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# The effect of adjusting LDL-cholesterol for Lp(a)-cholesterol on the diagnosis of familial hypercholesterolaemia

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**Background:** Familial hypercholesterolaemia (FH) diagnostic tools help prioritise patients for genetic testing and include LDL-C estimates commonly calculated using the Friedewald equation. However, cholesterol contributions from lipoprotein(a) (Lp(a)) can overestimate 'true' LDL-C, leading to potentially inappropriate clinical FH diagnosis.

**Objective:** To assess how adjusting LDL-C for Lp(a)-cholesterol affects FH diagnoses using Simon Broome (SB) and Dutch Lipid Clinic Network (DLCN) criteria.

**Methods:** Adults referred to a tertiary lipid clinic in London, UK were included if they had undergone FH genetic testing based on SB or DLCN criteria. LDL-C was adjusted for Lp(a)-cholesterol using estimated cholesterol contents of 17.3%, 30% and 45%, and the effects of these adjustments on reclassification to 'unlikely' FH and diagnostic accuracy were determined.

**Results:** Depending on the estimated cholesterol content applied, LDL-C adjustment reclassified 8-23% and 6-17% of patients to 'unlikely' FH using SB and DLCN criteria, respectively. The highest reclassification rates were observed following 45% adjustment in mutation-negative patients with higher Lp(a) levels. This led to an improvement in diagnostic accuracy (46% to 57% with SB, and 32% to 44% with DLCN following 45% adjustment) through increased specificity. However all adjustment factors led to erroneous reclassification of mutation-positive patients to 'unlikely' FH.

**Conclusion:** LDL-C adjustment for Lp(a)-cholesterol improves the accuracy of clinical FH diagnostic tools. Adopting this approach would reduce unnecessary genetic testing but also incorrectly reclassify mutation-positive patients. Health economic analysis is needed to balance the risks of over- and under-diagnosis before LDL-C adjustments for Lp(a) can be recommended.

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## Introduction

Familial hypercholesterolaemia (FH) is a common monogenic disorder of low-density-lipoprotein cholesterol (LDL-C) associated with premature cardiovascular disease (CVD).[1] Untreated, the cumulative incidence of ischaemic

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heart disease in men and women is 50% by age 50, and 60% by age 60, respectively.[2] The 2013 consensus panel statement from the European Atherosclerosis Society highlights that FH is underdiagnosed and undertreated, and a 'call to arms' was recently issued to address screening, diagnosis, and treatment, supported by development of a global FH registry: the European Atherosclerosis Society FH Studies Collaboration.[3,4]

Tools commonly used to aid FH diagnosis include the Dutch Lipid Clinic Network (DLCN) and Simon Broome (SB) criteria. Using these criteria, patients are classified as 'unlikely', 'possible', 'probable' (DLCN-only), or 'definite' FH according to lipid levels, clinical signs, and family or personal history of CVD or dyslipidaemia. These have long been used to make a clinical diagnosis of FH but, in the era of widespread molecular diagnostics, also serve to prioritise patients for genetic testing.[5] Currently, mutation detection rates among patients with a clinical diagnosis of FH vary from 15-50%.[6] This suggests the presence of either unidentified monogenic FH-causing variants or, more likely, polygenic sources of hypercholesterolaemia.

The serum LDL-C concentration is a major component of all FH screening tools and is commonly estimated using the Friedewald equation.[7] However, it is under-appreciated that LDL-C estimation includes a contribution from the cholesterol-rich molecule lipoprotein (a) (Lp(a)), an atherogenic and prothrombotic molecule often measured as part of FH assessment to allow more accurate risk stratification.[8,9] Plasma levels of Lp(a) are genetically determined and vary considerably between individuals in an inverse relationship relative to apo(a) isoform size,[10,11] so patients with very high Lp(a) concentrations may erroneously be thought to have FH as their Lp(a)-associated cholesterol mass leads to overestimation of the "true" LDL-C levels. Of note, it has traditionally been estimated that 30-45% of the molecular mass of mature Lp(a) is cholesterol,[12,13] but a recent study suggested a median value of 17.3%, with wide variability between individuals ranging from 5.8 to 57.6%.[14] This lower median estimate may partly reflect methodological differences, as the earlier papers measured both esterified and unesterified cholesterol but the paper by Yeang *et al.* included only the unesterified form. As cholesterol assays used in clinical laboratories are calibrated against unesterified cholesterol, this lower estimate may in fact be more appropriate.

In addition to monogenic FH, a large number of genomic variants can have individually small but cumulatively large effects on LDL-C levels. This has led to the development of several polygenic risk scores (PRS) derived from LDL-C-raising single nucleotide polymorphisms (SNPs) identified by the Global Lipid Genetics Consortium.[15–17] These scores may be used to identify the likelihood of polygenic hypercholesterolaemia in patients with an FH phenotype but no identifiable mutation. This has important implications for management given that cardiovascular risk remains high in patients with polygenic hypercholesterolaemia, albeit to a lesser extent than monogenic FH[18] and when compared to cardiovascular risk conferred by non-LDL-based cardiovas-

cular PRS.[19] Of note, apo(a)-encoding *LPA* risk genotypes are more frequent among patients with clinical FH, independent of FH-causing mutations.[20]

Recent studies have investigated Lp(a) measurement as a means to improve accuracy of FH clinical diagnosis, and have shown that LDL-C adjustment using traditional Lp(a) cholesterol content estimates (e.g. 30%) can lead to individuals being reclassified from FH to unlikely FH. In these studies, the reclassification rate varied from 8% to 25%.[20,21] To build on these recent studies, we present results from our analysis of the contribution of Lp(a) to FH diagnosis in a tertiary lipid clinic in London, UK, using a range of adjustment factors to account for more recent estimates of Lp(a) cholesterol content. We have additionally assessed how this effect can be modulated by LDL-C PRS.

## Materials and methods

A retrospective analysis was undertaken of patients aged ≥18 years referred to a tertiary lipid clinic at Hammersmith Hospital, London, UK. Patients were included if they had a clinical diagnosis of at least 'possible' FH using modified SB or DLCN criteria and had undergone both Lp(a) measurement and FH molecular genetic testing. In our study the SB criteria were modified to exclude total cholesterol and DNA testing results as qualifying parameters, referred to here as "SB[LDL-C]" criteria to reflect the use of LDL-C levels only as the biochemical qualifying parameter, in addition to standard clinical qualifying parameters, i.e. family history and tendon xanthomata, which were still included.[22] Using SB[LDL-C] criteria, patients were classified as 'unlikely', 'possible', or 'definite' FH, with possible/definite defined as screen positive. Using DLCN criteria, patients were classified as 'unlikely', 'possible', 'probable', or 'definite' FH, with possible/probable/definite FH (DLCN score ≥3) defined as screen positive. Additional patients who did not meet SB[LDL-C] or DLCN criteria for FH diagnosis, but who had undergone FH genetic testing at the discretion of managing clinicians, were included in screening test accuracy calculations. Patients referred for cascade screening were not included in our analysis.

Biochemical tests were performed using routine assays at the North West London Pathology Blood Sciences laboratories, with all assays registered and monitored for quality assurance through the UK National External Quality Assessment Service (UK NEQAS) scheme. Lipid profile, including total cholesterol (TC), HDL-cholesterol, and triglycerides, were measured using Abbott Architect or Alinity analysers; LDL-C was estimated using the Friedewald equation.[7] When multiple lipid profiles were available, the highest LDL-C level was used for analysis. Prior to April 2018, serum Lp(a) was measured using the IMMAGE Beckman Coulter assay, calibrated in mg/L, associated with a within-run precision of ≤5%. From April 2018, serum Lp(a) was measured using the Randox assay, calibrated in mg/L and nmol/L, the lat-

ter of which is traceable to the WHO/IFCC reference material (IFCC SRM 2B). This methodological change was implemented to improve the performance of Lp(a) measurement as the Randox Lp(a) assay uses Denka based calibration to minimise apo(a) isoform size-related variations. This assay was associated with a within run precision of ≤2.54%. Our initial analysis only included patients with serum Lp(a) measurements using IMMAGE assay (mg/L). We subsequently replicated our analysis for patients with serum Lp(a) measurements using Randox assay (nmol/L). For combined analyses, we opted for the mass unit, mg/L (using an assay-specific conversion factor of 5 mg/nmol), as both IMMAGE and Randox assays are calibrated in this measurement. When multiple Lp(a) measurements were available we used the latest value to reflect the higher performance of Randox Lp(a) assay adopted later in the study period.

Genetic testing for the four gene panel available at the time (*LDLR, APOB, PCSK9 and LDLRAP1*) was performed at the Bristol Genetics Laboratory, UK, using next generation sequencing (NGS). Patients with a pathogenic FH-causing mutation were defined as 'confirmed' FH (mutation positive (M+)) while patients without a pathogenic FH mutation, or with a variant of uncertain clinical significance (VUS), were defined as 'not confirmed' FH (M-). A PRS was also reported for M- patients using the LDL-C PRS developed by Talmud *et al.*[15] This stratified patients into high, intermediate, or low likelihood of having polygenic hypercholesterolaemia. PRS were not reported for M+ patients.

LDL-C was adjusted for Lp(a) using estimated Lp(a) cholesterol contents of 17.3%, 30% and 45%[12-14] using the following formula: adjusted LDL-C = LDL-C (mmol/L) – [Lp(a) (mg/L) x 0.002586 × 0.173 (or 0.30, or 0.45)], where 0.002586 is the conversion factor for LDL-C from mg/L to mmol/L.[23] Other adjustment factors were applied using the same approach. Patients were assessed using SB$^{LDL-C}$ and DLCN criteria before and after LDL-C adjustment. Patients classified as 'unlikely' FH at baseline for a given diagnostic tool were not included in reclassification analysis for that tool. Data analysis was performed on the whole cohort and separately for IMMAGE and Randox assay cohorts using R v3.6.2.[24] Data were analysed according to Lp(a) subgroups: <300 mg/L, 300-500 mg/L, >500-1000 mg/L and >1000 mg/L, representing groups of increasing cardiovascular risk.[25]

Between-assay demographic data were compared using Fisher's Exact, Chi-squared, and Wilcoxon tests; TC and LDL-C were considered non-parametric data for these comparisons. Correlation analyses were performed using Spearman's rank test. Reclassification rates to 'unlikely' FH were assessed among M+ and M- patients and compared using the Fisher's Exact Test. Statistical significance for all comparisons was defined as *p*-value <0.05. Diagnostic tool sensitivity, specificity, and accuracy were compared before and after LDL-C adjustment, using FH molecular genetic results as the gold standard. Figures were generated using R and Prism v9.4.0 (Graphpad Software).

## Results

### Patient characteristics

513 patients meeting SB$^{LDL-C}$ or DLCN FH criteria of at least 'possible' FH were included in this study, of whom 239 and 274 had Lp(a) levels measured using the IMMAGE and Randox assays, respectively. There were no significant differences in patient characteristics between assay subgroups with the exception of Lp(a) levels (after unit interconversion; see Table 1). Therefore, all subsequent results are presented from combined IMMAGE and Randox cohorts, with assay-specific cohort results presented separately in supplementary material.

The median (IQR) age of the combined cohort was 49 (40-57) years with 57.1% female. Median (IQR) TC, unadjusted LDL-C, and Lp(a) were 8.3 (7.8-9.1) mmol/L, 5.9 (5.4-6.6) mmol/L, and 288 (108-755) mg/L, respectively. FH mutations were identified in 21.1% of patients (M+). Of M- patients, 51.6%, 23.7%, and 19.3% had a high, intermediate, or low PRS for hypercholesterolemia, respectively. Before adjustment, most patients had a 'possible' clinical FH diagnosis (66.7% and 67.8% using SB$^{LDL-C}$ and DLCN criteria, respectively).

### LDL-C adjustment

A weak positive correlation was observed between unadjusted LDL-C and Lp(a) concentration (Supplementary Figure 1A, R=0.15, *p*=0.00048). However, this was not significant in the IMMAGE assay cohort (Supplementary Figure 1B, R=0.084, *p*=0.20). As our clinical assay does not provide individualised measurement of Lp(a)-associated cholesterol, we used estimates of 17.3%, 30% and 45% Lp(a) cholesterol mass, based on published literature, to adjust LDL-C. Across the whole cohort, median LDL-C levels reduced by 3.4% (5.9 to 5.7 mmol/L) to 8.5% (5.9 to 5.4 mmol/L) when the lower and higher Lp(a) cholesterol estimates were used, respectively. Unsurprisingly, higher Lp(a) concentrations were associated with larger LDL-C adjustments, e.g. following 45% adjustment, median LDL-C levels were reduced by 29% (6.3 to 4.5 mmol/L) in patients with Lp(a) >1000 mg/L, compared to just 1.7% (5.8 to 5.7 mmol/L) following 17.3% adjustment in patients with <300 mg/L. Similar reductions were seen with the IMMAGE and Randox assay subgroups (Supplementary Table 1).

### Reclassification rates: effect of adjustment factors

We wanted to determine the impact of adjusting LDL-C for Lp(a) on FH clinical category, as reclassification to 'unlikely' FH could lead to a genetic test not being performed. For patients meeting 'possible' or 'definite' SB$^{LDL-C}$ criteria, 7.7%, 15.1% and 22.8% were reclassified to 'unlikely' FH following 17.3%, 30% and 45% Lp(a) adjustment, respectively (Table 2). As expected, patients were more likely to be reclassified when they had higher Lp(a) levels and

**Table 1** Patient characteristics by Lp(a) assay.

| | IMMAGE assay (mg/L) (n= 239) | Randox assay (nmol/L) (n= 274) | All patients (n= 513) | *p*-value |
|---|---|---|---|---|
| | | Characteristics | | |
| F sex n/N (%) | 141/239 (59.0) | 152/274 (55.5) | 293/513 (57.1) | 0.47[b] |
| Age at 1st clinic, median (IQR) | 49 [39 - 56] | 49 [41 - 58] | 49 [40 -57] | 0.27[c] |
| Ethnicity n/N (%) | | | | |
| White | 122/239 (51.0) | 126/274 (46.0) | 248/513 (48.3) | |
| Black | 13/239 (5.4) | 7/274 (2.6) | 20/513 (3.9) | 0.06[d] |
| Asian | 23/239 (9.6) | 21/274 (7.7) | 44/513 (8.6) | |
| Mixed | 4/239 (1.7) | 2/274 (0.7) | 6/513 (1.2) | |
| Other | 20/239 (8.4) | 21/274 (7.7) | 41/513 (8.0) | |
| Unknown | 57/239 (23.8) | 97/274 (35.4) | 154/513 (30.0) | |
| | | Biochemistry, *median (IQR)* | | |
| TC (mmol/L) | 8.4 [7.9 - 9.2] | 8.2 [7.8 - 9.1] | 8.3 [7.8 - 9.1] | 0.29[c] |
| LDL-C (mmol/L) | 6.0 [5.5 - 6.6] | 5.8 [5.4 - 6.6] | 5.9 [5.4 - 6.6] | 0.08[c] |
| Lp(a) | | | | |
| mg/L | 365 [122 - 862] | 210 [100 - 632][a] | 288 [108 - 755] | 0.01[c] |
| nmol/L | 73 [24.4 – 172.4][a] | 42 [20 - 126.4] | 57.6 [21.6 -151] | |
| | | FH Mutation status, n/N (%) | | |
| Mutation + (M+) | 48/239 (20.1) | 60/274 (21.9) | 108/513 (21.1) | 0.66[b] |
| Mutation – (M-) | 191/239 (79.9) | 214/274 (78.1) | 405/513 (78.9) | |
| *VUS* | 13/191 (6.8) | 8/214 (3.7) | 21/405 (5.2) | |
| LDLR | 6/13 (46.2) | 4/8 (50.0) | 10/21 (47.6) | 1.00[b] |
| APOB | 5/13 (38.5) | 3/8 (37.5) | 8/21 (38.1) | |
| PCSK9 | 2/13 (15.4) | 1/8 (12.5) | 3/21 (14.3) | |
| | | Polygenic Risk Score (M- only), n/N (%) | | |
| High likelihood | 94/191 (49.2) | 115/214 (53.7) | 209/405 (51.6) | 0.83[d] |
| Intermediate likelihood | 43/191 (22.5) | 53/214 (24.8) | 96/405 (23.7) | |
| Low likelihood | 38/191 (19.9) | 40/214 (18.7) | 78/405 (19.3) | |
| NA | 16/191 (8.4) | 6/214 (2.8) | 22/405 (5.4) | |
| | | FH category, n/N (%) | | |
| *Simon Broome* | | | | |
| Definite | 15/239 (6.3) | 20/274 (7.3) | 35/513 (6.8) | 0.81[d] |
| Possible | 158/239 (66.1) | 184/274 (67.2) | 342/513 (66.7) | |
| Unlikely | 66/239 (27.6) | 70/274 (25.5) | 136/513 (26.5) | |
| *Dutch Lipid Clinic Network* | | | | |
| Definite | 22/239 (9.2) | 33/274 (12.0) | 55/513 (10.7) | 0.43[d] |
| Probable | 55/239 (23.0) | 49/274 (17.9) | 104/513 (20.3) | |
| Possible | 159/239 (66.5) | 189/274 (69.0) | 348/513 (67.8) | |
| Unlikely | 3/239 (1.3) | 3/274 (1.1) | 6/513 (1.2) | |
| | | Treatment status, n/N (%) | | |
| Pre- treatment | 197/239 (82.4) | 234/274 (85.4) | 431/513 (84.0) | 0.06[d] |
| On treatment | 27/239 (11.3) | 34/274 (12.4) | 61/513 (11.9) | |
| Unknown | 15/239 (6.3) | 6/274 (2.2) | 21/513 (4.1) | |

[a]Converted value using ratio 5 mg/L: 1 nmol/L Statistical comparison between IMMAGE and Randox assay subgroups using [b]Fisher's exact test, [c]Wilcoxon signed-rank test and [d]Chi-squared test.

when higher adjustment factors were applied, e.g. for patients with Lp(a) >1000 mg/L, 64.7% were reclassified using the 45% adjustment, compared to 1% following 17.3% adjustment in patients with Lp(a) <300 mg/L. Similar trends were observed using DLCN criteria (Table 2), albeit with a smaller proportion of patients reclassified as 'unlikely', which reached statistical significance using the 45% adjustment factor only. Results were similar when the IMMAGE

and Randox subgroups were analysed separately (Supplementary Tables 2-3).

It is important to know the eventual genetic status of patients reclassified as 'unlikely' FH – reclassification of M- patients is 'appropriate' as it could reduce unnecessary genetic testing, whereas M+ patient reclassification is 'inappropriate' as it could lead to a missed FH diagnosis. Using SB[LDL-C] criteria, a greater proportion of M- than M+ pa-

**Table 2** Reclassification rates to 'unlikely' FH using SB[LDL-C] and DLCN criteria after adjusting for Lp(a)-cholesterol content, assuming either 17.3%, 30% or 45% mass.

| Lp(a) concentration (mg/L) | Before adjustment | | | Reclassified using 17.3% adjustment | | | | Reclassified using 30% adjustment | | | | Reclassified using 45% adjustment | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lp(a) concentration (mg/L) | All | M+ | M- | All | M+ | M- | p-value (M- vs M+) | All | M+ | M- | p-value (M- vs M+) | All | M+ | M- | p-value (M- vs M+) |
| SB[LDL-C] criteria (n= 377, n/N (%)) | | | | | | | | | | | | | | | |
| <300 | 199 | 42 | 157 | 2/199 (1.0) | 0/42 (0.0) | 2/157 (1.3) | 1.00 | 8/199 (4.0) | 0/42 (0.0) | 8/157 (5.1) | 0.21 | 13/199 (6.5) | 0/42 (0.0) | 13/157 (8.3) | 0.07 |
| 300-500 | 46 | 16 | 30 | 2/46 (4.3) | 0/16 (0.0) | 2/30 (6.7) | 0.53 | 4/46 (8.7) | 1/16 (6.3) | 3/30 (10.0) | 1.00 | 6/46 (13.0) | 1/16 (6.3) | 5/30 (16.7) | 0.65 |
| >500-1000 | 64 | 15 | 49 | 10/64 (15.6) | 2/15 (13.3) | 8/49 (16.3) | 1.00 | 15/64 (23.4) | 3/15 (20.0) | 12/49 (24.5) | 1.00 | 23/64 (35.9) | 4/15 (26.7) | 19/49 (38.8) | 0.54 |
| >1000 | 68 | 12 | 56 | 15/68 (22.1) | 1/12 (8.3) | 14/56 (25.0) | 0.28 | 30/68 (44.1) | 3/12 (25.0) | 27/56 (48.2) | 0.20 | 44/68 (64.7) | 5/12 (41.7) | 39/56 (69.6) | 0.10 |
| Overall reclassification rate to 'unlikely' | | | | 29/377 (7.7) | 3/85 (3.5) | 26/292 (8.9) | 0.11 | 57/377 (15.1) | 7/85 (8.2) | 50/292 (17.1) | 0.06 | 86/377 (22.8) | 10/85 (11.8) | 76/292 (26.0) | 0.01 |
| DLCN criteria (n= 507, n/N (%)) | | | | | | | | | | | | | | | |
| <300 | 259 | 51 | 208 | 5/259 (1.9) | 0/51 (0.0) | 5/208 (2.4) | 0.59 | 8/259 (3.1) | 0/51 (0.0) | 8/208 (3.8) | 0.36 | 12/259 (4.6) | 1/51 (2.0) | 11/208 (5.3) | 0.47 |
| 300-500 | 71 | 21 | 50 | 2/71 (2.8) | 0/21 (0.0) | 2/50 (4.0) | 1.00 | 2/71 (2.8) | 0/21 (0.0) | 2/50 (4.0) | 1.00 | 5/71 (7.0) | 0/21 (0.0) | 5/50 (10.0) | 0.31 |
| >500-1000 | 86 | 21 | 65 | 10/86 (11.6) | 1/21 (4.8) | 9/65 (13.8) | 0.44 | 15/86 (17.4) | 1/21 (4.8) | 14/65 (21.5) | 0.10 | 19/86 (22.1) | 2/21 (9.5) | 17/65 (26.2) | 0.14 |
| >1000 | 91 | 15 | 76 | 12/91 (13.2) | 0/15 (0.0) | 12/76 (15.8) | 0.20 | 30/91 (33.0) | 3/15 (20.0) | 27/76 (35.5) | 0.37 | 49/91 (53.8) | 4/15 (26.7) | 45/76 (59.2) | 0.03 |
| Overall reclassification rate to 'unlikely' | | | | 29/507 (5.7) | 1/108 (0.9) | 28/399 (7.0) | 0.01 | 55/507 (10.8) | 4/108 (3.7) | 51/399 (12.8) | 0.01 | 85/507 (16.8) | 7/108 (6.5) | 78/399 (19.5) | <0.01 |
| p-value (SB[LDL-C] vs DLCN) | | | | 0.27 | 0.32 | 0.39 | | 0.07 | 0.22 | 0.13 | | 0.03 | 0.21 | 0.05 | |
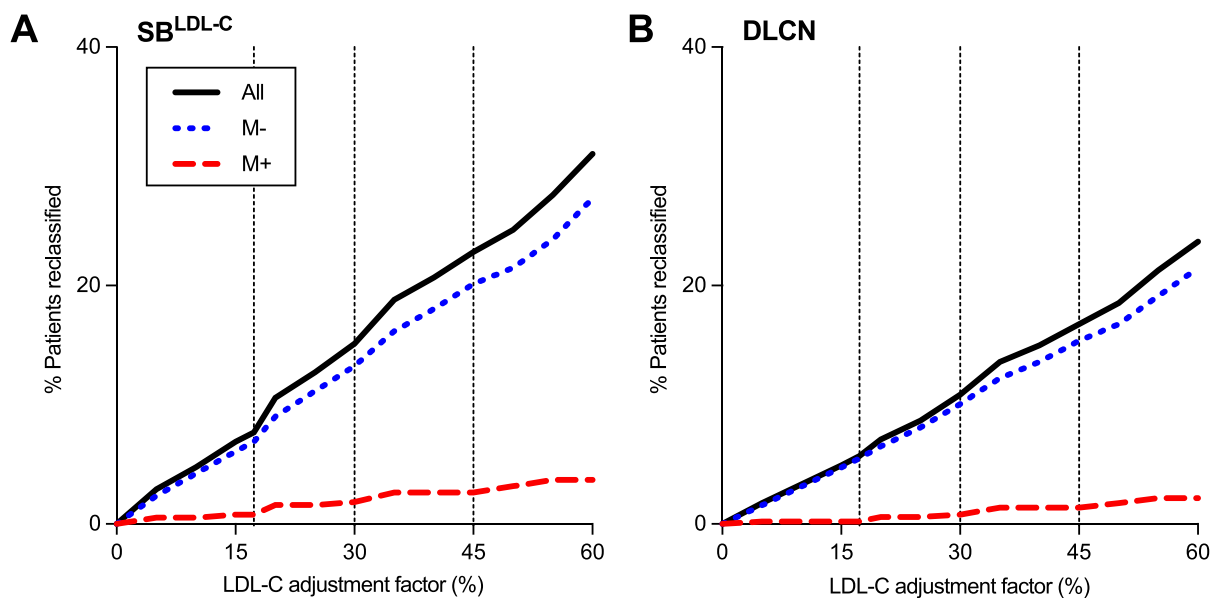
**Fig. 1** Reclassification to 'unlikely' FH across a range of LDL-C factors, assuming Lp(a) cholesterol mass between 5% and 60%. The y-axes represent the percentage of patients that were reclassified and their eventual mutation status. Note that this differs to Table 2, wherein the % reported refers to the % of M- or M+ patients that were reclassified.

tients were reclassified to 'unlikely' FH using all three adjustment factors, albeit reaching statistical significance only after 45% adjustment (Table 2). However, erroneous reclassification of M+ patients was seen at all adjustment factors, amounting to 3.5%, 8.2% and 11.8% of M+ patients using 17.3%, 30% and 45% adjustment factors, respectively. Of interest, a smaller proportion of M+ patients were reclassified using the DLCN than SB$^{LDL-C}$ criteria (e.g. only one M+ patient (0.9%) after 17.3% adjustment), although this difference was not statistically significant. Similar trends were seen with the Randox and IMMAGE cohorts (Supplementary Tables 2-3). Bearing in mind the inter-individual variability in Lp(a)-associated cholesterol, we also considered a wider range of adjustment factors ranging from 5% to 60% (Fig. 1). This confirmed that M- patients consistently accounted for the majority of reclassifications, but that even the smallest adjustment factor (5%) for both SB$^{LDL-C}$ and DLCN incorrectly reclassified at least one M+ patient as 'unlikely' FH.

Importantly, inappropriate M+ reclassifications were not exclusively seen at higher Lp(a) levels, with some instances observed with Lp(a) levels <500 mg/L using both SB$^{LDL-C}$ and DLCN criteria (Table 2). For example, using SB$^{LDL-C}$ criteria, one M+ patient with an Lp(a) of 427 mg/L and LDL-C of 5.1 mmol/L was reclassified following 30% and 45% (but not 17.3%) adjustment. Using DLCN criteria, one M+ patient with an Lp(a) of 95 mg/L and LDL-C level of 4.03 mmol/L was reclassified following 45% adjustment.

Most reclassified patients had a 'possible' clinical FH status prior to LDL-C adjustment, irrespective of eventual mutation status. Detail on the reclassification of patients in different FH clinical categories is shown in Supplementary Tables 4-5 and summarised in Supplementary Figure 2. No

patients were reclassified from 'definite' to 'unlikely' after 17.3% adjustment using SB$^{LDL-C}$, although one 'definite' patient was reclassified using 30% adjustment (M- with Lp(a) 1469 mg/L) and an additional two following 45% adjustment (one M- patient with Lp(a) 1010 mg/L and one M+ patient with Lp(a) 580 mg/L). By contrast, no patients were reclassified from 'definite' to 'unlikely' FH using DLCN criteria. However, three were reclassified from 'probable' FH (all M-, with Lp(a) >1000 mg/L).

Mutation details and biochemical features of the M+ patients who were erroneously reclassified to 'unlikely FH' are summarised in Supplementary Table 6. Overall, 11 patients had an *LDLR* mutation while two had an *APOB* mutation; 10 patients had a single nucleotide variant, while two had a duplication and one had an indel variant.

## Polygenic score distribution

Table 3 shows the distribution of PRS among reclassified M- patients using SB$^{LDL-C}$ and DLCN criteria. Overall, higher reclassification rates were seen among patients with low PRS compared to patients with high or intermediate scores. Using SB$^{LDL-C}$ criteria, 15% of patients with low PRS were reclassified following 17.3% Lp(a) adjustment compared to 8% of patients with high-intermediate scores. Similar trends were seen at 30% and 45% adjustments, and using DLCN criteria. There was no significant correlation between raw PRS and LDL-C levels before (R=0.016, p=0.77) or after Lp(a) adjustment (R=0.020, p=0.71; R=0.042, p=0.43; and R=0.044, p=0.42 following 17.3%, 30% and 45% Lp(a) adjustment, respectively).

**Table 3**  Distribution of polygenic risk scores among reclassified M- patients using SB$^{LDL-C}$ and DLCN criteria after adjusting for Lp(a)-cholesterol content, assuming 17.3%, 30% or 45% mass.

| Lp(a) concentration (mg/L) | Before adjustment | | | Reclassified using 17.3% adjustment | | | Reclassified using 30% adjustment | | | Reclassified using 45% adjustment | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | High-intermediate | Low | All | High-intermediate | Low | All | High-intermediate | Low | All | High-intermediate | Low |
| SB$^{LDL-C}$ criteria (n= 273, n/N (%)) | | | | | | | | | | | | |
| <300 | 146 | 116 | 30 | 2/146 (1.4) | 1/116 (0.9) | 1/30 (3.3) | 7/146 (4.8) | 5/116 (4.3) | 2/30 (6.7) | 12/146 (8.2) | 8/116 (6.9) | 4/30 (13.3) |
| 300-500 | 27 | 24 | 3 | 2/27 (7.4) | 2/24 (8.3) | 0/3 (0.0) | 3/27 (11.1) | 3/24 (12.5) | 0/3 (0.0) | 5/27 (18.5) | 5/24 (20.8) | 0/3 (0.0) |
| >500-1000 | 46 | 41 | 5 | 8/46 (17.4) | 7/41 (17.1) | 1/5 (20.0) | 12/46 (26.1) | 11/41 (26.8) | 1/5 (20.0) | 19/46 (41.3) | 16/41 (39.0) | 3/5 (60.0) |
| >1000 | 54 | 40 | 14 | 14/54 (25.9) | 8/40 (20.0) | 6/14 (42.9) | 27/54 (50.0) | 17/40 (42.5) | 10/14 (71.4) | 39/54 (72.2) | 27/40 (67.5) | 12/14 (85.7) |
| Overall reclassification rate to 'unlikely' | | | | 26/273 (9.5) | 18/221 (8.1) | 8/52 (15.4) | 49/273 (17.9) | 36/221 (16.3) | 13/52 (25.0) | 75/273 (27.5) | 56/221 (25.3) | 19/52 (36.5) |
| DLCN criteria (n= 377, n/N (%)) | | | | | | | | | | | | |
| <300 | 195 | 154 | 41 | 4/195 (2.1) | 3/154 (1.9) | 1/41 (2.4) | 7/195 (3.6) | 4/154 (2.6) | 3/41 (7.3) | 10/195 (5.1) | 7/154 (4.5) | 3/41 (7.3) |
| 300-500 | 47 | 39 | 8 | 2/47 (4.3) | 2/39 (5.1) | 0/8 (0.0) | 2/47 (4.3) | 2/39 (5.1) | 0/8 (0.0) | 5/47 (10.6) | 4/39 (10.3) | 1/8 (12.5) |
| >500-1000 | 62 | 53 | 9 | 9/62 (14.5) | 8/53 (15.1) | 1/9 (11.1) | 14/62 (22.6) | 13/53 (24.5) | 1/9 (11.1) | 17/62 (27.4) | 15/53 (28.3) | 2/9 (22.2) |
| >1000 | 73 | 55 | 18 | 12/73 (16.4) | 8/55 (14.5) | 4/18 (22.2) | 27/73 (37.0) | 17/55 (30.9) | 10/18 (55.6) | 45/73 (61.6) | 33/55 (60.0) | 12/18 (66.7) |
| Overall reclassification rate to 'unlikely' | | | | 27/377 (7.2) | 21/301 (7.0) | 6/76 (7.9) | 50/377 (13.3) | 36/301 (12.0) | 14/76 (18.4) | 77/377 (20.4) | 59/301 (19.6) | 18/76 (23.7) |

## Sensitivity, specificity, and accuracy

608 patients were included in diagnostic test performance calculations. These included patients who were excluded from the main analysis due to an 'unlikely' FH diagnosis using both SB$^{LDL-C}$ and DLCN criteria, but had undergone genetic testing at the discretion of the managing clinician. Before LDL-C adjustment, SB$^{LDL-C}$ showed a sensitivity, specificity, and diagnostic accuracy of 70.8%, 40.2% and 46.2% respectively. By contrast, DLCN showed a sensitivity, specificity, and diagnostic accuracy of 90.0%, 18.2%, and 32.4% respectively (Table 4). These compare with 'standard' (TC or LDL-C) SB criteria which showed a sensitivity, specificity, and diagnostic accuracy of 78.3%, 31.6%, and 40.8% respectively when applied to our cohort. Overall, with the usual LDL-C thresholds retained, LDL-C adjustment for Lp(a) improved the diagnostic accuracy of both SB$^{LDL-C}$ and DLCN criteria. While SB$^{LDL-C}$ accuracy increased to 50.0% and 57.1% with 17.3 and 45% adjustment respectively, DLCN accuracy increased to 36.8% and 44.1% respectively. These trends were consistent across Lp(a) subgroups (with greatest effects seen at Lp(a) levels >500 mg/L following 45% adjustment) and across the two different Lp(a) assay subgroups. It is important to note that these increases were due to improved specificity; the inclusion of Lp(a) adjustment led to reduced sensitivity, meaning that patients with an FH mutation could have been missed.

## Discussion

Untreated FH carries significant morbidity, particularly for younger adults and, as a common autosomal dominant condition, has important implications for the wider population.[22] There are currently several diagnostic tools used to prioritise patients with suspected FH for genetic testing. Both the SB and DLCN criteria inform secondary care referral, and include LDL-C levels, which are commonly estimated in laboratories using the Friedewald equation. Lp(a), which is gaining wider attention as an important biomarker in cardiovascular risk stratification, but is not frequently measured outside specialist lipid or cardiovascular risk clinics, contains a significant amount of cholesterol and can thus skew LDL-C levels and impact on clinical FH diagnosis.

In this retrospective study of 513 adult patients, we demonstrate that LDL-C adjustment for Lp(a) can lead to reclassification of clinical FH status using both DLCN and SB$^{LDL-C}$ criteria. This was particularly the case for individuals with high Lp(a) levels and when the larger 45% adjustment factor was employed. Importantly, we observed significantly higher reclassification rates among M- patients compared to M+ patients. Our findings are therefore consistent with results from a 2019 study by Chan et al. who showed that, following 30% LDL-C adjustment for Lp(a), 22.8% and 8.2% of patients were reclassified to 'unlikely' FH using SB and DLCN criteria respectively. As was also the case in our study, the greatest reclassification rates were demonstrated among M- patients with the highest Lp(a) levels.[21] Moreover, in a large prospective cohort study using data from the Copenhagen General Population Study, LDL-C adjustment for Lp(a)-associated cholesterol revealed that approximately 25% of clinical FH diagnoses were partly attributable to raised Lp(a) levels.[20]

Our results showed a small number of M+ patients were erroneously reclassified to 'unlikely' FH following LDL-C adjustment. Of the 13 reclassified M+ patients in this study, the majority carried missense variants in LDLR or APOB, as opposed to more destructive frameshift, large deletion, or duplication mutations, which usually confer a more severe biochemical phenotype.[26] Accordingly, all but two patients had a peak recorded LDL-C of <6 mmol/L. Similarly, all but two patients in this subgroup had an Lp(a) >500 mg/L, suggesting that reclassifications were largely due to the combination of low unadjusted LDL-C and high Lp(a) levels, the former of which may reflect milder disease phenotypes. However, as inappropriate reclassification of M+ patients was seen across the spectrum of LDL-C and Lp(a) concentrations, it is difficult to establish biochemical categories in our dataset within which Lp(a)-driven LDL-C adjustment does not result in the potential for missed FH diagnosis. However, it is worth underlining that, although a molecular FH diagnosis could have been missed in this cohort of reclassified patients by incorporating an Lp(a)-driven LDL-C adjustment factor, measuring Lp(a) still presents an opportunity to ensure optimal treatment is given. This was recently demonstrated in a study by Hedegaard et al.[27] who reported the risk equivalence of Lp(a) and LDL-C in clinical and genetic FH. This risk is also stressed in the 2022 European Atherosclerosis Society consensus statement which highlights the continuous relationship between Lp(a) and cardiovascular risk, even at low LDL-C concentrations.[28]

It has recently been shown that Lp(a) cholesterol content is lower than previously thought – at least as pertaining to LDL-C adjustment, when the unesterified fraction (as measured by Yeang et al.) is most relevant.[14] We therefore included a 17.3% adjustment factor in addition to the traditional 30% and 45% estimates. This lower adjustment resulted in an overall reclassification rate of 7.7% (29/377) and 5.7% (29/507) using SB$^{LDL-C}$ and DLCN criteria respectively. Importantly, fewer M+ patients were reclassified compared to 30-45% Lp(a) adjustment (3.5% and 0.9% using SB$^{LDL-C}$ and DLCN criteria respectively). Nevertheless, it could be argued that missing even small numbers of FH diagnoses is an unacceptable trade-off, given the high lifetime risk of CVD and resultant healthcare costs.

In our study, 78.9% of patients meeting FH clinical criteria were M-, with DNA analysis identifying a PRS suggestive of hypercholesterolaemia of polygenic origin in a majority of cases. Following LDL-C adjustment for Lp(a), greater reclassification rates were observed among M- patients with low PRS, compared to those with combined high-intermediate PRS. This was the case for both SB$^{LDL-C}$ and DLCN criteria with higher rates seen at greater Lp(a) levels

**Table 4** Sensitivity, specificity, and accuracy of SB$^{LDL-C}$ and DLCN criteria after adjusting for Lp(a)-cholesterol content, assuming 17.3%, 30% or 45% mass (n= 608, n/N (%)).

| | SB$^{LDL-C}$ criteria | | | | DLCN criteria | | | |
|---|---|---|---|---|---|---|---|---|
| | Before adjustment | After 17.3% adjustment | After 30% adjustment | After 45% adjustment | Before adjustment | After 17.3% adjustment | After 30% adjustment | After 45% adjustment |
| **Lp(a) <300 mg/L** | | | | | | | | |
| Sensitivity | 42/60 (70.0) | 42/60 (70.0) | 42/60 (70.0) | 42/60 (70.0) | 51/60 (85.0) | 51/60 (85.0) | 51/60 (85.0) | 50/60 (83.3) |
| Specificity | 102/259 (39.4) | 104/259 (40.2) | 110/259 (42.5) | 115/259 (44.4) | 51/259 (19.7) | 56/259 (21.6) | 59/259 (22.8) | 62/259 (23.9) |
| Accuracy | 144/319 (45.1) | 146/319 (45.8) | 152/319 (47.6) | 157/319 (49.2) | 102/319 (32.0) | 107/319 (33.5) | 110/319 (34.5) | 112/319 (35.1) |
| **Lp(a) 300-500 mg/L** | | | | | | | | |
| Sensitivity | 16/23 (69.6) | 16/23 (69.6) | 15/23 (65.2) | 15/23 (65.2) | 21/23 (91.3) | 21/23 (91.3) | 21/23 (91.3) | 21/23 (91.3) |
| Specificity | 29/59 (49.2) | 31/59 (52.5) | 32/59 (54.2) | 34/59 (57.6) | 9/59 (15.3) | 11/59 (18.6) | 11/59 (18.6) | 14/59 (23.7) |
| Accuracy | 45/82 (54.9) | 47/82 (57.3) | 47/82 (57.3) | 49/82 (59.8) | 30/82 (36.6) | 32/82 (39.0) | 32/82 (39.0) | 35/82 (42.7) |
| **Lp(a) >500-1000 mg/L** | | | | | | | | |
| Sensitivity | 15/21 (71.4) | 13/21 (61.9) | 12/21 (57.1) | 11/21 (52.4) | 21/21 (100.0) | 20/21 (95.2) | 20/21 (95.2) | 19/21 (90.5) |
| Specificity | 28/77 (36.4) | 36/77 (46.8) | 40/77 (51.9) | 47/77 (61.0) | 12/77 (15.6) | 21/77 (27.3) | 26/77 (33.8) | 29/77 (37.7) |
| Accuracy | 43/98 (43.9) | 49/98 (50.0) | 52/98 (53.1) | 58/98 (59.2) | 33/98 (33.7) | 41/98 (41.8) | 46/98 (46.9) | 48/98 (49.0) |
| **Lp(a) >1000 mg/L** | | | | | | | | |
| Sensitivity | 12/16 (75.0) | 11/16 (68.8) | 9/16 (56.3) | 7/16 (43.8) | 15/16 (93.8) | 15/16 (93.8) | 12/16 (75.0) | 11/16 (68.8) |
| Specificity | 37/93 (39.8) | 51/93 (54.8) | 64/93 (68.8) | 76/93 (81.7) | 17/93 (18.3) | 29/93 (31.2) | 44/93 (47.3) | 62/93 (66.7) |
| Accuracy | 49/109 (45.0) | 62/109 (56.9) | 73/109 (67.0) | 83/109 (76.1) | 32/109 (29.4) | 44/109 (40.4) | 56/109 (51.4) | 73/109 (67.0) |
| **All Lp(a) concentration (mg/L)** | | | | | | | | |
| Sensitivity | 85/120 (70.8) | 82/120 (68.3) | 78/120 (65.0) | 75/120 (62.5) | 108/120 (90.0) | 107/120 (89.2) | 104/120 (86.7) | 101/120 (84.2) |
| Specificity | 196/488 (40.2) | 222/488 (45.5) | 246/488 (50.4) | 272/488 (55.7) | 89/488 (18.2) | 117/488 (24.0) | 140/488 (28.7) | 167/488 (34.2) |
| Accuracy | 281/608 (46.2) | 304/608 (50.0) | 324/608 (53.3) | 347/608 (57.1) | 197/608 (32.4) | 224/608 (36.8) | 244/608 (40.1) | 268/608 (44.1) |

and following 45% adjustment. This is in keeping with the apparently high "LDL-C" in these patients, despite no evidence of monogenic or polygenic origin, being partly driven by Lp(a)-associated cholesterol.

At baseline, DLCN criteria had the highest sensitivity for identifying FH-causing mutations compared to SB$^{LDL-C}$, which had the higher specificity. While DLCN values were consistent with results from other studies, SB$^{LDL-C}$ sensitivity was lower, with a higher specificity.[29] This may be explained by the fact that the literature only cites 'standard' SB criteria in accuracy analyses and is supported by our observations when 'standard' SB criteria were applied to our data. Following LDL-C adjustment for Lp(a), the accuracy of both SB$^{LDL-C}$ and DLCN criteria increased when the usual LDL-C thresholds were applied, with the greatest effects seen at higher Lp(a) levels and following 45% adjustment. This corresponded to a rise in specificity at the expense of sensitivity, as has been demonstrated in other studies.[21] To our knowledge, no previous study has reported accuracy data on SB$^{LDL-C}$ criteria. Our results suggest that this variation of the SB criteria may be better at identifying true negative cases compared to standard SB criteria, however, this is at the risk of potentially missing M+ cases. While increased specificity is desirable to minimise anxiety and costs associated with unnecessary molecular genetic testing, decreased sensitivity carries the potentially more harmful risk of missed diagnoses and treatment in index and cascaded cases.

In the UK, it costs approximately £250/patient per NGS diagnostic test for FH. LDL-C adjustment for Lp(a) therefore has the potential to reduce unnecessary FH genetic testing and therefore save costs. For example, following 30% Lp(a) adjustment, 57/377 patients were reclassified to 'unlikely' FH using SB$^{LDL-C}$. This would hypothetically have saved £14,250 in genetic testing costs.[30] However, this would be at the risk of missing up to 7 M+ patients. Given our findings that LDL-C adjustment for Lp(a) improves the accuracy of both SB$^{LDL-C}$ and DLCN diagnostic criteria, how much could a health system save with these improved tools? The answer may differ between countries due to considerable variation in the cost of NGS. To answer this, health economic analysis is needed as savings from NGS may be offset by the morbidity and mortality associated with cardiovascular events resulting from untreated high-risk individuals with genetically determined hypercholesterolemia. It is therefore not possible to recommend use of LDL-C adjusted diagnostic criteria without a complete risk-benefit cost analysis. As Lp(a)-driven adjustment inevitably reduces LDL-C, re-evaluation of the LDL-C thresholds used in FH scoring systems would need to be considered as part of this analysis.

There are a number of limitations with our study. Firstly, we have presented a combined cohort comprised of patients with Lp(a) levels measured using two different commercially available assays, widely used globally. However, the principal findings and conclusions from our combined cohort were similar to those from assay subgroup analyses. Second, we assumed 17.3-45% of Lp(a) mass is cholesterol, but it is now known that Lp(a) cholesterol content is highly variable between individuals, ranging from approximately 5 to 60%. While more accurate measurements could be made using recently described assays that specifically quantify Lp(a)-cholesterol, these are not widely available in clinical laboratories.[14]

## Conclusion

Our study shows that LDL-C adjustment for Lp(a) improves the diagnostic accuracy of SB$^{LDL-C}$ and DLCN criteria with potential cost-savings from reduced genetic testing. However, adjustments also lead to inappropriate reclassification of M+ patients, primarily (but not exclusively) in patients with high Lp(a) or borderline LDL-C. Health economic analysis is therefore needed to balance cost-savings with clinical consequences of untreated hypercholesterolaemia in index and cascaded FH cases before LDL-C adjustments for Lp(a) can be recommended in clinical practice.

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## Author contributions

J.C., A.D. and B.J. designed and supervised this study. A.D., D.T. and A.P.T.T. collected data. D.T., A.P.T.T. and S.E. performed data analyses. D.T. wrote the first draft of the manuscript, which was reviewed and approved by all authors prior to submission.

## Declaration of Competing Interest

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jacl.2023.01.006.

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