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**Technology Assessment Unit of the  
McGill University Health Centre  
(MUHC)**

**Evaluating the value of apolipoprotein B  
testing for the assessment and management  
of atherosclerotic cardiovascular disease at  
the MUHC RUISSS**

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**Report prepared for the Technology  
Assessment Unit (TAU) of the McGill  
University Health Centre (MUHC)**

**by**

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**Approved by the Committee of the TAU on June 2<sup>nd</sup>, 2020**

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## REPORT REQUESTOR

This report was requested by Dr. Andre Dascal, Chief, Department of Clinical Laboratory Medicine, MUHC and Medical Director - OPTILAB Montreal-MUHC Cluster, on March 18, 2018, and will be presented to him on completion.

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## ABSTRACT

- There are several clinical markers of atherogenic risk:
  - LDL cholesterol (LDL-C), corresponding to the mass of **cholesterol within LDL particles** and the traditional target for cardiovascular disease (CVD) risk assessment and management;
  - Non-high density lipoprotein cholesterol (non-HDL-C), a measure of the **mass of cholesterol within all atherogenic lipid particles**; and
  - Apolipoprotein B (apoB), which corresponds to the total **number of all atherogenic particles** because each lipoprotein particle contains one apoB molecule.
- While all three markers are highly correlated, there is considerable debate surrounding which measure is the ideal lipoprotein test, with American guidelines continuing to support the sole use of LDL-C for CVD risk assessment.
- The debate has intensified with recent evidence of inter-individual variability in cholesterol mass, with some individuals having cholesterol-depleted particles (high apoB/ low cholesterol mass) and others having cholesterol-enriched particles (low apoB/ high cholesterol mass). This discordance can lead to the under- or over-treatment of patients if only cholesterol mass (LDL-C or non-HDL-C) is used as a marker.
- The objective of this report was to evaluate the value of integrating apoB testing at the MUHC RUISSS for risk assessment and risk management of CVD, particularly in discordant populations. Currently, LDL-C is the standard lipid test at the MUHC.
- Discordance analysis studies restrict evaluation of the ability of apoB vs LDL-C to predict CVD risk to only those individuals with discordant levels of lipid measures. We identified 8 discordance analysis studies of risk assessment, 3 discordance studies of risk management, and 2 Mendelian randomization studies.
- These studies were conducted in a diverse group of people, and all consistently found that particle number (apoB) had a stronger association with CVD risk than LDL-C in discordant populations. Furthermore, participants with discordantly higher apoB than LDL-C were more likely to have several CVD risk factors such as obesity, diabetes and hypertension. Hence, when particle number and cholesterol mass disagree, CVD risk appears to track with particle number.
- Mendelian randomization studies underscored that lowering apoB plays an additional role in lowering CVD risk, over and above lowering LDL-C.

- Barriers to the uptake of apoB include concerns over cost, test accuracy, and disruption of clinical practice over introduction of a test equivalent to LDL-C. A thorough review by the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) reported that apoB tests are accurate, reliable and relatively inexpensive. ApoB tests are independent of variations in triglyceride concentrations, and hence can be measured in non-fasting samples.
- Although the American and Canadian guideline continue to recommend LDL-C as the first-line test for CVD risk, the most recent European guidelines have recommended apoB testing in high risk groups likely to have discordant apoB vs LDL-C levels.

## RÉSUMÉ

- Il existe plusieurs marqueurs cliniques concernant les risques athérogéniques :
  - Le LDL du cholestérol (LDL-C), qui correspond à la **masse de cholestérol dans les particules LDL** et qui est le paramètre classique pour l'évaluation et la gestion des risques de maladies cardiovasculaires ;
  - Le cholestérol à lipoprotéines de basse densité (non-HDL-C), qui correspond à la **masse de cholestérol à l'intérieur de toutes les particules lipidiques athérogéniques** ; et
  - L'apolipoprotéine B (apoB), qui correspond au **nombre total de toutes les particules athérogéniques** car chaque particule lipoprotéinique contient une molécule apoB.
- Bien que la corrélation de ces trois marqueurs soit très élevée, il existe un important débat concernant le test idéal pour la lipoprotéine, où les lignes directrices américaines supportent toujours l'utilisation unique du LDL-C pour l'évaluation des risques de maladies cardiovasculaires.
- Le débat s'est intensifié avec la preuve récente de la variabilité inter-individuelle de la masse de cholestérol, avec certains individus ayant des particules appauvries en cholestérol (apoB élevé / faible masse de cholestérol), et d'autres ayant des particules enrichies en cholestérol (apoB faible / masse élevée de cholestérol). Cette discordance peut mener à un traitement insuffisant ou un traitement excessif pour les patients, si seule la masse de cholestérol (LDL-C ou non-HDL-C) est utilisée comme marqueur.
- L'objectif de ce rapport était d'évaluer les avantages d'intégrer le test de l'apoB pour l'évaluation et la gestion des risques de maladies cardiovasculaires au RUISSS du CUSM, particulièrement chez les populations discordantes. Actuellement, le LDL-C est le test standard lipidique au CUSM.
- Les études d'analyse de discordance restreignent l'évaluation de la capacité de l'apoB vs le LDL-C, à prédire les risques de maladies cardiovasculaires chez les individus montrant des mesures lipidiques discordantes. Nous avons identifié 8 études d'analyse de discordance pour l'évaluation des risques, 3 études de discordance pour la gestion des risques et 2 études de randomisation mendélienne.
- Ces études ont été menées chez divers groupes d'individus et toutes ont trouvé systématiquement que le nombre de particules apoB avait un lien plus fort avec les risques de maladies cardiovasculaires que le LDL-C chez les populations discordantes. De plus, les participants avec des valeurs discordantes plus élevées d'apoB que de LDL-

C, étaient plus susceptibles d'avoir plusieurs facteurs de risque tel que l'obésité, le diabète et l'hypertension. Ainsi, lorsque le nombre de particules et la masse de cholestérol ne concordent pas, les risques de maladies cardiovasculaires semblent suivre le nombre de particules.

- Les études de randomisation mendélienne soulignent qu'une réduction de l'apoB joue un rôle supplémentaire pour diminuer les risques de maladies cardiovasculaires, en plus de la réduction due à la diminution du LDL-C.
- Les obstacles à l'adoption de l'apoB incluent les coûts supplémentaires, l'exactitude des tests et la perturbation de la pratique clinique concernant l'introduction d'un test équivalent au LDL-C. Un examen approfondi réalisé par *l'European Atherosclerosis Society (EAS)* et *l'European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)* a souligné que les tests de l'apoB sont exacts, fiables, et relativement peu coûteux. Les tests de l'apoB sont indépendants des variations de concentration des triglycérines et peuvent ainsi être réalisés à partir d'échantillons pris chez des patients non à jeun.
- Même si les lignes directrices américaines et canadiennes recommandent toujours le test LDL-C comme test de première ligne pour les risques de maladies cardiovasculaires, les plus récentes lignes directrices européennes ont recommandé le test de l'apoB chez les groupes à risques élevés, susceptibles d'avoir des valeurs discordantes de l'apoB et du LDL-C.

## EXECUTIVE SUMMARY

### BACKGROUND

Apolipoprotein B (apoB) is the primary apolipoprotein of atherogenic lipid particles and thus corresponds to the total burden of atherogenic particles in plasma. LDL cholesterol (LDL-C), which represents the mass of cholesterol within LDL particles, has been the traditional target for the assessment and management of cardiovascular disease (CVD) risk. There has been considerable debate about which measure is the ideal lipoprotein test. The standard perception is that both markers are equivalent in risk prediction. However, this view does not take into account inter-individual heterogeneity, leading to discordantly elevated apoB levels despite normal cholesterol levels. ApoB may have added utility in identifying such discordant individuals.

### OBJECTIVE

The objectives of this report are to:

- Evaluate the benefit of integrating apoB testing across the MUHC RUISSS for the purpose of:
  - Risk assessment, i.e. the ability of apoB to identify patients at risk of adverse cardiovascular outcomes, particularly those with discordant apoB and LDL-C levels;
  - Risk management in statin-treated patients, i.e. the ability of apoB to identify residual risk of adverse cardiovascular outcomes, particularly in patients with discordant apoB and LDL-C levels;
- Describe current practice at the MUHC.

### METHODS

We limited our literature search to studies of discordant populations comparing the ability of apoB versus traditional lipid markers to identify adverse cardiovascular outcomes. We reviewed available evidence on other perceived barriers to the use of apoB, including analytical performance and cost. We also identified and summarized recommendations from relevant guidelines for the treatment of dyslipidemia.

### RESULTS

Discordance analysis evaluates the association between lipid measures and future CVD risk by restricting the analysis to only those individuals with discordant levels, with the aim of evaluating the added value of these markers when they disagree.

### Risk assessment:

- We identified 8 discordance analysis studies that evaluated the association between discordance in apoB and cholesterol content measures, and risk of cardiovascular events. Discordance between apoB and LDL-C measures ranged from 18% to 20% of the population analysed.
- These studies (n=2794 to 63,520), which were conducted in a diverse range of populations, consistently found particle number (apoB or LDL-P) to have a stronger association with CVD risk than LDL-C in discordant populations. Patients with higher levels of apoB, irrespective of levels of LDL-C, had consistently worse outcomes than those with lower levels of apoB. In a discordance analysis of the largest study (UK biobank study), only apoB was associated with the composite CVD score in adjusted analyses [HR per 1 standard deviation: 1.23 (1.12, 1.35)].
- Hence, when particle number and cholesterol mass disagree, CVD risk appears to track with particle number. These results highlight the utility of apoB in identifying patients at risk despite low LDL-C levels.
- These studies also indicated that participants with discordantly higher apoB than LDL-C were more likely to have several CVD risk factors including higher BMI, blood pressure, and fasting glucose, and elevated triglycerides.

### Risk management:

Some patients continue to have residual risk for CVD events despite treatment with lipid-lowering therapies. This could be because statins lower apoB and LDL-C to differing degrees.

- We identified 3 studies that evaluated discordance in lipid measures and CVD outcomes among statin-treated patients (n=4957 to 21,465).
- The largest study found that participants with discordantly elevated apoB relative to cholesterol content (i.e. those with cholesterol-depleted particles) had a higher risk of acute myocardial infarction relative to the concordant group (OR: 1.48; 95% CI: 1.38, 1.58).
- These studies confirmed that risk of CVD was elevated in discordant populations, i.e. patients who were at or below target goals for LDL-C or non-HDL-C, but remained above goal of apoB.
- Most studies found that patients with cholesterol-depleted particles were more likely to be diabetic or have risk factors for metabolic syndrome.

### Mendelian analyses:

Mendelian randomization studies rely on naturally occurring genetic variants that are randomly distributed across the population to evaluate the associated phenotypes; the random distribution ensures that confounding factors are evenly balanced between groups, thus approximating a randomized controlled trial.

- 2 large Mendelian randomization studies attempted to disentangle the roles of apoB vs. LDL-C and triglycerides in impacting CVD risk. These studies conclude that lowering apoB i.e. particle number drive the clinical benefit of lowering triglycerides or LDL-C; this clinical benefit of lipid-lowering therapies is proportional to the absolute reduction in apoB, irrespective of changes in lipid concentrations.

### Analytical performance and cost:

A very in-depth review of the evidence pertaining to analytical performance and clinical effectiveness was conducted by the The European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) in 2018. It concludes that apoB and non-HDL-C tests are more accurate than direct and calculated measurements of LDL-C, particularly in non-fasting, hypertriglyceridemic samples, or those with very low LDL-C concentrations. Furthermore, it notes that apoB tests can be easily integrated into available platforms, and are widely accessible at reasonable operating costs.

### Guidelines:

In addition to the EAS/EFLM afore mentioned consensus statement, we identified recent Canadian, American and European guidelines.

- **The American Heart Association/American College of Cardiology guidelines:** The 2018 AHA/ACC guidelines, while acknowledging that apoB is a 'stronger indicator of atherogenicity than LDL-C alone' continue to recommend only LDL-C as the routine measure of risk assessment. The guidelines do not cite any of the literature in discordant populations or the Mendelian randomization studies.
- **Canadian Cardiovascular Society guidelines:** The 2016 Canadian Cardiovascular Society guidelines continue to recommend LDL-C as the primary target for risk assessment of dyslipidemia, "because clinicians are most familiar with LDL-C.... but anticipate a shift to preferential use of non-HDL-C or apoB in the future." apoB and non-HDL-C are currently recommended as optional secondary targets.

- **The European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) guidelines:** In contrast to the AHA/ACC guidelines, the EAS/ EFLM 2018 consensus statement on quantifying atherogenic lipoproteins, extensively reviewed all the evidence, including the value of apoB in discordant populations. In terms of analytical performance, they conclude that apoB more accurately reflects particle number. With respect to clinical performance, the guidelines state that discordance analysis shows that apoB improves risk assessment. However, in terms of cost-effectiveness, the guidelines conclude that there is no evidence yet that apoB or non-HDL-C are cost-effective measures in comparison to LDL-C, and hence they recommend the use of apoB only as a secondary target, with LDL-C remaining the standard measure. Overall, they recommend apoB for dyslipidemia characterization, and as an optional measure for CVD risk estimation and treatment target, but not recommended for treatment choice.
- **European Society of Cardiology/European Atherosclerosis Society guidelines:** The 2019 European Society of Cardiology/European Atherosclerosis Society guidelines also differ from the 2018 AHA guidelines. They included evidence from the Mendelian randomization studies and the discordance analysis studies, and thus recommend that "ApoB analysis is recommended for risk assessment, particularly in people with high TG levels, diabetes, obesity, metabolic syndrome, or very low LDL-C levels. It can be used as an alternative to LDL-C, if available, as the primary measurement for screening, diagnosis, and management, and may be preferred over non-HDL-C in people with high TG levels, diabetes, obesity, or very low LDL-C levels." (Class I, Level C evidence)

## EXPERIENCE AT THE MUHC

Currently, the McGill RUISSS (which includes the MUHC) uses the standard lipid panel as the standard measure for dyslipidemia assessment and management, with apoB ordered in hypertriglyceridemia cases.

A total of 10,775 tests for apoB were done at the MUHC, compared to 96,840 tests for total cholesterol.

## COSTS

The cost of laboratory tests is set by the province of Quebec. For 2018-19, an apoB test cost \$3.60, while a standard lipid panel (total cholesterol, HDL-C, and triglycerides) cost \$2.40. Assuming 100,000 lipid panel tests are performed annually at the MUHC, replacing



the standard panel test with apoB would result in an additional cost to the hospital of \$120,000. However, as the average cost is a function of volume, as the use of apoB becomes more widespread, its cost will decrease.

## CONCLUSIONS

- There is considerable evidence indicating that apoB is *at least* as good as LDL-C in predicting CVD risk in the general population, and is superior to current markers (LDL-C and non-HDL-C) in predicting risk in discordant populations.
- Studies indicate that approximately 20% of the population may have elevated particle number but low or normal LDL-C, and are thus missed by traditional lipid measures. Furthermore, patients with discordantly elevated particle number relative to cholesterol content are more likely to have CVD risk factors associated with metabolic syndrome. Hence, apoB has the potential to improve risk prediction, both in primary and secondary prevention, by identifying those at high risk.
- ApoB assays are precise, accurate, and easily accessible at reasonable operating costs. In addition, these assays avoid the disadvantages associated with LDL-C measurements, such as having to use fasting samples, and imprecise measures at very low LDL-C concentrations.
- The main barriers to the uptake of apoB has been a reluctance to disrupt practice if the risk prediction associated with apoB is similar to LDL-C. However, very little attention has been accorded to apoB's utility in discordant populations, and if this aspect is factored into the equation, apoB becomes clinically more relevant than LDL-C as the routine measure. In addition, apoB has the ability to simplify physician workload by replacing 5 measures with a single one.
- There appears to be a turning of the tide with guidelines, such as the recent European guidelines, slowly adopting new recommendations endorsing the use of apoB in a routine setting.

## RECOMMENDATION

- Given that apoB is equivalent to LDL-C in predicting CVD risk in the general population and superior to LDL-C in discordant populations, we recommend that apoB testing be [Approved](#) for use as an alternative to LDL-C testing for the assessment and management of CVD risk in:
  - patients with CVD risk factors such as high triglyceride levels, diabetes, obesity, and metabolic syndrome;

- patients with very low LDL-C levels.
- To avoid unnecessary duplication of testing at the MUHC RUISSS, we recommend that apoB replace LDL-C testing, at the discretion of the treating physician.
- An educational programme for physicians will be developed with the collaboration of Dr. Sniderman and relevant stakeholders to promote better understanding of the advantages of apoB.
- This recommendation will be reassessed in 1 year after evaluation of local data and/or new evidence in the scientific literature.

## SOMMAIRE

### CONTEXTE

L'apolipoprotéine B (apoB) est l'apolipoprotéine principale des particules lipidiques athérogènes et correspond ainsi à la charge totale des particules athérogéniques dans le plasma. Le cholestérol LDL (LDL-C) qui représente la quantité de cholestérol dans les particules LDL, a été le paramètre classique pour l'évaluation et le management des risques de maladies cardiovasculaires (RCV). Un débat important a eu lieu concernant le test idéal pour la mesure de la lipoprotéine. Le sentiment actuel est que les deux marqueurs s'équivalent pour la prédiction des risques. Cependant, cette vision ne tient pas compte de l'hétérogénéité inter-individuelle menant à une discordance élevée des niveaux de l'apoB malgré des niveaux normaux de cholestérol. L'ApoB pourrait avoir une valeur ajoutée pour identifier ces individus discordants.

### OBJECTIFS

Les objectifs de ce rapport sont :

- Évaluer le bénéfice d'intégrer les tests de l'apoB au niveau du RUISSS du CUSM dans le but :
  - De faire une évaluation des risques, c'est-à-dire la capacité de l'apoB pour identifier les patients sujets à développer des séquelles cardiovasculaires, particulièrement chez ceux avec des niveaux de l'apoB et de LDL-C discordants ;
  - De gérer les risques chez les patients traités avec les statines, c'est-à-dire la capacité de l'apoB d'identifier les risques résiduels des séquelles cardiovasculaires, particulièrement chez les patients avec des niveaux de l'apoB et de LDL-C discordants.
- Décrire la pratique actuelle au CUSM.

### MÉTHODOLOGIE

Nous avons limité notre recherche de la littérature aux études de populations discordantes comparant la capacité de l'apoB versus les marqueurs lipidiques traditionnels, pour identifier les séquelles cardiovasculaires. Nous avons aussi revu les preuves des autres obstacles à l'utilisation de l'apoB, incluant les études de performance et de coûts. Enfin, nous avons aussi identifié et résumé les recommandations provenant des lignes directrices pertinentes pour le traitement de la dyslipidémie.

## RÉSULTATS

L'analyse de discordance évalue le lien entre les mesures lipidiques et les risques futurs de maladies cardiovasculaires en limitant l'analyse uniquement chez les individus montrant des niveaux discordants, dans le but d'évaluer la valeur ajoutée de ces marqueurs lors de désaccords.

### Évaluation des risques :

- Nous avons identifié 8 études d'analyse de discordance qui évaluaient le lien entre la discordance des mesures de l'apoB et celles du contenu en cholestérol, et les risques d'événements cardiovasculaires. La discordance entre les mesures de l'apoB et du LDL-C variaient de 18% à 20% chez la population étudiée.
- Ces études (n=2,794 à 63,520) qui avaient été réalisées chez un éventail diversifié de populations, avaient systématiquement trouvé un nombre de particules (apoB ou LDL-P) qui avaient un lien plus fort avec les risques de maladies cardiovasculaires que le LDL-C chez les populations discordantes. Les patients avec des niveaux élevés de l'apoB, indépendamment des niveaux de LDL-C, avaient systématiquement de moins bons résultats que ceux ayant de plus faibles niveaux de l'apoB. Dans une analyse de discordance de la plus vaste étude (étude UK biobank), seule la mesure de l'apoB était associée avec le résultat combiné des analyses ajustées pour les maladies cardiovasculaires [HR pour 1 déviation standard : 1.23 (1.12, 1.35)].
- Ainsi, lorsque le nombre de particules et la masse du cholestérol ne correspondent pas, les risques de maladies cardiovasculaires semblent liés au nombre de particules. Ces résultats mettent en lumière la capacité de l'apoB pour identifier les patients à risque, malgré de faibles niveaux de LDL-C.
- De même, ces études indiquaient que les participants présentant des valeurs discordantes plus élevées de l'apoB que de LDL-C, étaient plus susceptibles d'avoir plusieurs facteurs de risque de maladies cardiovasculaires, incluant un indice de masse corporelle plus élevé, une pression sanguine plus élevée, un glucose à jeun plus élevé ainsi qu'un niveau de triglycérides élevé.

### Gestion des risques :

Certains patients présentent toujours des risques résiduels de développer des maladies cardiovasculaires, malgré des traitements pour abaisser les lipides. Ceci pourrait être dû aux statines qui abaisseraient l'apoB et le LDL-C à des degrés différents.

- Nous avons identifié 3 études qui évaluaient la discordance entre les mesures lipidiques et les résultats de maladies cardiovasculaires chez les patients traités avec des statines (n=4,957 à 21,465).
- La plus importante étude montra que les participants avec des mesures discordantes au niveau de l'apoB et du cholestérol (c'est-à-dire celles avec des particules appauvries en cholestérol) avaient un niveau de risques plus élevé d'infarctus du myocarde, comparativement au groupe avec des mesures concordantes (OR : 1.48 ; 95% CI : 1.38,1.58).
- Ces études ont confirmé que les risques de maladies cardiovasculaires étaient plus élevés chez les populations discordantes, c'est-à-dire chez les patients où les mesures étaient égales ou inférieures aux objectifs visés pour le LDL-C ou le non-HDL-C, mais demeuraient au-dessus de l'objectif pour l'apoB.
- La plupart des études montrèrent que les patients avec des particules appauvries en cholestérol étaient plus susceptibles d'être diabétiques ou d'avoir des facteurs de risques liés à un syndrome métabolique.

#### Analyses mendéliennes :

Les études de randomisation mendélienne reposent sur des variantes génétiques naturelles qui sont distribuées de façon aléatoire parmi la population pour évaluer les phénotypes associés ; la distribution randomisée nous assure que les facteurs de confusion sont également distribués entre les groupes, se rapprochant ainsi d'une étude randomisée.

- Deux vastes études de randomisation mendélienne ont tenté de démêler les rôles de l'apoB vs le LDL-C et les triglycérides, concernant leur impact sur les maladies cardiovasculaires. Ces études démontrent que abaisser l'apoB, c'est-à-dire le nombre de particules, améliore les avantages cliniques de la réduction des triglycérides ou de LDL-C ; cet avantage clinique des thérapies pour abaisser les lipides est proportionnel à la diminution absolue en apoB, indépendamment des variations de la concentration des lipides.

#### Performance analytique et coûts :

Une revue en profondeur des preuves concernant la performance analytique et l'efficacité clinique fut menée par « *The European Atherosclerosis Society (EAS)* » et « *The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)* » en 2018. Elle conclut que les tests de l'apoB et du non-HDL-C sont plus exactes que les mesures

directes et calculées de LDL-C, particulièrement chez les échantillons hypertriglycéridémiques de patients non à jeun ou ceux avec une très faible concentration de LDL-C. De plus, cette revue note que les tests de l'apoB peuvent aisément être intégrés dans les plateformes disponibles et sont largement accessibles à des coûts d'opération raisonnables.

### Lignes directrices :

En plus de la déclaration de consensus EAS/EFLM susmentionnée, nous avons identifié de récentes lignes directrices canadiennes, américaines et européennes.

- **Lignes directrices de « The American Heart Association/American College of Cardiology »:** Les lignes directrices de 2018 de l'AHA/ACC, tout en reconnaissant que l'apoB est un plus fort indicateur de l'athérogénicité que le LDL-C seul, recommande toujours uniquement le LDL-C comme mesure de routine pour l'évaluation des risques. Les lignes directrices ne mentionnent aucun article sur les populations discordantes ou sur les études de randomisation mendélienne.
- **Lignes directrices de la « Canadian Cardiovascular Society »:** Les lignes directrices de 2016 de la « *Canadian Cardiovascular Society* » recommandent toujours le LDL-C comme paramètre principal pour l'évaluation des risques de dyslipidémie « *parce que les cliniciens sont plus familiers avec le LDL-C...mais anticipent un virage vers l'utilisation privilégiée du non-HDL-C ou de l'apoB dans le futur* ». L'apoB et le non HDL-C sont couramment recommandés comme paramètres secondaires optionnelles.
- **Lignes directrices de « The European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) »:** Contrastant avec les lignes directrices de l'AHA/ACC, la déclaration de consensus de 2018 de l'EAS/EFLM sur la quantification des lipoprotéines athérogéniques a revu en profondeur toutes les preuves, incluant les avantages de l'apoB chez les populations discordantes. En termes de performance analytique, ils ont conclu que l'apoB reflète plus précisément le nombre de particules. En regard de la performance clinique, les lignes directrices soulignent que l'analyse de discordance montre que l'apoB améliore l'évaluation des risques. Cependant, en termes de coût efficacité, les lignes directrices concluent qu'il n'y a pas encore de preuve que l'apoB ou le non-HDL-C sont des mesures rentables par comparaison au LDL-C et recommandent ainsi l'utilisation de l'apoB seulement comme paramètre secondaire, le LDL-C demeurant la mesure standard. De façon générale, ils recommandent l'apoB pour la caractérisation de la dyslipidémie et

comme une mesure optionnelle pour l'évaluation des risques de maladies cardiovasculaires et le but du traitement, mais non pour le choix du traitement.

- **Lignes directrices de l'« European Society of Cardiology/European Atherosclerosis Society »:** Les lignes directrices de 2019 de l'*European Society of Cardiology/European Atherosclerosis Society* diffèrent aussi des lignes directrices de 2018 de l'AHA. Elles incluaient les preuves des études de randomisation mendélienne et des études d'analyse de discordance, et soulignent ainsi que *« l'analyse de l'apoB est recommandée pour l'évaluation des risques, particulièrement chez les personnes avec des niveaux élevés de triglycérides, diabétiques, obèses, souffrant d'un syndrome métabolique ou avec des niveaux très faibles de LDL-C. Cette analyse peut être utilisée comme alternative au LDL-C, si disponible, comme mesure principale pour l'échantillonnage, le diagnostic, la gestion et peut être préférée, par comparaison au non-HDL-C, chez les personnes avec des niveaux élevés de triglycérides, diabétiques, obèses ou avec des niveaux très faibles de LDL-C. »* (Preuves Niveau C, Classe I).

## EXPÉRIENCE AU CUSM

Actuellement, le RUISSS de McGill (incluant le CUSM) utilise le bilan lipidique standard comme mesure standard pour l'évaluation et la gestion de la dyslipidémie, avec ajout de l'apoB lors de cas d'hypertriglycéridémie.

Un nombre total de 10,775 tests pour l'apoB ont été complétés au CUSM, comparativement à un total de 96,840 tests pour le cholestérol.

## Coûts

Le coût des tests de laboratoire est déterminé par la province de Québec. Pour l'année 2018-19, un test pour l'apoB coûtait 3,60 \$, comparativement au bilan lipidique standard (soit le cholestérol total, le HDL-C et les triglycérides) dont le coût était de 2,40 \$. En supposant que 100,000 tests lipidiques sont effectués annuellement au CUSM, le remplacement des tests standards par des tests de l'apoB se traduirait par un coût additionnel de 120,000 \$ pour l'hôpital. Cependant, puisque le coût moyen est lié au volume de tests, ce coût diminuera avec un usage croissant.

## CONCLUSIONS

- Il y a énormément de preuves indiquant que l'apoB est au moins aussi bon que le LDL-C pour prédire les risques de maladies cardiovasculaires dans la population

en général, et supérieur aux marqueurs courants (LDL-C, et non-HDL-C) pour prédire ces risques chez les populations discordantes.

- Les études indiquent qu'approximativement 20% de la population peut avoir un nombre élevé de particules mais avec un LDL-C faible ou normal, et échappent donc aux mesures lipidiques classiques. De plus, les patients avec un nombre discordant élevé de particules, relativement au contenu en cholestérol, sont plus susceptibles d'avoir des facteurs de risque de maladies cardiovasculaires associés à un syndrome métabolique. Ainsi, l'apoB peut améliorer la prédiction des risques, à la fois dans la prévention primaire et secondaire, en identifiant les patients à haut risque.
- Les tests de l'apoB sont précis, exacts, et facilement accessibles à des coûts d'opération raisonnables. De plus, ces tests évitent les désavantages associés aux mesures de LDL-C, tel que la nécessité d'utiliser des échantillons à jeun et les mesures imprécises à des concentrations très faibles de LDL-C.
- Le principal obstacle à l'adoption de l'apoB a été une réticence à perturber la pratique médicale si la prédiction des risques associés à l'apoB est identique à celle du LDL-C. Cependant, très peu d'attention a été accordée aux avantages de l'apoB chez les populations discordantes et si nous prenons en considération ce facteur, l'apoB devient cliniquement plus pertinent que le LDL-C comme mesure de routine. En outre, l'apoB peut simplifier la charge de travail du médecin en remplaçant 5 tests par un seul.
- Il semble y avoir un virage concernant les lignes directrices si l'on en juge par les récentes lignes directrices européennes qui adoptent lentement de nouvelles recommandations approuvant l'utilisation de l'apoB de routine.

## RECOMMANDATION

- Étant donné que l'apoB est équivalent au LDL-C pour prédire les risques de maladies cardiovasculaires chez la population en général, et supérieur au LDL-C chez la population discordante, nous recommandons que l'utilisation du test de l'apoB soit approuvée comme alternative au test du LDL-C pour l'évaluation et la gestion des risques de maladies cardiovasculaires :
  - chez les patients avec des facteurs de risque de maladies cardiovasculaires tels que des niveaux élevés de triglycérides, le diabète, l'obésité et le syndrome métabolique ;
  - chez les patients avec des valeurs LDL-C très faibles.



- Pour éviter une duplication des tests au RUISSS du CUSM, nous recommandons que le test de l'apoB remplace le test du LDL-C, à la discrétion du médecin traitant.
- Un programme éducatif pour les médecins sera développé par le Dr Sniderman et les acteurs concernés, pour favoriser une meilleure compréhension des avantages de l'apoB.
- Cette recommandation sera réévaluée dans un an, après l'évaluation des données recueillies et/ou la publication de nouvelles preuves dans la littérature scientifique.

## LIST OF ABBREVIATIONS

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ApoB	Apolipoprotein B
ASCVD	Atherosclerotic cardiovascular disease
BMI	Body mass index
BMI	Body mass index
BP	Blood pressure
CARDIA	The Coronary Artery Risk Development in Young Adults study
CETP	Cholesteryl Ester Transfer Protein
CHD	Coronary heart disease
CVD	Cardiovascular disease
ERFC	Emerging Risk Factor Collaboration
HDL-C	High density lipoprotein cholesterol
HMGCR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase,
HR	Hazard ratio
IDL	Intermediate lipoprotein
IHD	Ischemic heart disease
LC-MS	Liquid chromatography–mass spectrometry
LDL-C	Low density lipoprotein cholesterol
LDLR	LDL receptor
LPL	Lipoprotein lipase
MACE	Major adverse cardiovascular event
MESA	The Multi-Ethnic Study of Atherosclerosis
MI	Myocardial infarction
MUHC	McGill University Health Centre
NHANES	The National Health and Nutrition Examination Survey
OR	Odds ratio
PAV	Percent atheroma volume
RUISSS	Réseau universitaire intégré de santé et services sociaux [Network to advance the university's mission to promote health care, teaching and research]
TAU	MUHC Technology Assessment Unit
TC	Total cholesterol
TG	Triglycerides
VLDL	Very low density lipoprotein

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# EVALUATING THE VALUE OF APOLIPOPROTEIN B TESTING FOR THE ASSESSMENT AND MANAGEMENT OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE AT THE MUHC RUISSS

## 1. BACKGROUND

### 1.1. Apolipoprotein B (apoB) and other measures of lipids

The cholesterol contained within lipoproteins has been the traditional target for the assessment and management of atherosclerosis risk. As cholesterol is insoluble in water, it is bundled into a hydrophilic lipoprotein particle that enables its transport in water-based media such as blood or lymph. Lipoproteins are thus composed of a cholesterol and triglyceride (fat) containing core that is enveloped by a phospholipid monolayer, through which traverses a single molecule of an apolipoprotein. Apolipoprotein B (apoB), one of the two major classes of apolipoproteins, is the primary apolipoprotein of atherogenic lipid particles including low density lipoprotein (LDL), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL), and chylomicrons ([Figure 1](#)). ApoB lipoprotein particles smaller than 70 nm can cross the endothelial barrier, and their deposition within the arterial wall leads to the formation of atherosclerotic plaques, increasing the risk for atherosclerotic cardiovascular disease (ASCVD).<sup>1,2</sup>

There are several clinical markers of atherogenic particles in plasma ([Figure 1](#)):

- LDL cholesterol (LDL-C), the biomarker used in traditional lipid panels, which corresponds to the **mass of cholesterol within LDL particles**;
- Non-high density lipoprotein cholesterol (non-HDL-C), calculated as total cholesterol minus HDL cholesterol, which is a measure of the **mass of cholesterol within all atherogenic lipid particles** (LDL, VLDL, IDL, lipoprotein (a), chylomicrons, remnant particles);
- apoB, the total **number of apoB particles**, which corresponds to the total burden of atherogenic particles because each lipoprotein particle contains one apoB molecule.

## 1.2. The debate

Although LDL-C, non-HDL-C and apoB are highly correlated, there has been considerable debate spanning several decades as to which measure is the ideal lipoprotein test.<sup>3,4</sup> LDL-C has been the traditional measure of cardiovascular disease (CVD) risk. Yet, there is a lack of consensus among the various guidelines, with the most recent American guidelines continuing to support the sole use of LDL-C as the standard measure for CVD risk assessment.<sup>5</sup> The Canadian guidelines acknowledge the added utility of apoB, and recommend its use as an add-on or optional test.<sup>6</sup> In what may prove to be a turning point in the debate, the 2019 European guidelines have endorsed the use of apoB as the primary measure in the screening, diagnosis, and management of patients with high triglycerides, diabetes, obesity or metabolic syndrome, or low LDL-C levels.<sup>7</sup>

While much of the past discourse has assumed that the three biomarkers are similarly involved in the etiology of CVD, and has thus tried to parse differences in their predictive ability for CVD risk, more recent research has tried to highlight the etiological differences in the biomarkers. The retention of atherogenic lipid particles (i.e. apoB particles) within the arterial wall is a key factor in the eventual development of atherosclerosis,<sup>1,2</sup> and hence advocates of apoB contend that using apoB to estimate the number of atherogenic lipid particles is more in line with the current understanding of the pathophysiology of atherosclerosis.

The etiological roles of apoB and LDL-C may be teased out by studying populations who have discordant values for these biomarkers relative to population standards. This discordance arises due to inter-individual variability of the cholesterol content within apoB particles, whereby some individuals have small, cholesterol-depleted particles ([Figure 2](#), Panel B), and others have large, cholesterol-enriched particles ([Figure 2](#), Panel D).<sup>8-10</sup> At the same LDL-C concentration, individuals with cholesterol-depleted particles will have a greater number of lipid particles ([Figure 2](#), Panels A & B). Proponents of apoB argue that, if the cholesterol content within lipid particles is variable, then the *number* of lipid particles as estimated by apoB is a more accurate measure of ASCVD risk in these discordant populations, rather than cholesterol *mass* as measured by LDL-C.<sup>8,10</sup> Studies have shown that patients with cholesterol-depleted particles, i.e. discordantly low LDL-C and high apoB are more likely to have high triglycerides, low HDL-C, high BMI, and diabetes.<sup>10-13</sup> Thus, a reliance on LDL-C alone will lead to the under treatment of patients with cholesterol-depleted particles, and the overtreatment of those with cholesterol-rich particles ([Figure 2](#), Panels B and D).

This discordance between cholesterol mass (as measured by LDL-C or non-HDL-C) and number of lipid particles (measured by apoB or LDL-P) can help elucidate the debate on the ideal lipid test, by answering the following questions:

- Which measure is most useful for **risk assessment** i.e. predicting risk of ASCVD, even in **discordant populations**;
- Which measure is most useful for **risk management** i.e. predicting residual risk in statin-treated patients, for e.g. in patients who have attained treatment targets of LDL-C, but may have discordantly elevated apoB levels.

In addition to identifying the ideal marker for risk assessment and risk management, other points of evaluation include which lipid marker can be measured more accurately (analytical performance) and which is more cost-effective.

### 1.3. Context of the current report

Currently, LDL-C is the standard lipid test at the MUHC for assessing ASCVD risk, as part of the standard lipid panel that provides results for five lipid measures: three that are measured directly (HDL-C, total cholesterol, and triglycerides); and two that are calculated from the previous three (LDL-C and non-HDL-C). ApoB testing is provided for patients with hypertriglyceridemia. Given the inter-individual variability in apoB and cholesterol mass, the objective of this report is to evaluate the incremental benefit of apoB over standard measures, particularly LDL-C and non-HDL-C, in risk assessment and risk management of patients at the MUHC [RUISSS](#). This report was requested by Dr. Andre Dascal, director of the McGill RUISSS cluster of Optilab, on March 18, 2018.

## 2. OBJECTIVES

The objectives of this report are to:

- Evaluate the benefit of integrating apoB testing across the MUHC [RUISSS](#) for the purpose of:
  - Risk assessment, i.e. the ability of apoB to identify patients at risk of adverse cardiovascular outcomes, particularly those with discordant apoB and LDL-C levels;
  - Risk management in statin-treated patients, i.e. the ability of apoB to identify residual risk of adverse cardiovascular outcomes, particularly in patients with discordant apoB and LDL-C levels;
- describe current practice at the MUHC.

## 3. METHODS

### 3.1. Literature search and quality assessment

Since the focus of this report is to evaluate the additional benefit of apoB in discordant populations, we limited our literature search to studies of discordant populations comparing the ability of apoB versus traditional lipid markers to identify adverse cardiovascular outcomes including cardiovascular disease (CVD), coronary heart disease (CHD), myocardial infarction (MI), and ischemic heart disease (IHD). We restricted our search to systematic reviews and recent major articles on the subject. We also identified relevant guidelines from international societies.

Relevant scientific articles were identified by searching electronic databases and websites, scanning citation lists of retrieved articles, and consultation with experts. We searched PubMed/MEDLINE (OVID) using the following main search terms: discordance analysis, apolipoprotein B, and LDL-C. The search was last updated in July 2019.

### 3.2. MUHC experience

We describe the current policy for lipid testing at the MUHC. Laboratory volume estimates were provided by Dr. David Blank, Director of the Division of Clinical Biochemistry at the MUHC. For comparison, we approached some other institutions where apoB testing is carried out routinely, including Centre Hospitalier Université-Laval and University of British Columbia. Current practice at University of British Columbia was obtained from Dr. John Mancini, Director, CardioRisk Clinic, Vancouver Hospital.

## 4. RESULTS OF LITERATURE REVIEW

### 4.1. Conflicting reviews of the predictive ability of LDL-C and apoB in the general population

In an attempt to distinguish between the predictive ability of apoB, LDL-C and non-HDL-C for ASCVD risk in the general population, two large meta-analyses came to different conclusions.<sup>3,4</sup>

In 2009, the Emerging Risk Factor Collaboration (ERFC) conducted an individual patient-level meta-analysis of 68 studies, of which 22 studies (n=91,307; events=4,499) evaluated apoB.<sup>3</sup> Using a random-effects model, adjusting for several potential confounders including age, sex, systolic BP, smoking, BMI, diabetes, and other lipid markers, the

authors reported that apoB and non-HDL-C were similar in their abilities to predict CHD: adjusted hazard ratio (HR) and 95 % CI of CHD for a 1 standard deviation increase in apoB (29 mg/dl) was 1.58 (1.39, 1.79) vs 1.59 (1.36, 1.85) for a 1 standard deviation increase in non-HDL-C (43 mg/dl). In the subset of patients with directly measured LDL-C, the HR of CHD was 1.38 (1.09, 1.73) for LDL-C, and 1.42 (1.06, 1.91) for non-HDL-C.

A second meta-analysis in 2011 used aggregate-level data from 12 studies (n=233,455; events=22,950) and evaluated a variety of outcomes including CHD, CVD, IHD, and MI.<sup>4</sup> There was high between-study heterogeneity. Using a random-effects model, the authors reported that the relative risk ratios (95% CI) of adverse vascular outcomes were 1.43 (1.35 to 1.51) for apoB; 1.34 (1.24 to 1.44) for non-HDL-C and; 1.25 (1.18 to 1.33) for LDL-C, concluding that apoB was superior to non-HDL-C, which in turn was superior to LDL-C in predicting adverse cardiovascular outcomes. Given that only 3 of the studies included in the ERFC analysis have published data on apoB and non-HDL-C, these 3 studies were the only ones including in the second meta-analysis, resulting in very little overlap in the 2 reviews. The strength of this review, which included adjusted risk estimates that were converted to standardized relative risk ratios (i.e. risk per 1 standard deviation of the lipid marker), was the large number of events.

Very recently, a prospective analysis of the UK Biobank study (n=346,686) by **Welsh et al.** revisited this debate by comparing the conventional lipoproteins with the apolipoproteins.<sup>14</sup> In adjusted analyses, they report that the associations of the three biomarkers apoB, direct LDL-C, and non-HDL-C with a composite score of fatal/non-fatal CVD events were similar: HR for every 1 standard deviation increase in apoB, non-HDL-C, directly measured LDL-C, and Friedewald LDL-C were 1.23 (1.20, 1.26); 1.21 (1.18, 1.24); 1.20 (1.17, 1.23); and 1.17 (1.14, 1.20), respectively. However, when the analysis was restricted to the discordant population, the authors found that apoB was the only atherogenic lipid significantly associated with risk (discussed further in Section 4.3). The authors also calculated the c-statistic, which is a measure of how well a new test or risk factor discriminates between cases and non-cases, and is indicative of the probability that the novel predictor is higher in cases than in non-cases.<sup>15</sup> They report that the addition of apoB or LDL-C to a CVD risk model already containing total cholesterol and HDL-C did not substantively improve the model. However, the use of the c-statistic, also known as the area under the receiver operator curve (AUC), has several limitations, particularly related to interpretation of the clinical relevance of its results, and its ability to distinguish between the predictive importance of several risk factors in the same model.<sup>15</sup>

The ERFC authors conclude that, given the similar predictive power of apoB and non-HDL-C, the debate on which lipid measure to use should focus on practical issues such as cost and standardization of assays, rather than on the strength of the association with

cardiovascular outcomes. However, the studies included in their analyses did not focus on discordant populations, and hence the review was not able to evaluate the potential utility of apoB in such populations.

## 4.2. Discordant populations and discordance analysis

[Figure 2](#) illustrates the lipid measures giving rise to discordant populations, and the risk of under- or over-treatment. Individuals with cholesterol-depleted lipid particles have normal cholesterol levels but high apoB concentration. Conversely, individuals with cholesterol-rich particles have high cholesterol and low apoB levels. As the retention of apoB particles within the arterial wall and thus the number of apoB particles is a key factor in the eventual development of atherosclerosis, patients with a large number of cholesterol-depleted particles may be undertreated if using LDL-C measures alone, while those with cholesterol-rich particles may be over-treated. There is now considerable research on the risk of cardiovascular events in these discordant groups, summarized below.

### Discordance analysis

Discordance analysis evaluates the association between lipid measures and future CVD risk by restricting the analysis to only those individuals with discordant levels of lipid measures.<sup>12</sup> The rationale behind such analysis is that this restriction sharpens the signal by excluding the noise from patients with concordant values. Such analyses seek to evaluate the added value of these markers when they disagree. Several discordant analyses comparing lipid particle number with lipid cholesterol content have now been conducted in a wide variety of populations demonstrating the added value of apoB over non-HDL-C and LDL-C in identifying patients at residual risk of CVD.

Studies have evaluated several lipid markers and their association with risk of cardiovascular events. These include:

<b>Measures of number of lipid particles</b>	<b>Equivalent measures of cholesterol content (mass)</b>
LDL-P	LDL-C
apoB	Non-HDL-C



### 4.3. Role of apoB in risk assessment: Evidence from discordance analysis

We identified 8 discordance analysis studies that evaluated the association between discordance in apoB and cholesterol content measures, and risk of cardiovascular events, summarized in [Table 1](#).

For studies comparing apoB with LDL-C and that used a median cutoff for defining discordant values, discordance in both groups (i.e. high apoB/low LDL-C and low LDL-C/high apoB) was 18-19% of the population analysed. In statistical analyses, most studies evaluated the association of each lipid marker with CVD events in separate models, since these variables are highly correlated. A standardized beta coefficient was used, which assessed change in risk for every 1 standard deviation change in the lipid marker, to ensure the different models were comparable.

In sensitivity analyses of the UK biobank study mentioned above,<sup>14</sup> **Welsh et al.** conducted a discordance analysis by identifying participants with a  $\geq 10\%$  difference in their baseline percentiles of apoB and LDL-C ( $n=63,520$  i.e. 18% of the study population). They reported adjusted (age, sex, ethnicity, SBP, DBP, antihypertensive medication, diabetes, and smoking) associations between various lipids and a composite CVD outcome in patients not taking statins and no history of CVD at baseline. When lipid markers were included as continuous variables in linear models, only apoB was associated with the composite CVD score [HR per 1 standard deviation: 1.23 (1.12, 1.35)] ([Table 1](#)). No associations were found for non-HDL-C [HR: 1.08 (0.89, 1.18)] or for direct or calculated LDL-C [HR: 1.00 (0.91, 1.10); and 1.00 (0.91, 1.09), respectively]. Similar associations were found using categorical variables (quintiles) instead of continuous measures for each biomarker.

In an analysis of the Women's Health Study by **Mora et al.**, 27,533 women were followed for a median of 17.2 years. The authors used a median cutoff to define discordance between LDL-C and 3 measures: LDL-P, non-HDL-C, and apoB, and determined time to a coronary event in these three discordant populations. This study also found that women with discordantly high particle number (as measured by apoB or LDL-P) and low LDL-C had unfavourable risk profiles including high BMI, elevated triglycerides, low HDL-C, and smaller LDL particles. They report that women with apoB  $\geq$  median and LDL-C  $<$  median had an increased risk of incident CHD events [age-adjusted HR: 2.48 (2.01, 3.07)] compared to the low/low concordant group. The HR in women with high non-HDL-C/low LDL-C was 2.92 (2.33, 3.67), and that for women with high LDL-P/low LDL-C was 2.32 (1.88, 2.85) ([Table 1](#)). Associations were attenuated but remained significant in fully-adjusted models additionally including randomized treatment assignment, hormone use, postmenopausal status, smoking, blood pressure, diabetes, body mass index, HDL

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cholesterol, triglycerides, high-sensitivity C-reactive protein, and parental history of premature MI.

**Pencina et al.** defined discordance as the difference between observed values of apoB vs those expected based on their LDL-C levels (i.e. residuals from linear regression of apoB and LDL-C), in 2966 participants of the Framingham Offspring study. Participants in the highest tertile (apoB discordantly higher than LDL-C) were more likely to be obese, diabetic and hypertensive, have lower HDL-C levels and higher triglycerides compared to those in the bottom tertile (apoB discordantly lower than LDL-C). In multivariable-adjusted survival analysis for the onset of new coronary heart disease, a one SD increase in the difference between observed and expected values of apoB (based on LDL-C) was associated with a HR of 1.26 (95% CI: 1.15, 1.37). Similar results were obtained when examined as tertiles, and when using non-HDL-C levels. ([Table 1](#))

**Lawler et al.** similarly defined discordance using residuals from linear regressions models of apoB and LDL-P vs non-HDL-C in a sample of 27, 533 women in the Women's Health Study. They report that 14% of the population had discordant apoB vs non-HDL-C values, and those with discordantly higher particle number vs non-HDL-C were more likely to have risk factors associated with metabolic syndrome compared to the concordant or discordantly lower particle number groups. In Cox proportional hazards models adjusted for CHD risk factors, women with discordantly high apoB had a higher risk of incident CHD vs the concordant group [HR: 1.22 (95% CI: 1.07,1.39)]. ([Table 1](#))

The earliest discordance study was conducted by **Sniderman et al.** in 2003 in 2,103 men without CAD enrolled in the Quebec Cardiovascular Study, who were followed for 5 years. The authors evaluated discordance between apoB and LDL-C, non-HDL-C, and total cholesterol by dividing the cohort into quintiles for each measure. Discordance was defined as a difference between 2 biomarkers of greater than 1 quintile; discordance of apoB with LDL-C in the 5 quintiles ranged from 34% to 67%, with the greatest discordance in the middle quintiles. Participants with discordantly higher apoB than LDL-C were more likely to have higher BMI, lower HDL-C, and higher triglycerides than those with discordantly higher LDL-C than apoB. In survival analysis of time to CAD, men with disproportionately higher apoB to LDL-C were 3.2 (95% CI: 1.2, 8.1) times more likely to have an event compared to men in the lowest quintiles for both apoB and LDL-C, while those with disproportionately higher LDL-C were 3.0 (95% CI: 1.1, 7.8) times more likely to develop CAD ([Table 1](#)).

Two studies, in different populations— one using participants in the Framingham offspring study, and the other using MESA, a racially diverse population— compared LDL-P with LDL-C in predicting incident CVD events. In the Framingham analysis by **Cromwell et al.** that was adjusted for adjusted for age, gender, systolic and diastolic blood pressure, smoking, and lipid medication use, the authors report an HR per 1 SD increase in LDL-P

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and LDL-C of 1.28 (1.17-1.39) and 1.11 (1.01-1.22), respectively ([Table 1](#)). In discordance analysis using a median cutoff, they report better event-free survival in the low LDL-P/high LDL-C group vs. the high LDL-P/low LDL-C discordant groups.

The MESA analysis by **Otvos et al.**, which was adjusted for age, gender and race, found that only LDL-P was a significant predictor of 1<sup>st</sup> incident CVD event in discordant groups ([Table 1](#)). Furthermore, participants with discordantly higher LDL-P vs LDL-C were more likely to have several risk factors for CVD including high glucose, insulin intolerance, obesity, elevated triglycerides, low HDL-C and small LDL size.

In a discordance analysis of young adults in the CARDIA study by **Wilkins et al.**, using a median cutoff, discordantly high apoB/low LDL-C was better able to predict year 25 coronary artery calcification (CAC) in fully adjusted models [OR: 1.55 (1.10,2.18)] vs. discordantly high LDL-C/low apoB [OR: 1.29 (0.91,1.83)] ([Table 1](#)). Furthermore, this analysis found a dose-response relationship between increasing apoB levels and year 25 CAC. Individuals in the highest apoB tertiles were more likely to have higher fasting glucose levels, blood pressure, BMI, and elevated triglycerides.

#### **4.3.1. Summary of findings**

- These studies were conducted in a diverse group of people (young adults, women, white men, racially diverse participants) and used a variety of cutoffs to define discordance, from median cut points to percentile differences. Study sample sizes ranged from 2794 to 63,520 persons, and outcomes included incident cardiovascular events, carotid intima thickness, and coronary artery calcification. All analyses adjusted for age, sex, and race at a minimum, and most included several other CVD risk factors.
- All studies consistently found particle number (apoB or LDL-P) to have a stronger association with CVD risk than LDL-C in discordant populations, including the largest study using the UK biobank data. Patients with higher levels of apoB, irrespective of levels of LDL-C, had consistently worse outcomes than those with lower levels of apoB. In discordant groups, participants with high apoB/low LDL-C had risk levels more similar to participants with concordant high apoB/high LDL-C measures.<sup>8,9,13</sup> Conversely, patients with discordantly low apoB/high LDL-C measures were more similar to the concordantly low apoB/low LDL-C group, with lower overall risk of events. **Hence, when particle number and cholesterol mass disagree, CVD risk appears to track with particle number.** These results highlight the utility of apoB in identifying patients at risk despite low LDL-C levels.
- A majority of studies demonstrated that participants with discordantly higher apoB than LDL-C were more likely to have several CVD risk factors including

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higher BMI, blood pressure, and fasting glucose, and elevated triglycerides. Furthermore, these individuals had smaller LDL particle size. These results indicate that the residual risk in such patients is due to the higher number of atherogenic particles.

- Finally, studies of the analytical performance of these tests have shown that the low coefficient of variation associated with apoB and cholesterol content measures indicates that the discordance between these biomarkers cannot be attributed to measurement variability or test imprecision.<sup>16</sup> Hence, this discordance indicates that apoB and cholesterol content are not identical markers of CVD risk in a large segment of the population.

#### 4.4. Role of apoB in risk management: Evidence from discordance analysis

A substantial subset of patients receiving lipid-lowering therapies continue to exhibit residual risk for CVD.<sup>17</sup> Statins lower LDL-C, non-HDL-C and apoB to differing degrees: a meta-analysis of 11 statin trials including 17,000 patients reported reductions of 43%, 39% and 33% for LDL-C, non-HDL-C, and apoB, respectively.<sup>18,19</sup> More recently, **Wong et al.** analysed 473 statin-treated adults who participated in the NHANES 2009-10 survey, and reported that 64% and 63% were at goal for LDL-C (target set at different levels based on risk profile) and non-HDL-C (targets set at 30mg/dl above LDL-C targets), respectively, but only 52% were at goal for apoB (set at the corresponding LDL-C percentiles).<sup>20</sup> Non-Hispanic blacks, and those with CVD or CHD were least likely to reach target goals for the three biomarkers. Up to 50% of participants, depending on the risk profile, had residual elevated apoB despite being on target for non-HDL-C.

The lesser reductions in apoB versus LDL-C and non-HDL-C could be because statins tend to lower larger cholesterol-enriched particles to a greater degree than smaller cholesterol-depleted particles.<sup>21</sup> Could the smaller reductions in apoB be associated with the residual CVD risk in statin-treated patients?

We identified 3 studies that evaluated discordance in lipid measures and CVD outcomes among statin-treated patients, one of which included an analysis of 9 clinical trials.<sup>22-24</sup> Results are summarized in [Table 2](#).

- A discordance analysis by **Sniderman et al.** of the INTERHEART study—an ethnically-diverse case-control investigation of acute MI—included 9345 cases and 12,120 age- and sex-matched controls.<sup>23</sup> Three equal groups of participants were created based on whether their population percentiles of apoB and non-HDL-C differed by at least 5%: non-HDL-C > apoB (cholesterol-enriched particles); non-HDL-C ~ apoB (concordant group); apoB > non-HDL-C (cholesterol-depleted particles). Participants with cholesterol-enriched particles were less likely to have

hypertension, diabetes, or be a current smoker than those with cholesterol-depleted particles. In addition, the non-HDL-C/apoB ratio, which is an indication of the cholesterol content per non-HDL-C particle, was higher in the non-HDL-C > apoB group, confirming that this group had a larger number of cholesterol-rich particles.

The authors report that the odds ratio of an acute MI was lower for participants with cholesterol-enriched particles (OR: 0.72; 95% CI: 0.67, 0.77) as compared to the concordant group, while the OR was higher in participants with cholesterol-depleted particles (OR: 1.48; 95% CI: 1.38, 1.58) ([Table 2](#)). The risk in MI tracked with the concentration of apoB rather than non-HDL-C levels. This relationship held even when stratified by various ethnic groups, and for a range of percentile differences from 1% to 10%: ORs for 1% to 10% percentile differences when non-HDL-C > apoB ranged from 0.67 to 0.76, and from 1.35 to 1.61 when apoB > non-HDL-C.

- In an analysis of 9 clinical trials of patients (n=4957) with coronary artery disease (CAD), **El Shazly et al.** evaluated the association between discordant levels of TC/HDL-C and apoB, LDL-C and non-HDL-C to assess percent atheroma volume (PAV) changes and major adverse cardiovascular events (MACE).<sup>22</sup> The authors report considerable discordance between TC/HDL-C and the 3 lipid measures: 20% with non-HDL-C, 26% with LDL-C, and 27% with apoB, despite treatment with statins. These 3 lipid measures were included in separate survival models for the incidence of MACE, and the authors reported a residual risk of MACE when these markers are below the median, and TC/HDL-C is above the median ([Table 2](#)). This study underscores the point that there remains a residual risk of CVD in statin-treated patients even when the targeted biomarker (LDL-C, or non-HDL-C, or apo-B) is low, indicating the need for the use of more than one biomarker, particularly in discordant populations.
- **Tehrani et al.** examined the association between discordant markers and CHD/CVD events in a population of statin-treated patients with diabetes (n=838) or metabolic syndrome (n=1596) versus those without these conditions (n=3983) in the MESA study.<sup>24</sup> Discordance as a continuous variable was defined as the difference between an individual's lipid particle and cholesterol mass percentiles (LDL-P% - LDL-C%). The authors reported that, on average, participants with diabetes or metabolic syndrome had a greater number of lipid particles relative to their cholesterol concentration (cholesterol-depleted particles), while the opposite was true for controls. In survival analysis, the LDL discordance variable (LDL-P > LDL-C) was significantly associated with CHD and CVD events only in the metabolic syndrome groups [HR for CHD: 1.21 (1.01, 1.47) and HR for CVD: 1.26

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(1.07, 1.47)]. In all participants combined, LDL-P and LDL-C similarly predicted risk for CHD [HR: 1.16 (1.05, 1.28) and 1.17 (1.06, 1.28), respectively] ([Table 2](#)).

#### **4.4.1. Summary of findings:**

- The above studies indicate that many patients, irrespective of risk group, still have residual risk of CVD despite statin therapy.
- Most studies found that patients with cholesterol-depleted particles, i.e. those with discordantly elevated particle number relative to cholesterol mass are more likely to be diabetic or have risk factors for metabolic syndrome.
- Risk of CVD was elevated particularly in discordant populations, i.e. patients who were at or below target goals for LDL-C or non-HDL-C, but remained above goal for apoB.

#### **4.5. Evidence from Mendelian randomization studies**

The results of the aforementioned observational studies were bolstered by two recent Mendelian randomized studies.<sup>25,26</sup> Mendelian randomization studies rely on naturally occurring genetic variants that are randomly distributed across the population to evaluate the associated phenotypes. The random distribution of these genetic variants assures that confounding factors are evenly balanced between the groups being compared, thus approximating a randomized controlled trial.

##### **4.5.1. Unravelling the role of apoB vs LDL-C in impacting CVD risk**

In the first study, **Ferrence et al.** aimed to tease out the roles of apoB and LDL-C in affecting CVD risk.<sup>25</sup> They included individual-patient data from 102,837 participants in studies contained in the National Centre for Biotechnology Information database of Genotypes and Phenotypes program. The authors analysed genetic variants of two genes involved in cholesterol metabolism: the CETP gene, which encodes the target of CETP inhibitor drugs that lower LDL-C; and the HMGCR gene which encodes the protein targeted by statins to lower cholesterol. The aim was to evaluate whether the mechanism by which LDL-C is lowered plays a role in the beneficial effect of lipid-lowering therapy on CVD risk.

- The authors created a genetic score for each participant that corresponded to the gene activity of their genetic variants, such that higher scores corresponded to more underactive versions of the protein encoded by the gene. Lower CETP or HMGCR gene activity (i.e. higher scores) is equivalent to receiving CETP inhibitor or statin therapy, respectively.

- In a 2x2 factorial design with scores dichotomized at the median, individuals with scores above the median for either CETP or HMCGR showed reductions in LDL-C and apoB, and had a lower risk of major cardiovascular events relative to participants with scores below the median ([Table 3](#)).
- Participants with higher scores for *both* genes (equivalent to receiving CETP inhibitor and statin combination therapy) had an additive decrease in LDL-C and an additive increase in HDL-C; however, the decrease in apoB was less than expected, and there was no further reduction in CVD risk compared to those with high scores only for HMCGR ([Table 3](#)). This finding suggests that lowering apoB plays an additional role in lowering CVD risk, over and above lowering LDL-C.

#### 4.5.2. Unravelling the roles of apoB, LDL-C and triglycerides in CVD risk

In a more recent Mendelian randomization analysis, the role of the *number* of atherogenic particles, as measured by apoB, versus the cholesterol or triglyceride content of these atherogenic particles in affecting CVD risk was further delineated.<sup>26</sup> This study sought to evaluate the clinical benefit of lowering triglycerides on cardiovascular outcomes. As with cholesterol-rich particles, triglyceride-rich particles including chylomicrons, chylomicron remnants, and VLDL each contain one molecule of apoB ([Figure 1](#)). Thus, the effect of lowering triglycerides on CVD risk can be directly compared to the effect of lowering LDL-C by comparing their effects per unit change in apoB.

- Participants in 63 cohort or case-control studies (n=654,783) conducted between 1948 and 2017 in Europe and North America were included. The authors created scores from genetic variants of two genes: variants in the lipoprotein lipase (LPL) gene corresponding to the effects of triglyceride-lowering therapies that increase LPL activity, and variants in the LDL receptor gene (LDLR), mimicking therapies that lower LDL-C by increasing LDLR activity.
- The authors report that higher genetic scores, corresponding to greater gene activity, for the LPL gene were associated with large reductions in triglycerides (-69.9 mg/dl; 95% CI: -68.3, -71.6), but no discernible change in LDL-C (0.7 mg/dl; 95% CI: 0.0, 1.4). In contrast, participants with higher scores for LDLR showed substantial declines in LDL-C (-14.2 mg/dl; 95% CI: -13.6, -14.8), but only small reductions in triglycerides (-1.9 mg/dl; 95% CI: -0.1, -3.9).
- Despite these differential effects on the two lipid measures, both scores were associated with similar decreases in CVD risk for every 10 mg/dl change in apoB (OR for LPL: 0.771 (0.741, 0.802) vs OR for LDLR: 0.773 (0.747, 0.801).

- In a 2x2 factorial design with scores dichotomized at the median, changes in triglycerides, LDL-C and apoB were additive for participants with higher scores for *both* genes ([Table 4](#)). However, the risk reduction in CVD appeared to be proportional to the absolute change in apoB, but not to changes in triglycerides or LDL-C.
- In a multivariable meta-regression analysis of 186 genetic variants associated with either LDL-C, triglycerides, or both, the addition of apoB to a model containing both triglycerides and LDL-C reduced the associations of these latter two lipids with CVD risk to null.

As triglyceride and cholesterol are transported within apoB particles, the authors conclude that the similar reductions in CVD risk seen in their study with decreases in triglyceride or cholesterol indicate that:

- apo-B containing particles i.e. triglyceride-rich VLDL and cholesterol-rich LDL, have a similar effect on CVD risk; and
- reductions in apoB i.e. particle number drive the clinical benefit of lowering triglycerides or LDL-C; this clinical benefit of lipid-lowering therapies is proportional to the absolute reduction in apoB, irrespective of changes in lipid concentrations.

#### 4.6. Reliability and accuracy of the lipid measurements

The European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), in a consensus statement on quantifying atherogenic lipoproteins, reviewed the evidence on the measurement reliability of all the atherogenic lipid measures, summarized below.<sup>16</sup> In terms of analytical performance, they conclude that apoB more accurately reflects particle number, is relatively inexpensive, and is more reliable than LDL-C measurements at low LDL-C levels.

##### 4.6.1. LDL-C

Direct measurement of LDL-C: LDL-C can be directly assayed using CDC reference methods ( $\beta$  quantification); assay kits from most manufacturers are standardized against a Cholesterol Reference Method Laboratory Network (CRMLN) laboratory.<sup>27</sup> However, recent data suggest that there is considerable heterogeneity between assays from different manufacturers, especially due to non-selectivity errors, i.e. variability in the definitions from different manufacturers to selectively isolate cholesterol.<sup>28-30</sup>

The National Cholesterol Education Program (NCEP) recommends that the total error (imprecision or reproducibility + systematic error or bias) in LDL-C measurements fall



within 12% and 13% of the true value obtained from a reference standard.<sup>27,31</sup> While these errors are minimized in normal patient samples, in patients with atypical lipid profiles, e.g. hypertriglyceridemia, diabetes, or chronic kidney disease, these errors can range from -26% to +32% for LDL-C, when compared with ultracentrifugation references.<sup>28</sup> Such errors normally arise from the high heterogeneity in the operational definitions of LDL fractions used by different assays, leading to non-specificity to different subclasses of LDL-C.

Calculated LDL-C: Most laboratories calculate LDL-C, using the Friedewald formula developed in 1972:

LDL-C = Total cholesterol – HDL-C – VLDL-C, where VLDL-C is estimated as TG/5 in mg/dl.<sup>32</sup>

However, this calculation is not without concerns<sup>16</sup>:

- The calculated value of LDL-C incorporates 3 direct measurements – total cholesterol, HDL-C and VLDL-C- thus amplifying the measurement error.
- The formula assumes a constant TG:cholesterol ester ratio, which may not be the case in non-fasting samples, thereby underestimating LDL-C at high TG concentrations. It has been demonstrated that the Friedewald formula underestimates LDL-C when compared to a different novel estimate, which assumes that the TG: VLDL ratio is an adjustable factor and not a constant.<sup>33</sup> The Friedewald equation is considered invalid at TG>400 mg/dl, and in type III dyslipoproteinemia, wherein LDL-C is overestimated.<sup>29,34</sup>
- The formula is less reliable and accurate at lower LDL-C concentrations, which is a particular problem with newer, highly effective LDL-C lowering therapies.<sup>35-38</sup> Underestimation of LDL-C with the Friedewald formula can lead to misclassification of patients based on a cut-off of  $\leq 70$  mg/dl ranging from 29% in normotriglyceridemic patients to 59% in a hypertriglyceridemic population, versus ultracentrifugation direct techniques.<sup>35,36</sup>

Clinical significance of LDL-C measurement errors: Between-method and between-laboratory differences may mask or erroneously attribute these difference to the benefits of lipid-lowering therapies. Guidelines thus recommend that patients be monitored over time with the same laboratory and method, because current recommendations target % reductions in LDL-C (e.g.  $\geq 50\%$ ), rather than achieving specific targets.<sup>39</sup>

#### **4.6.2. ApoB**

According to a joint consensus statement from the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine

(EFLM), apoB and non-HDL-C tests are more accurate than direct and calculated measurements of LDL-C, particularly in non-fasting, hypertriglyceridemic samples, or those with very low LDL-C concentrations.<sup>16</sup>

As apoB is a clearly defined protein, standardized measurement techniques are available based on the International Federation of Clinical Chemistry (IFCC)/WHO reference standards.<sup>40</sup> ApoB can be quantified more accurately than direct measures of LDL-C or HDL-C, and inter-laboratory variability has been reduced substantially with uniform calibration techniques.

Some of the other advantages of apoB over other measures include:

- There is less inter-individual variability in apoB, as compared to non-HDL-C
- It is independent of variations in TG concentrations, and hence can be measured in non-fasting samples
- Improvements in new methods, such as LC-MS/MS-based quantification of apolipoproteins will allow for wider uptake of apoB testing, while also allowing for production of a complete apolipoprotein profile (apoC and ApoE) for more accurate diagnosis and management of dyslipidemia.

#### **4.7. Cutoff (thresholds) for initiating therapy and for treatment targets**

One of the arguments against the incorporation of apoB in lipid testing is that there is no established cut point for initiating therapy, or achieving treatment goals. However, the same can be said of the other lipid markers, because thresholds for most biomarkers are arbitrarily established by a consensus of experts or societies. For example, the most recent European guidelines have recommended lower thresholds for all lipid measures ([Table 5](#)).

##### **4.7.1. LDL-C and non-HDL-C cutoffs**

Treatment target values for very high-risk patients are an LDL-C below 70 mg/dl (1.8 mmol/L) or a non-HDL-C below 100 mg/dl (2.59 mmol/L), which correspond to the 10<sup>th</sup> percentile of the American population. Non-HDL-C target values have been arbitrarily defined by consensus guidelines to be 30 mg/dl above LDL-C cut-offs, based on the assumption that VLDL-C values are normal at TG values below 150 mg/dl (i.e. a VLDL-C of 30 mg/dl).<sup>16</sup>

#### 4.7.2. apoB cutoffs

The consensus report from the American Diabetes Association and the American College of Cardiology was the first to recommend a treatment target for apoB, which they set at 80 mg/dl for very high risk subjects and 90 mg/dl for high risk subjects based on their consensus evaluation of the available evidence.<sup>41</sup>

If the rationale was to set a population-equivalent cutoff for apoB corresponding to the 10<sup>th</sup> percentile of the population, then a treatment target of 80 mg/dl is too high, according to recent research. In an analysis of 2518 nationally representative participants in the NHANES survey, and 126,092 participants in the VLDL study that comprised patients referred for lipid testing, the authors found that 80 mg/dl corresponded to approximately the 31<sup>st</sup> to 36<sup>th</sup> percentile of the American population (i.e. LDL-C of 100 mg/dl).<sup>42</sup> They report that the population-percentile equivalent apoB value that corresponds to an LDL-C value of 70mg/dl was approximately 60 mg/dl (59 mg/dl in NHANES and 63 mg/dl in the VLDL database, equivalent to the 7<sup>th</sup> and 9<sup>th</sup> percentile of the population). Another analysis of the NHANES data reported similar results, where weighted linear regression estimated the respective apoB targets corresponding to the same percentiles for LDL-C targets. The authors found that LDL-C targets of 70 mg/dl and 100 mg/dl corresponded to apoB values of 62.3 mg/dl and 80.6 mg/dl, respectively.<sup>20</sup>

As statins lower LDL-C and non-HDL-C more than they do apoB, targeting a goal of 80 mg/dl for apoB would leave patients exposed to residual CVD risk, particularly in the discordant populations.<sup>43</sup> Thus the new ESC guidelines recommend the use of apoB <65 mg/dL, <80 mg/dL, and <100 mg/dL in very-high, high, and moderate CVD risk populations, respectively.<sup>7</sup>

#### 4.8. Target attainment and effect on CVD risk

The European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) consensus statement concluded that the evidence base for intensifying treatment to further lower apoB to the above targets is incomplete. There are indications that lowering apoB to lower targets may have additional benefit versus lowering LDL-C. **Thanassoulis et al.** estimated the additional benefits of attaining non-HDL-C or apoB targets instead of LDL-C targets.<sup>44</sup> Using the overall risk reduction per standard deviation obtained from a meta-analysis of 7 statin trials, the authors showed that reducing biomarker levels to LDL-C, non-HDL-C and apoB targets of 70 mg/dl, 90 mg/dl, and 65 mg/dl, respectively, would result in proportionally greater reductions in CHD risk for apoB than the other markers (12% per 1-SD decrease in LDL-C vs. 19% per 1-SD decrease in apoB), and that these reductions would be even greater for higher baseline levels of these markers. For example, a 42% reduction in LDL-

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C levels (from 120 mg/dl to 70 mg/dl) would result in a risk reduction of 32%, while a 42% reduction in apoB values (from 112 mg/dl to 65 mg/dl) would result in a risk reduction of 39%.

#### 4.9. Cost of various tests

Average costs are a function of volume, and as apoB tests become more widespread, average costs will decrease. The current cost of apoB relative to the lipid panel is slightly higher (see section [5.2](#) for costs at the MUHC). According to the 2018 Clinical Diagnostic Laboratory Fee Schedule of the American Medical Association, a lipid panel test costs Medicare USD 13.39, while apolipoprotein assays cost USD 21.09.<sup>45</sup>

In Europe, the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) consensus statement noted that apoB tests are available at a relatively low cost, and given apoB's superiority in analytical performance over direct or calculated LDL-C, the cost of apoB is immaterial.<sup>16</sup>

#### 4.10. Guidelines/consensus statements

- **Canadian Cardiovascular Society guidelines:** The 2016 Canadian Cardiovascular Society guidelines continue to recommend LDL-C as the primary target for risk assessment of dyslipidemia, "because clinicians are most familiar with LDL-C... but anticipate a shift to preferential use of non-HDL-C or apoB in the future." apoB and non-HDL-C are currently recommended as optional secondary targets.<sup>6</sup>
- **The American Heart Association/American College of Cardiology guidelines:** The most recent AHA/ACC guidelines released in October 2018 acknowledge that apoB is a 'stronger indicator of atherogenicity than LDL-C alone'.<sup>5</sup> However, they conclude that the greater expense and unreliable laboratory measurement of apoB preclude it from being used as a routine measure of risk assessment in primary prevention; rather, the guidelines recommend it be used as a risk-enhancing factor in selected patients whose TG levels  $\geq 200$ mg/dl. The guidelines do not cite any of the above substantial literature in discordant populations, or the Mendelian randomization studies, which suggest that apoB is able to identify populations at risk of CVD despite normal LDL-C levels. The guidelines also do not give any reason for excluding this evidence. Furthermore, as shown in sections [4.6](#) and [4.9](#) above, apoB was determined to have the same or better analytical performance than LDL-C, at reasonable operating costs.
- **The European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) guidelines:** In contrast to

the AHA/ACC guidelines, the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), in a May 2018 consensus statement on quantifying atherogenic lipoproteins, extensively reviewed all the evidence, including the value of apoB in discordant populations, and measurement reliability of apoB versus other lipids.<sup>16</sup> In terms of analytical performance, they conclude that apoB more accurately reflects particle number. With respect to clinical performance, the guidelines state that discordance analysis shows that apoB improves risk assessment. However, in terms of cost-effectiveness, the guidelines conclude that there is no evidence yet that apoB or non-HDL-C are cost-effective measures in comparison to LDL-C, and hence they recommend the use of apoB only as a secondary target, with LDL-C remaining the standard measure. Overall, they recommend apoB for dyslipidemia characterization, and as optional measure for CVD risk estimation and treatment target, but not recommended for treatment choice.

- **European Society of Cardiology/European Atherosclerosis Society guidelines:** The 2019 European Society of Cardiology/European Atherosclerosis Society guidelines also differ from the 2018 AHA guidelines.<sup>7</sup> They included evidence from the Mendelian randomization studies and the discordance analysis discussed above, and thus recommend that “ApoB analysis is recommended for risk assessment, particularly in people with high TG levels, diabetes, obesity, metabolic syndrome, or very low LDL-C levels. It can be used as an alternative to LDL-C, if available, as the primary measurement for screening, diagnosis, and management, and may be preferred over non-HDL-C in people with high TG levels, diabetes, obesity, or very low LDL-C levels.” (Class I, Level C evidence)

## 5. EXPERIENCE AT THE MUHC

### 5.1. Current practice

Currently, the standard test to measure lipids at the MUHC RUISSS is the standard lipid panel. Apo B is ordered in the case of hypertriglyceridemia. [Table 6](#) shows the volume of tests at the MUHC for the 2017-2018 fiscal year. A total of 10,775 tests for apoB were done at the MUHC, compared to 96,840 tests for total cholesterol.

In other Canadian jurisdictions, current practice at the Centre Hospitalier Universitaire de Quebec-Université Laval is to order apoB along with the standard lipid panel. At the Vancouver Coastal Health network of hospitals, apoB is ordered along with the standard

lipid panel in cases of suspected complex dyslipidemia (such as hypertriglyceridemia); apoB is ordered as the only test for follow-up (personal communication).

## 5.2. Cost

The cost of the various lipid tests across the province of Quebec for 2019-2020, which are published by the provincial health ministry, are shown in [Table 6](#).<sup>46</sup> The cost of an apoB test is \$3.60, while that for a standard lipid panel (total cholesterol, HDL-C, and triglycerides) is \$2.40. Assuming 100,000 lipid panel tests are performed annually at the MUHC, replacing the standard panel test with apoB would result in an additional cost to the hospital of \$120,000. However, as the average cost is a function of volume, as the use of apoB becomes more widespread, its cost will decrease.

## 6. DISCUSSION

### 6.1. Current evidence for use of apoB

ApoB has several advantages over other lipid markers as shown in [Table 7](#). Furthermore, the use of apoB or non-HDL-C simplifies the testing process by replacing 5 lipid measures with a single marker.

#### 6.1.1. Clinical effectiveness

The European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) consensus statement summarized the current evidence for various criteria with respect to the different lipid markers ([Table 7](#)).

The authors of this statement conclude that apoB is probably superior to LDL-C in predicting risk in the general population.<sup>16</sup> Furthermore, based on our review of the numerous studies in discordant populations summarized above, there is considerable evidence showing that apoB is superior to LDL-C in assessing residual risk in primary and secondary prevention of CVD. In particular, apoB contributes novel information beyond existing markers in populations with discordant levels of apoB and LDL-C/non-HDL-C. These studies have demonstrated that patients with discordantly elevated particle number versus cholesterol content are more likely to have CVD risk factors associated with metabolic syndrome. Thus the use of apoB has the potential to improve risk prediction, both in primary and secondary prevention, by identifying those at high risk.

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### **6.1.2. Analytical performance**

Based on an extensive review of the current evidence, the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) consensus statement concluded that apoB assays are both precise and accurate, and are widely accessible at reasonable operating costs.<sup>16</sup> The statement notes that apoB tests are fully automated and can be easily integrated into available platforms.

### **6.1.3. Population impact**

Based on the discordance studies cited in this report, approximately 20% of the population may have discordantly elevated apoB particles despite low or normal cholesterol levels.

As an exercise to illustrate the additional cases of CVD events that would be prevented by using apoB, an analysis by **Sniderman et al.** applied the relative risks obtained from their systematic review for apoB, non-HDL-C and LDL-C to the NHANES 2005-2006 survey population, which is representative of the adult US population.<sup>4</sup> This analysis determined that using non-HDL-C would identify an additional 300,000 cases compared to LDL-C (1.8 million vs 1.5 million), while targeting apoB would identify an excess 500,000 patients (2.3 million vs 1.5 million cases).

## **6.2. Barriers to adoption**

Despite the advantages described above, there are significant barriers to the adoption of apoB as the primary measure for dyslipidemia risk assessment and management:

### **6.2.1. Maintaining the status quo**

There is a long-held assumption that apoB is equivalent to LDL-C in predicting risk of CVD events, and hence there is no benefit in disrupting current practice to educate clinicians and patients about a novel measure for dyslipidemia.

However, such a view ignores the considerable swathe of the population with discordant particle number relative to cholesterol content, in whom risk is elevated but who would be missed by current markers. Some guidelines recommend apoB as a secondary marker in these populations, but if apoB is equivalent to LDL-C in concordant populations and superior in discordant populations, then replacing this cumbersome 2-step procedure with a single apoB test rather than an add-on test makes clinical sense. apoB is hence a good candidate in this era of personalized medicine, where patients can be offered therapy targeted to their individual condition.<sup>16</sup>

### 6.2.2. Patient and physician reticence and systemic challenges

As LDL-C and HDL-C are strongly ingrained in the mainstream consciousness, and there is a general lack of awareness of the added utility of apoB, both physicians and patients may be reluctant to adopt apoB.

However, some Canadian jurisdictions that have integrated apoB testing have found that patients have adapted well to the change, with little resistance to the new test once they received an explanation for why the standard lipid panel was replaced by apoB. They also report that primary care physicians are well acquainted with the benefits of apoB, and their major obstacle to ordering the test is lack of coverage by insurance companies (personal communication). Most insurance companies do not accept non-HDL or apoB for access to PCSK9 inhibitors, a situation that may change only when apoB has been endorsed by major guidelines. These issues were echoed by experts within the McGill RUISSS (*Réseau Universitaire Intégré de Santé et Services Sociaux*) [see [Appendix](#)].

In other jurisdictions like the US, a barrier to implementation is the delayed availability of apoB results relative to the other lipid measures. This is because apoB samples are often analysed in external laboratories, due to the low volume of apoB tests ordered (personal communication).

### 6.2.3. Lack of evidence for proven clinical benefit and cost-effectiveness

Research indicates that apoB levels remain elevated despite attainment of LDL-C target goals with therapy, and these increased apoB levels may confer residual risk of CVD events. The most recent ESC guidelines have recommended new lower targets for apoB (<65 mg/dL in very high-risk patients).<sup>7</sup> However, the evidence base on whether lowering apoB to these levels will further prevent CVD events remains incomplete. The effect of novel therapies to attain these lower targets and their cost-effectiveness also need to be further evaluated.

## 7. CONCLUSIONS

- There is considerable evidence indicating that apoB is *at least* as good as LDL-C in predicting CVD risk in the general population, and is superior to current markers (LDL-C and non-HDL-C) in predicting risk in discordant populations.
- Studies indicate that approximately 20% of the population may have elevated particle number but low or normal LDL-C, and are thus missed by traditional lipid measures. Furthermore, patients with discordantly elevated particle number



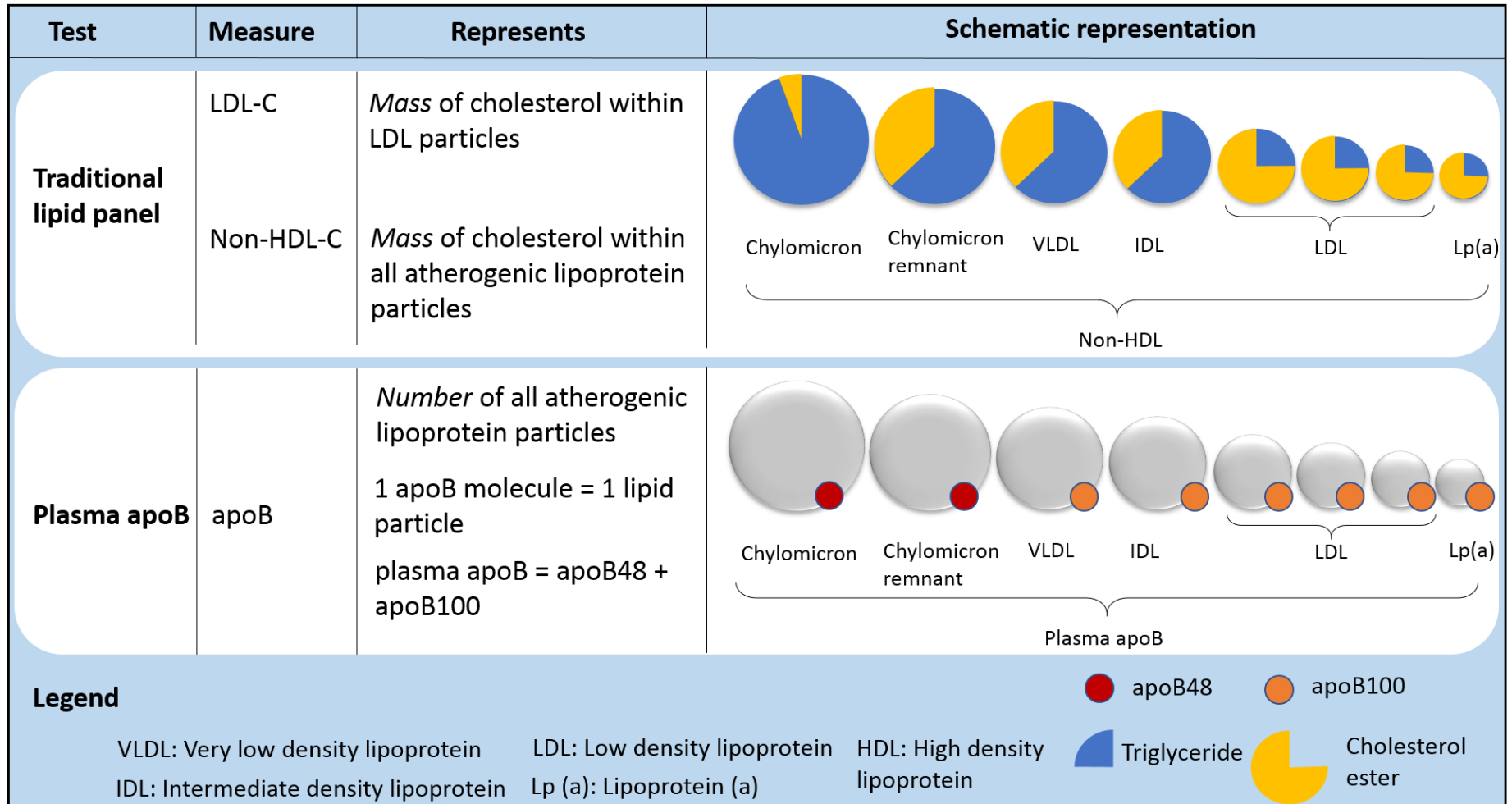
relative to cholesterol content are more likely to have CVD risk factors associated with metabolic syndrome. Hence, apoB has the potential to improve risk prediction, both in primary and secondary prevention, by identifying those at high risk.

- apoB assays are precise, accurate, and easily accessible at reasonable operating costs. In addition, these assays avoid the disadvantages associated with LDL-C measurements, such as having to use fasting samples, and imprecise measures at very low LDL-C concentrations.
- The main barriers to the uptake of apoB has been a reluctance to disrupt practice if the risk prediction associated with apoB is similar to LDL-C. However, very little attention has been accorded to apoB's utility in discordant populations, and if this aspect is factored into the equation, apoB becomes clinically more relevant than LDL-C as the routine measure. In addition, apoB has the ability to simplify physician workload by replacing 5 measures with a single one.
- There appears to be a turning of the tide with guidelines, such as the recent European guidelines, slowly adopting new recommendations endorsing the use of apoB in a routine setting.

## 8. RECOMMENDATIONS

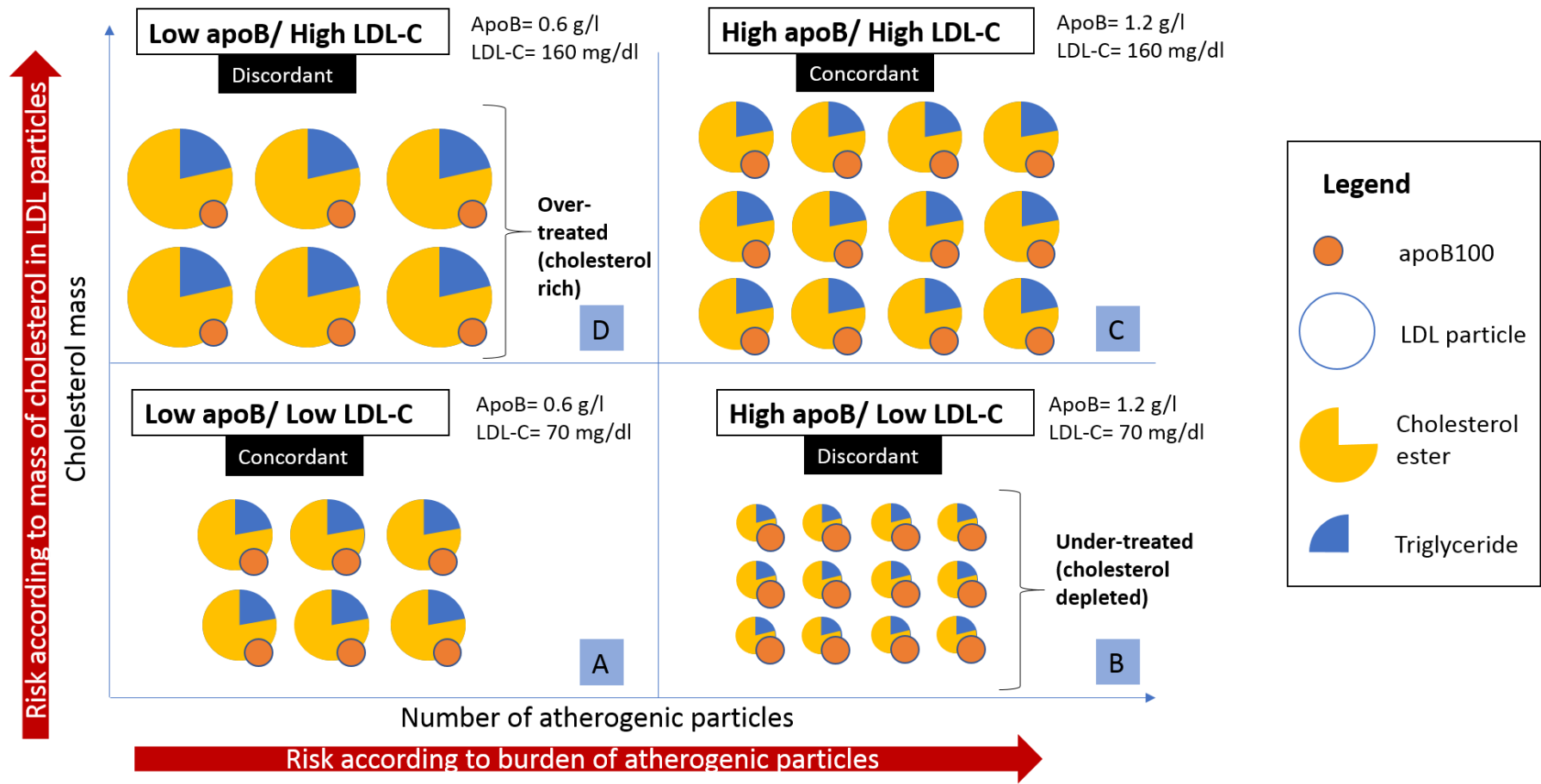
- Given that apoB is equivalent to LDL-C in predicting CVD risk in the general population and superior to LDL-C in discordant populations, we recommend that apoB testing be [Approved](#) for use as an alternative to LDL-C testing for the assessment and management of CVD risk in:
  - patients with CVD risk factors such as high triglyceride levels, diabetes, obesity, and metabolic syndrome;
  - patients with very low LDL-C levels.
- To avoid unnecessary duplication of testing at the MUHC RUISSS, we recommend that apoB replace LDL-C testing, at the discretion of the treating physician.
- An educational programme for physicians will be developed with the collaboration of Dr. Sniderman and relevant stakeholders to promote better understanding of the advantages of apoB.
- This recommendation will be reassessed in 1 year after evaluation of local data and/or new evidence in the scientific literature.

FIGURES



Adapted from Vaverkova H. LDL-C or apoB as the best target for reducing coronary heart disease: should apoB be implemented into clinical practice? *Clinical Lipidology*. 2011;6(1):35-48.

**Figure 1. Schematic representation of atherogenic burden as measured by LDL-C, non-HDL-C and apoB**



Panels A shows an individual with normal levels of apoB and LDL-C. Panel B shows an individual with the same level of LDL-C as in Panel A, but with abnormal apoB (discordance), because of a greater number of smaller, dense LDL particles. Panel C represents an individual with abnormal levels of apoB and LDL-C. In Panel D, the individual has the same level of LDL-C as in Panel C, but normal apoB because of fewer, cholesterol-enriched particles.

**Figure 2. Representation of inter-individual variation in cholesterol content and number of particles**

## TABLES

Table 1. Summary of discordance analysis studies for risk assessment

Study	Population	Particle no. measure	Cholesterol content measure	Measurement method	Cutoff to define discordance	% discordant	Outcome	Results	
								Continuous HR (95% CI)	Categorical HR (95% CI)
<b>Welsh et al. 2019</b>	N=63,520 mostly white participants from the UK Biobank study; Median f/up: 8.9 years	<ul style="list-style-type: none"> <li>• apoB</li> <li>• apoA1</li> </ul>	<ul style="list-style-type: none"> <li>• LDL-C (3 measurement types)</li> <li>• Non-HDL-C</li> <li>• HDL-C</li> </ul>	<ul style="list-style-type: none"> <li>• apoB and apoA1: Immunoturbidimetric</li> <li>• direct LDL-C: Enzymatic selective protection</li> <li>• calculated LDL-C: Friedewald and Martin/Hopkins</li> </ul>	≥10% difference in percentiles between biomarkers	18%	Composite fatal and non-fatal CVD events	HR for 1 SD increase in biomarker: <ul style="list-style-type: none"> <li>• apoB: 1.23 (1.12-1.35)</li> <li>• non-HDL-C: 1.08 (0.98-1.18)</li> <li>• LDL-C (direct): 1.00 (0.91-1.10)</li> <li>• LDL-C (Friedewald): 1.00 (0.91-1.09)</li> <li>• LDL-C (Martin/Hopkins): 1.00 (0.91-1.10)</li> </ul>	Adjusted HR of biomarker in highest quintile vs. third quintile: <ul style="list-style-type: none"> <li>• apoB: 1.29 (1.10, 1.52)</li> <li>• non-HDL-C: 1.10 (0.92, 1.31)</li> <li>• LDL-C (direct): 1.09 (0.90-1.30)</li> <li>• LDL-C (Friedewald): 1.01 (0.84-1.22)</li> <li>• LDL-C (Martin/Hopkins): 1.09 (0.91-1.31)</li> </ul>
<b>Lawler et al. 2017</b>	N= 27,533 healthy women ≥45 years in Women's Health Study;	<ul style="list-style-type: none"> <li>• apoB</li> <li>• LDL-P</li> </ul>	<ul style="list-style-type: none"> <li>• Non-HDL-C</li> </ul>	<ul style="list-style-type: none"> <li>• apoB: Immunoturbidimetric</li> <li>• LDL-P: proton nuclear magnetic resonance</li> <li>• Non-HDL-C: calculated</li> </ul>	<ul style="list-style-type: none"> <li>• Median</li> <li>• Quartiles of residuals</li> </ul>	Discordance vs non-HDL-C: 14% for apoB 20% for LDL-P	Incident CHD event (composite of MI, coronary revascularization, or CHD)		Adjusted-HR for top and bottom quartiles vs 2 middle quartiles: <ul style="list-style-type: none"> <li>• high apoB/high non-LDL-C: 1.22 (1.07,1.39)</li> <li>• low apoB/high non-LDL-C: 0.97 (0.83,1.14)</li> <li>• high LDL-P/high non-LDL-C: 1.13 (0.99,1.29)</li> <li>• low LDL-P/high non-LDL-C: 1.04 (0.89,1.22)</li> </ul>
<b>Wilkins et al. 2016</b>	N=2794 young adults (18-30 yrs); black and white, men and women, urban	<ul style="list-style-type: none"> <li>• apoB</li> </ul>	<ul style="list-style-type: none"> <li>• LDL-C</li> <li>• Non-HDL-C</li> </ul>	<ul style="list-style-type: none"> <li>• LDL-C: Friedewald</li> <li>• apoB: immunoassay</li> </ul>	median	18 %	Year 25 Measured coronary artery calcium		OR vs low/low referent: <ul style="list-style-type: none"> <li>• Low apoB/high LDL-C: 1.29 (0.91,1.83)</li> <li>• High apoB/low LDL-C: 1.55 (1.10,2.18)</li> <li>• high/high: 1.94 (1.58,2.40)</li> </ul>

Study	Population	Particle no. measure	Cholesterol content measure	Measurement method	Cutoff to define discordance	% discordant	Outcome	Results	
								Continuous HR (95% CI)	Categorical HR (95% CI)
<b>Pencina et al. 2015</b>	N=2966 adults from Framingham Offspring Cohort; mean f/up: 19 years	• apoB	• LDL-C • Non-HDL-C	• apoB: Immunoturbidimetric • Non-HDL-C: calculated	Tertiles	Created 3 equal tertiles of residuals: Highest tertile: apoB>LDL-C, Middle tertile: Concordant; Bottom tertile: LDL-C>apoB	Incident CHD (coronary death, recognized or unrecognized myocardial infarction, angina pectoris or coronary insufficiency)	HR for 1 SD increase in difference between observed and expected apoB vs: • LDL-C: 1.26 (1.15-1.37) • non-HDL-C: 1.20 (1.11-1.29)	vs concordant tertile: • high apoB/low LDL-C: 1.46 (1.14,1.86) • low apoB/high LDL-C: 0.70 (0.51,0.96) • high apoB/low non-LDL-C: 1.62 (1.27,2.07) • low apoB/high non-LDL-C: 0.99 (0.75,1.32)
<b>Mora et al. 2014</b>	N=27,533 women; Women's Health Study; Median f/up: 17.2 years	• LDL-P • apoB	• LDL-C • Non-HDL-C	• LDL-P: NMR spectroscopy • LDL-C: direct • apoB: immunoassay • Non-HDL-C: calculated	median	Discordance of LDL-C vs • NHDL-C: 11.6% • apoB: 18.9% • LDL-P: 24.3%	Incident coronary event		Age-adjusted HR comparing medians vs low/low concordant group: • Low LDL-C/high NHDL-C: 2.92 (2.33-3.67) • Low LDL-C/high apoB: 2.48 (2.01-3.07) • Low LDL-C/high LDL-P: 2.32 (1.88-2.85)
<b>Otvos et al. 2011</b>	N=5598; MESA cohort; diverse racial groups (39% white); Mean f/up: 5.5 years	LDL-P	LDL-C	• LDL-P: NMR spectroscopy • LDL-C : Friedewald formula	≥ 12 percentile points difference	50% (engineered to have equal numbers in the categories)	Incident CVD event; IMT	HR (full population): • LDL-P: 1.32 (1.19-1.47) • LDL-C: 1.20 (1.08-1.34) HR (discordant population): • LDL-P: 1.45 (1.19-1.78) • LDL-C: 1.07 (0.88-1.30)	Adjusted CVD event rate per 1000 person years: • Concordant: 10.1 • LDL-P> LDL-C :12.5 • LDL-C>LDL-P :7.3
<b>Cromwell et al. 2007</b>	N=3066 white participants from	• LDL-P • VLDL-P	• LDL-C • Non-HDL-C	• LDL-P: NMR spectroscopy	Median	19%	Incidence of first CVD event	HR for 1 SD increase in biomarker: • LDL-P: 1.28 (1.17-1.39)	Event-free survival: • High LDL-P/Low LDL-C (median cut-off): 0.78

Study	Population	Particle no. measure	Cholesterol content measure	Measurement method	Cutoff to define discordance	% discordant	Outcome	Results	
								Continuous HR (95% CI)	Categorical HR (95% CI)
	Framingham offspring study; Median f/up: 14.8 years			• LDL-C : Friedewald formula				• LDL-C: 1.11 (1.01-1.22)	• Low LDL-P/high LDL-C: 0.88
<b>Sniderman 2003</b>	N=2103; Men 46-76 yrs w/o CAD; Quebec Cardiovascular Study; 5 yr-f/up	• apoB	• LDL-C • Non-HDL-C • Total C	LDL-C: Friedewald;	≥1 Quintile difference between biomarkers	51% (discordance in each quintile ranged from 34% to 67%)	Incident coronary artery disease		HR versus lowest quintile of both apoB and LDL-C: • apoB>LDL-C: 3.2 (1.2, 8.1) • LDL-C>apoB : 3.0 (1.1, 7.8)

Table 2. Summary of discordance analysis studies for risk management

Study	Population	Particle no. measure	Cholesterol content measure	Measurement method	Cutoff	% discordant	Outcome	Results	
								Continuous HR (95% CI)	Categorical HR (95% CI)
<b>Tehrani; 2016</b>	N=6417; MESA cohort; pts with diabetes and metabolic syndrome	<ul style="list-style-type: none"> <li>• HDL-P</li> <li>• LDL-P</li> </ul>	<ul style="list-style-type: none"> <li>• HDL-C</li> <li>• LDL-C</li> </ul>	<ul style="list-style-type: none"> <li>• HDL-C: measured</li> <li>• LDL-C: Friedewald</li> <li>• HDL-P and LDL-P: NMR</li> </ul>	Continuous percentile difference; and median cutoff	Not reported	Incident CHD & CVD events	<p><b>CHD events:</b></p> <p><u>Metabolic Syndrome:</u></p> <ul style="list-style-type: none"> <li>• LDL discordance (LDL-P – LDL-C percentile): 1.21 (1.01-1.47)</li> <li>• LDL-C: 0.80 (0.59-1.08)</li> <li>• LDL-P: 1.34 (1.01-1.78)</li> </ul> <p><u>Diabetes</u></p> <ul style="list-style-type: none"> <li>• LDL discordance: 0.88 (0.71-1.10)</li> <li>• LDL-C: 1.47 (1.07-2.03)</li> <li>• LDL-P: 0.82 (0.58-1.17)</li> </ul> <p><u>Neither disease</u></p> <ul style="list-style-type: none"> <li>• LDL discordance: 0.87 (0.74-1.02)</li> <li>• LDL-C: 1.27 (1.01-1.59)</li> <li>• LDL-P: 0.93 (0.71-1.22)</li> </ul> <p><b>CVD events:</b></p> <p><u>Metabolic Syndrome:</u></p> <ul style="list-style-type: none"> <li>• LDL discordance: 1.26 (1.07-1.47)</li> <li>• LDL-C: 0.77 (0.60-1.00)</li> <li>• LDL-P: 1.39 (1.09-1.75)</li> </ul> <p><u>Diabetes</u></p> <ul style="list-style-type: none"> <li>• LDL discordance: 0.88 (0.74-1.06)</li> <li>• LDL-C: 1.41 (1.08-1.84)</li> <li>• LDL-P: 0.76 (0.57-1.02)</li> </ul> <p><u>Neither disease</u></p> <ul style="list-style-type: none"> <li>• LDL discordance: 0.91 (0.79-1.04)</li> <li>• LDL-C: 1.17 (0.96-1.42)</li> <li>• LDL-P: 0.99 (0.79-1.24)</li> </ul>	<p><b>CHD:</b></p> <p>LDL-C above median/ LDL-P below-median: adjusted HRs: 0.30 to 0.82 vs concordant groups.</p> <p><b>CVD:</b></p> <p>LDL-C above median/ LDL-P below-median: adjusted HRs 0.52 to 0.88 vs concordant groups.</p>

Study	Population	Particle no. measure	Cholesterol content measure	Measurement method	Cutoff	% discordant	Outcome	Results	
								Continuous HR (95% CI)	Categorical HR (95% CI)
<b>El Shazly 2016</b>	N=4957 (9 trials); Patients w/ CAD	<ul style="list-style-type: none"> <li>apoB</li> <li>TC/HDL-C</li> </ul>	<ul style="list-style-type: none"> <li>LDL-C</li> <li>Non-HDL-C</li> </ul>		<ul style="list-style-type: none"> <li>Median</li> <li>Very low percentile equivalent cutoffs: <ul style="list-style-type: none"> <li>LDL-C:&lt;70mg/dl</li> <li>TC/HDL-C: 2.5</li> <li>non-HDL-C: 89 mg/dL</li> <li>apoB: 59 mg/dL.</li> </ul> </li> </ul>	Discordance of TC/HDL-C vs <ul style="list-style-type: none"> <li>LDL-C: 26%</li> <li>apoB: 27%</li> <li>non-HDL-C: 20%</li> </ul>	Change in percent atheroma volume and 2-year major adverse cardiovascular event (MACE) rates		Cumulative incidence of MACE: <ul style="list-style-type: none"> <li>Low apoB/high TC:HDL-C: 19.8</li> <li>Low apoB/low TC:HDL-C: 12.8</li> <li>High apoB/low TC:HDL-C: 19.9</li> <li>High apoB/high TC:HDL-C: 26.4</li> </ul> <ul style="list-style-type: none"> <li>Low LDL-C/high TC:HDL-C: 18.9</li> <li>Low LDL-C /low TC:HDL-C: 14.4</li> <li>High LDL-C /low TC:HDL-C: 15.0</li> <li>High LDL-C /high TC:HDL-C: 24.7</li> </ul>
<b>Sniderman 2012</b>	N=9345 cases with incident acute MI; 12120 matched controls; INTERHEART case control study	apoB	non-HDL-C	<ul style="list-style-type: none"> <li>ApoB: immunoassay</li> <li>Non-HDLC: calculated</li> </ul>	>5% difference in population percentiles	Created 3 equal groups: Concordant; Discordant apoB>non-HDLC; Discordant non-HDLC>apoB	First acute MI		OR of MI vs concordant group (apoB~non-HDL-C): <ul style="list-style-type: none"> <li>Non-HDLC&gt;apoB: 0.72 (0.67, 0.77)</li> <li>apoB&gt;non-HDLC : 1.48 (1.38, 1.58)</li> </ul>



**Table 3. 2x2 factorial results of HMGR and CETP genetic scores in a Mendelian randomization analysis to untangle the roles of apoB versus LDL-C in affecting CVD risk.**

Group	Change in lipid measure, mean (95% CI), mg/dl			OR (95%CI)
	HDL-C	LDL-C	apoB	
<b>Both scores <math>\geq</math> median</b>	5.40 (4.16, 6.64)	-5.29 (-7.29, -3.29)	-3.33 (-5.19, -1.46)	0.920 (0.869, 0.976)
<b>HMGR <math>\geq</math> median</b>	0.83 (0.11, 1.56)	-3.27 (-5.08, -1.46)	-2.74 (-4.31, -1.07)	0.929 (0.880, 0.981)
<b>CETP score <math>\geq</math> median</b>	4.64 (3.44, 5.83)	-2.16 (-3.69, -0.63)	-1.93 (-3.27, -0.57)	0.952 (0.910, 0.995)
<b>Both score <math>&lt;</math> median</b>	Reference			

\* From Ferrence et al. Association of Genetic Variants Related to CETP Inhibitors and Statins With Lipoprotein Levels and Cardiovascular Risk. JAMA. 2017

**Table 4. 2x2 factorial results of LPL and LDLR genetic scores in a Mendelian randomization analysis to untangle the roles of apoB versus LDL-C and triglycerides in affecting CVD risk.**

Group	Change in lipid measure, mean (95% CI), mg/dl			OR (95%CI)
	Triglycerides	LDL-C	apoB	
<b>Both scores <math>\geq</math> median</b>	-24.3 (-32.4, -16.2)	-4.9 (-7.7, -2.1)	-6.4 (-8.5, -4.4)	0.842 (0.811, 0.874)
<b>LPL score <math>\geq</math> median</b>	-20.1 (-28.8, -13.3)	-0.1 (-0.5, 0.3)	-3.0 (-4.9, -1.2)	0.924 (0.889, 0.960)
<b>LDLR score <math>\geq</math> median</b>	-3.8 (-15.1, 7.3)	-4.8 (-7.6, -2.0)	-3.4 (-5.2, -1.5)	0.921 (0.885, 0.958)
<b>Both score <math>&lt;</math> median</b>	Reference			

\* From Ferrence et al. Association of Triglyceride-Lowering LPL Variants and LDL-C-Lowering LDLR Variants With Risk of Coronary Heart Disease. JAMA. 2019

**Table 5. Treatment targets set by the Canadian, American and European guidelines**

	LDL-C treatment targets			Non-HDL-C treatment targets			apoB treatment targets		
	CCS 2016	AHA 2018	ESC 2019	CCS 2016	AHA 2018	ESC 2019	CCS 2016	AHA 2018	ESC 2019
<b>Very high risk</b>	70		55	100		85		80	65
<b>High risk</b>	100		70	130		100		90	80
<b>Moderate risk</b>	115		100	145		135			100

**Table 6. Volume and cost of lipid marker tests at the MUHC**

Test	Volume of tests done at the MUHC	Cost per test (weighted average across Quebec)
<b>ApoB</b>	10,775	\$3.60
<b>Total cholesterol</b>	96,840	\$0.70
<b>HDL-C</b>	86,572	\$1.00
<b>LDL-C direct assay</b>		\$2.90
<b>Triglycerides</b>	91,615	\$0.70

**Table 7. Comparison of LDL-C, non-HDL-C and apoB in terms of risk prediction, clinical and analytical performance, and cost-effectiveness**

Criterion	LDL-C	Non-HDL-C	apoB
<b>Clinical effectiveness</b>			
Superiority to existing tests			
Predicting risk in general population	Reference	Probably	Probably
Predicting risk in discordant populations	Reference	Yes	Yes
Modifiable risk association	Yes	Yes	Yes
Therapy reduces CVD risk	Yes	Probably	Probably
<b>Analytical Performance</b>			
Precise assays	Yes	Yes	Yes
Accurate assays (method independency)	No	No	Yes
Nonfasting measurement possible	With TG <4.5 mmol/L	Yes	Yes
Widely accessible assays	Yes	Yes	Yes
High throughput and rapid turnaround	Yes	Yes	Yes
Reasonable operational costs	Yes	No extra cost	Yes
<b>Clinical Performance</b>			
Robust associations with incident CVD	Yes	Yes	Yes
Novel information beyond existing markers	Reference	Yes	Yes
Validated cutoffs	No	No	No
<b>Cost-effectiveness</b>			
Biomarker-guided treatment saves healthcare costs	Yes	Unknown	Unknown

\*Adapted from Langlois MR, Chapman MJ, Cobbaert C, et al. Quantifying Atherogenic Lipoproteins: Current and Future Challenges in the Era of Personalized Medicine and Very Low Concentrations of LDL Cholesterol. A Consensus Statement from EAS and EFLM. *Clinical Chemistry*. 2018;64(7):1006-1033

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**APPENDIX: COMMENTS ON REPORT FROM EXPERTS IN OTHER HEALTH CENTRES WITHIN THE MCGILL RUISSS****Terres cries de la Baie James**

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Dr. Romina Pace, General Internal Medicine Service Chief for Region 18, Terres cries de la Baie James

The findings in the report look good.

Main issues would be

1. Is it feasible in the region to run this test?
2. It would need to be discussed with the local family physicians to assess if they can adapt their practices to this new test in order to avoid similar issues to the hsTrop. There are clear cut off targets and screening with apo B and I don't think it would be an issue but important to get the groups opinion first before any changes.
3. Don't think any of the validation studies looked at LDL vs apo B targets in indigenous populations. I don't think there would be a huge difference but this may be interesting to do before implementing in the region.
4. As they mention in the report cost-effectiveness and whether targeting Apo -B vs LDL leads to better clinical CVD outcomes is not yet clear so we need to weigh the risks and benefits of implementing it across the region.

Dr. Gang He, Medical Biochemist, MUHC

Chief for Medical Biological Services, Cree Board of Health and Social Services of James Bay

I agree with Dr Pace's opinions.

In addition, I would like to add two points for discussion.

1. The current commonly used treatment target for apob is 0.8g/L, but the cited new one is 0.65g/L. Should we choose 0.65g/L?
2. If not at target, some effective new drugs lowering apob (PCSK-9 antibodies, Lomitapide and Mipomersen (this one, from what I know is only approved in US)) are very expensive. For reimbursement, PCSK-9 antibodies are only approved for FH or high risk prevention (especially secondary prevention after MI, Stroke...) in statin

intolerant or statin ineffective cases with already maximal dose of statin. I have the impression this reimbursement approval is based mainly, on a LDL level, I am not sure if we use apob as reference, RAMQ will reimburse as well (for my own patients, I currently use LDL as reference for PCSK-9 antibody approval).