting enzyme in the metabolism of the chemotherapeutic drug 5-fluorouracil (5-FU), thus affecting its pharmacokinetics, efficacy, and toxicity (1). Application of 5-FU is restricted by a narrow therapeutic index because of severe toxicity of WHO grades III–IV (2). Polymorphisms within the DPYD gene have been reported, with deficiency in enzyme activity leading to severe 5-FU-related toxicity in cancer patients (3). The so-called exon 14-skipping mutation at the 5'-splice donor site of exon 14 (1905 +1G→A) has been detected in ~25% of affected patients (4). To identify patients at increased risk for severe 5-FU-induced toxicity, many medical centers routinely screen for the exon 14-skipping mutation before starting chemotherapy.

We directly compared DNA sequencing with a fluorescence-based technology, namely, the LightCycler. For this purpose, we used genomic DNA from a homozygous wild-type individual (sample A) and two DNA samples from persons believed to be heterozygous for the exon 14-skipping mutation from earlier LightCycler analyses (samples B and C). The LightCycler method was used as described previously (5) except that the transition from the annealing phase to the elongation phase was 5 °C/s (amplification was not always successful at a temperature transition rate of 20 °C/s). PCR conditions of the direct sequencing method are available from the authors on request.

Melting curve analysis of the homozygous sample A revealed a single peak at 60.5 °C in accordance with published data (5) (Fig. 1). The result has been confirmed by direct sequencing.

The melting curve profiles of samples B and C both exhibited a melting point at 60.5 °C for the wild-type allele. However, the lower melting temperature peaks were different (55.1 and 51.7 °C), indicating two different sequence alterations (6). Direct sequencing confirmed that sample C was heterozygous for the exon 14-skipping mutation. However, direct sequencing of sample B did not reveal the expected 1905 +1G→A polymorphism but a novel 1905C→T mutation 1 bp away from the guanidine of the 5'-splice donor site. According to our knowledge of splicing mechanisms to date, the 1905C→T nucleotide substitution presumably does not affect RNA processing and is supposed to be silent (7).

Melting curve profiles may be influenced by several factors, such as the concentrations of fluorophores, the rate of temperature transition during final denaturation, initial copy number, and product yield (8). The mentioned conditions were equal for all samples. Nonetheless, unequal amplification efficiency for both strands cannot always be ruled out.

The data demonstrate shortcomings in the unambiguous identification of mutations with hybridization probe methods on the basis of published melting curve profiles (5) and calculations based on the nearest-neighbor method (9). Rather, our data emphasize the use of external standards verified by direct sequencing during the validation process. Increased attention is required for detection of the exon 14-skipping mutation of the DPYD gene in routine diagnostics because of at least one existing nearby polymorphism.

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Fig. 1. Derivative melting curve plots of samples A–C obtained by LightCycler analysis of the exon 14-skipping mutation according to Nauck et al. (5).

Data are shown for sample A ( — ), believed to be homozygous for the wild-type allele, and for samples B (— — —) and C ( — — ), believed to be heterozygous for 1905 +1G→A from previous LightCycler analysis. A no-template control was performed as a negative control ( — — ). Melting points were calculated by MeltCalc software (9) as 61.4 and 55.6 °C for the 1905 +1G→A polymorphism and 61.4 and 52.5 °C for the 1905C→T mutation.