Are our HDL-C methods fit for purpose?

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Introduction

The term HDL-Cholesterol (HDL-C) is used to describe a range of complex lipoproteins composed of lipids and proteins in different proportions, varying in size, composition and function. HDL-C particles facilitate a process known as reverse cholesterol transport, by which they transport cholesterol from peripheral tissues to the liver, where the cholesterol is excreted in bile and faeces. This process is considered to be anti-atherogenic and there is overwhelming evidence which shows an inverse relationship between serum HDL-C concentrations and the risk of cardiovascular disease (CVD).

The main clinical application of lipid measurement is in the management of CVD, both to calculate CVD risk and low density lipoprotein cholesterol (LDL-C) concentrations. The NICE Lipid Modification guidelines (CG67) recommend that either Framingham, QRISK or ASSIGN risk calculations can be used to calculate CVD risk.[1] A meta analysis of randomised controlled trials showed that for every 0.026 mmol/L increase in HDL-C concentration there is a 3% reduction in CVD events in women and 2% reduction in men. A companion poster is also presented which investigates the effect of HDL-C on the Framingham and QRISK equations further.

The reference method for HDL-C uses ultracentrifugation, followed by precipitation with a heparin-manganese reagent and quantification with the Abell-Kendell reference method for cholesterol. Historically, precipitation based methods have been used to separate lipoproteins. Around the year 2000, these methods were deemed to not be suitable for the high and increasing workloads of HDL-C that were and are currently being seen in clinical biochemistry laboratories. As such new methods were developed which could easily be automated, do not require any off-line pre-treatment and separation, with analysis being undertaken in a single cuvette. These are known as homogeneous assays. Non HDL-C particles are blocked without precipitation and the cholesterol content is determined using enzymatic reagents. There are many different ways of blocking non HDL-C lipoprotein fractions, and each manufacturer employs different practices.

There are also large inter-individual variations in HDL-C composition which is particularly relevant in different disease states, and previous publications have shown methodological discrepancies in these situations.[2]

Methods

Audit of monthly patient means of HDL-Cholesterol

The Laboratory Information Management Systems (LIMS) – Telepath – were interrogated for data relating to HDL-C requests from 1st January 1999 to 31st December 2009 for both Birmingham Heartlands Hospital and Good Hope Hospital. An identical search was carried out using the LIMS at Ninewells Hospital. All HDL-C requests were included in the search criteria. Data was then sorted to calculate a monthly patient mean. A Pearson Correlation of the HDL-C between the three hospitals was used to test the significances between the results.

For the majority of the time audited, HDL-C analysis was undertaken using a direct HDL-C assay on the Roche Modular at all three hospitals. Three generation HDL-C assays were supplied by Roche during this time – generation 1 (2002-2005), generation 2 (2005-2007) and generation 3 (2007-current). Prior to 2002, both Birmingham Heartlands Hospital and Good Hope Hospital used different precipitation methods.

UK NEQAS Survey

Nine single collections of fresh human serum were distributed to laboratories in the UK as part of the UK NEQAS for Lipid Investigations Scheme (Distributions 105X, 109X and 111 X) over a six month time period. Both non-diseased and diseased (diagnosed lipid disorders or on lipid lowering medication) subjects were chosen to cover a range of HDL-C concentrations. All subjects fasted overnight (except 111D and 111F) before blood was collected using standard phlebotomy techniques into a sterile 500 mL dry transfer pack with no additives. The blood was anonymised at the point of collection. Serum was obtained within four hours of blood collection. Approximately 600 μL of the serum was aliquoted into screw-top vials and three aliquots were sent at each distribution by first class post to 158 UK participants (Distribution 105X) and 130 UK participants (Distributions 109X and 111X). Participants were advised to analyse the samples upon receipt and not to refrigerate or freeze samples. Samples were also sent to the CDC laboratory in Atlanta for measurement by the reference method (β-Quantification). These samples were batched and sent frozen after distributions 111X, therefore subjected to at least one freeze-thaw cycle.

Results

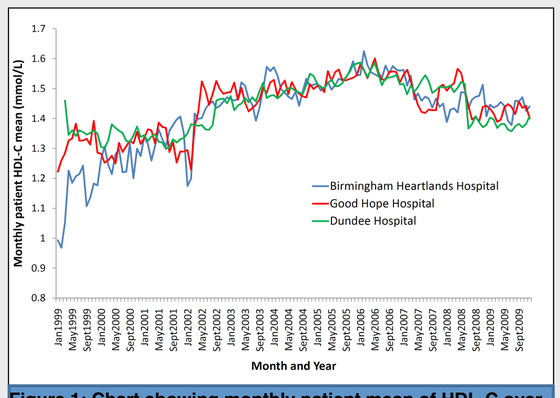

Figure 1: Chart showing monthly patient mean of HDL-C over the ten year period 1st January 1999 to 31st December 2009 All three laboratories used Roche Modular HDL-C reagents from approximately 2000 onwards

The monthly patient mean for HDL-C for all three hospitals is shown in Figure 1. All three laboratories show a similar phenomenon of an increase in monthly patient mean HDL-C, and a Pearson correlation of the three data sets show them not to be significantly different ($p<0.0001$) (Pearson $r=0.84$, Good Hope and Birmingham Heartlands), ($r=0.83$, Good Hope and Ninewells) and ($r=0.74$, Birmingham Heartlands and Ninewells).

There was a steady increase in the % of patients with an HDL-C concentration of ≥ 2.0 mmol/L, 3.4% (1999) to 15.9% (2006) – Birmingham Heartlands, 5.2% (1999) to 13.4% (2006) – Good Hope and 6.5% (2001) to 16.6% (2004) – Ninewells. HDL-C was normally distributed throughout this time period.

UK NEQAS Survey

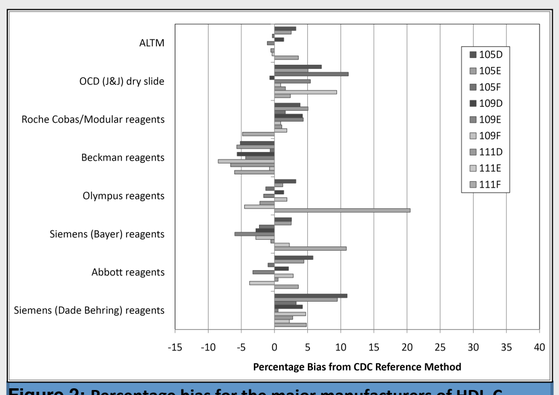

Figure 2: Percentage bias for the major manufacturers of HDL-C assays compared to the CDC reference method in Atlanta, for three EQA distributions 105X, 109X and 111X. (ALTM = All Laboratory Trimmed Mean)
Reference method HDL-C values for each specimen are as follows 105D = 1.55 mmol/L, 105E = 1.58 mmol/L, 105F = 3.05 mmol/L, 109D = 1.42 mmol/L, 109E = 1.84 mmol/L, 109F = 1.06 mmol/L, 111D = 1.81 mmol/L, 111E = 2.66 mmol/L and 111F = 0.83 mmol/L

Figure 2 shows a % bias plot of the HDL-C for the major manufacturers and ALTM (all laboratory trimmed mean) compared to the CDC reference method for fresh individual donations of human serum. The CDC reference results are very comparable to the ALTM and are not statistically significant (t-test $p=0.2731$). However, OCD (J&J) dry slide and Siemens (Dade Behring) reagents show a positive bias on all samples with statistically significant results when method means are compared with the reference results (t-test $p=0.0327$ and $p=0.039$ respectively), whereas Beckman reagents show a negative bias on all samples (t-test $p=<0.0001$).

Discussion & Conclusion

A gradual increase in patient mean HDL-C concentration over a decade was observed by three independent laboratories – (38% at Birmingham Heartlands Hospital, 24% for Good Hope Hospital and 21% for Ninewells Hospital). There was an increase in the proportion of female patients being tested in 2009 from 45% to 49% at Birmingham Heartlands Hospital. The mean age of the patient changed from 56.7 to 59.5 years (female) and 55.8 to 58.2 years (male). We do not consider it clinically plausible that a patient mean should increase to this extent over the past ten years as past/current medication or lifestyle changes alone would not cause this. If anything the prevalence of obesity has increased over the last decade and though niacin is the most potent HDL-C raising drug (up to 30%); however, this is not well tolerated and statins are more commonly used. These only increase HDL-C by 5-10% and would not account for the observed rise in HDL-C. The UK NEQAS study has shown that the majority of current HDL-C methods are showing results that are comparable to the CDC reference method. However, three out of the seven methods showed statistically different results when comparing method mean to the reference method. One important point to note is that the samples analysed by the reference method were subjected to at least one freeze thaw cycle. It has previously been reported by Cramb et al. that freeze thaw cycles can increase the HDL-C concentration on pooled serum samples.[3] This would apply to controls, calibrators and EQA materials. There are many implications of introducing a bias into HDL-C results, not only in the interpretation of HDL-C itself but it is used as a denominator in CVD risk equations, therefore any small change in HDL-C will have a large impact on the CVD risk, which is a significant issue with regards to NHS health check programs. Point of care devices are increasingly being used but have not been included here.

Our findings are supportive of previous work and show lipid EQA results for the first time from a single human donation.

Declaration & Acknowledgements

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