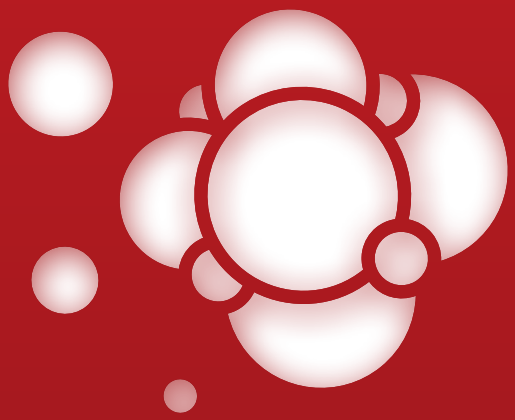
RANDOX

SMALL DENSE LDL CHOLESTEROL (SDLDL-C)



SIZE MATTERS: THE TRUE WEIGHT OF RISK IN LIPID PROFILING

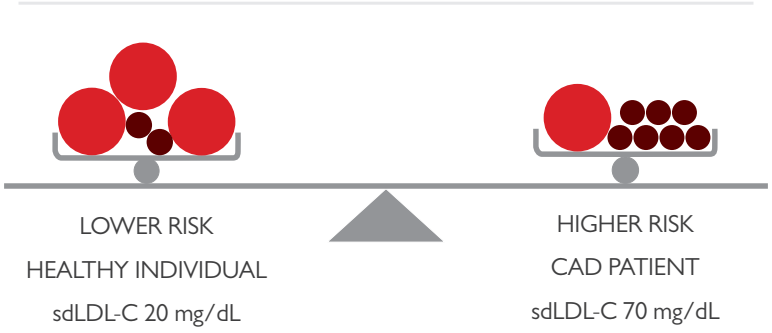
Background

Cardiovascular disease (CVD) is recognised as a leading cause of death, with approximately 17.7million deaths per year, an estimated 31% of all deaths worldwide. Furthermore, 80% of all CVD deaths are due to heart attacks and strokes[10]. There is a global commitment to reduce the probability of premature CVD deaths by 25% by 2025; a target set by the United Nations member states[9]. Globally, the mortality rate for CVD has dramatically declined over the past 20 years, however, in low and middle-income regions, the number of lives lost to CVD is increasing[9]. The global distribution of CVD is complex and defined by national and regional characteristics as much as by global disease trends. Even with the differences between regions, CVD remains a dominant cause of death, even in those who are under the age of 40. This indicates the need for superior CVD risk markers to include methods that account for uncertainty and heterogeneity.

Clinical Significance of small dense LDL Cholesterol (sdLDL-C)

When measuring LDL cholesterol (LDL-C), it is the cholesterol mass within the LDL particles that is being measured. The LDL particle population within LDL is heterogeneous - meaning that the size, density & composition of each particle will be different. sdLDL-C is a subfraction of low density lipoprotein (LDL) with smaller particle size and higher density than larger more buoyant LDL. They all transport triglycerides and cholesterol to the tissues, but their atherogenesis varies according to their size. sdLDL-C will more readily permeate the inner arterial wall. sdLDL-C is more susceptible to oxidation and has a lower affinity to the hepatic LDL receptor, and as such circulates in the blood longer.

Figure 1: Predominance of sdLDL particles in comparison to LDL-C particles



Even though the overall LDL-C mass is the same for each patient, by measuring sdLDL-C, there are very different treatment considerations required.

Table 1: Size and density comparison between lbLDL-C and sdLDL-C

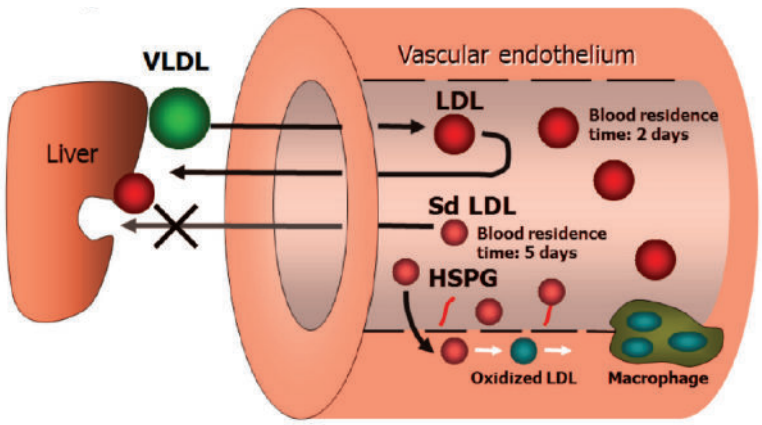
Lipoprotein	lbLDL-C	sdLDL-C
Average (nm)	25.5 - 28.0	22.0 - 25.5
Density (g/cm^3)	1.019 - 1.044	1.044 - 1.063

Risk Assessment

As sdLDL-C is particularly atherogenic, a person with elevated sdLDL-C levels has a **3-fold increased risk of myocardial infarction (MI).**[2]

sdLDL-C measurement therefore provides a more comprehensive understanding of cardiovascular disease (CVD) risk compared to traditional LDL-C tests. These factors provide evidence for sdLDL as a valuable screening tool for predicting future cardiovascular events and in the secondary prevention of subtle coronary artery disease (CAD).

Figure 2: Atherogenic mechanism of sdLDL-C[5]



sdLDL-C has a lower affinity to the hepatic LDL-C receptor, thus circulates in the blood longer than larger LDL-C. sdLDL-C has a stronger affinity to vessel wall heparin sulphate proteoglycans (HSPGs), which means that sdLDL-C can more readily permeate the arterial wall. sdLDL-C is also liable to oxidation from its physicochemical properties which leads to foam cell formation.

sdLDL-C Measurement vs Calculation

A recent paper investigated if sdLDL-C provided an independent atherosclerotic cardiovascular disease (ASCVD) risk factor in various subgroups and set out to determine if there were significant differences in sdLDL-C concentration when measured directly or determined through a calculation method[7].

Findings showed that men were at an increased risk of ASCVD, CHD and stroke when compared to women. Unsurprisingly, they also reported that risk of ASCVD, CHD and stroke increased with age[7].

A significant difference between direct and calculated sdLDL-C concentrations was observed. Correlation between these results had an r^2 value of 0.674, suggesting calculation of sdLDL-C is an inferior method when compared to the quantitated measurement of sdLDL-C. Subjects with a direct sdLDL-C concentration of >50mg/dl were considered to be at increased risk of ASCVD and CHD[7].

Schaefer, et al., report that not only is direct sdLDL-C related to ASCVD and CHD risk but measuring direct sdLDL-C can provide additional information on risk even when all other cholesterol-related risk factors had been controlled. This paper shows that risk of ASCVD increases proportionally to sdLDL-CL concentration. Through their multivariant analysis, the authors show this is not the case for calculated sdLDL-C, again displaying the superiority of direct sdLDL-C quantification[7].

Finally, this investigation states that a sdLDL-C concentration of >50mg/dl increases the risk of ASCVD and CHD by 50%, on top of the previously established risk factors, regardless of sample group. The authors claim the high atherogenic nature of sdLDL-C is due to its small size which increases its' penetrative potential and the extended residence period granting a higher probability of oxidation and modification[7].

In conclusion, this research paper provides strong evidence in support of a direct method for the quantification of sdLDL-C, particularly when screening for ASCVD, CHD, or stroke risk[7].

Table 2 : Comparison of results for sdLDL concentration when determined through measured and calculated methods and the associated hazard ratio relating to CVD risk.

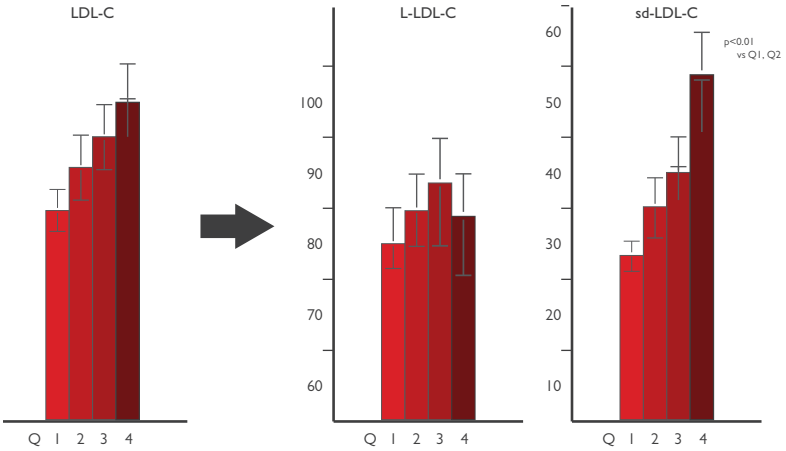
		Quartile 1		Quartile 2		Quartile 3		Quartile 4	
Cohort	sdLDL-C Analysis	mg/dL	HR_{adj} (95% CI)	mg/dL	HR_{adj} (95% CI)	mg/dL	HR_{adj} (95% CI)	mg/dL	HR_{adj} (95% CI)
All Subjects	Direct	<28.1	1.00 (ref)	28.1-<39.3	1.12 (1.07-1.17)	39.3-<54.2	1.27 (1.16-1.39)	54.2-214.8	1.56 (1.31-1.85)
	Calculated	<33.3	1.00 (ref)	33.3-<42.0	1.06 (0.88-1.14)	42.0-<51.8	1.11 (0.96-1.28)	51.8-211.0	1.19 (0.99-1.51)

Methods of Detection

sdLDL-C can be easily implemented in the routine biochemistry lab using the Randox IT assay.

The only direct automated sdLDL-C kit on the market, the Randox sdLDL-C test is a direct method for the quantitative determinationof sdLDL-C using automated chemistry analysers capable of accommodating two-reagent assays. The assay consists of two steps and is based on the use of well-characterised surfactants and enzymes that selectively react with certain groups of lipoproteins.

Figure 3: The Gensini score (Non-diabetic Stable CHD)[2]

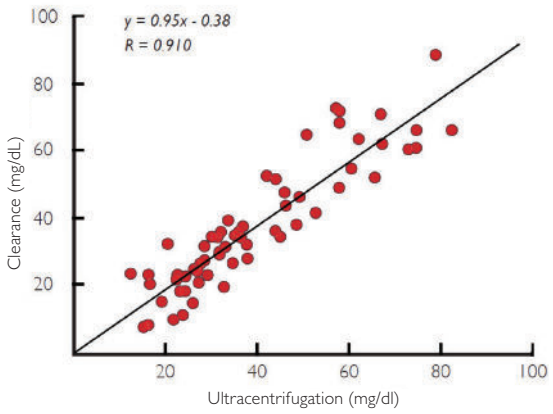


sdLDL-C level increases along with the development of arteriosclerosis. The impact and difference between the different quartiles of risk of large buoyant LDL and sdLDL-C are very clear. The higher the quartile of sdLDL-C the higher the risk of arteriosclerosis. Whilst, any quartile of large buoyant LDL Cholesterol (lbLDL-C) has minimal impact.

Key Features of the Randox sdLDL-C Assay

- **Direct, automated test** for convenience and efficiency
- **Rapid analysis** results can be produced in as little as ten minutes, facilitating faster patient diagnosis and treatment plan implementation
- **Good correlation** to the gold standard ultracentrifugation method (see figure 4)
- **Liquid ready-to-use reagents** for convenience and ease of use
- **Applications available** detailing instrument specific settings for a wide range of clinical chemistry analysers
- **Clearance method -** Ultracentrifugation is laborious and time consuming. The clearance method consists of two main reaction steps that selectively react with certain groups of lipoproteins.
- **sdLDL-C controls and calibrator available**

Figure 4: Correlation of Ultracentrifugation & Clearance methods[6]



The Randox automated sdLDL-C assay correlates well with the gold standard ultracentrifugation method.

Ordering Details

Description	Cat. No.	Size
Direct sdLDL-C kit	CH8153	R1 1 x 16.2ml R2 1 x 8.2ml
Direct sdLDL-C kit	562616	R1 1 x 19.8ml R2 1 x 8.6ml

Controls and Calibrators for Direct sdLDL-C Kit

Description	Cat. No.	Size
sdLDL-C Calibrator	CH5050	3 x 1ml
sdLDL-C Control Level 1	LE5013	3 x 1ml
sdLDL-C Control Level 2	LE5014	3 x 1ml
sdLDL-C Control Level 3	LE5015	3 x 1ml

Conclusions

CVD is a leading cause of death around the world. Although mortality rates associated with CVD have been in decline for the past 20 years, this is not evident in low to middle income countries and many are living with severe side effects related to these diseases. Therefore, it is essential to review the traditional methods of lipid quantification to enable clinician's to gain a more comprehensive view of CVD risk, allowing more appropriate preventative measures to be taken.

The current lipid panel consists of testing:

- Total Cholesterol
- HDL Cholesterol
- LDL Cholesterol
- Triglycerides
- Risk factors (including age, diet, smoking, QRISK, co-morbidities to view risk and management of risk)

The mission of NLA "is to enhance the practice of lipid management in clinical medicine". NLA advocate advancing the current lipid testing profile as the traditional tests only detect approximately 20% of all ASCVD patients. Advanced lipid testing is recommended to optimise patient care, which can be achieved through the addition of sdLDL-C 6.

References

1. *Hirano. T, et al, 2005, "Measurement of small dense low-density lipoprotein particles". J Atheroscler Thromb, 12, 67*
2. *Austin. MA, et at, "Low-density lipoprotein subclass patterns and risk of MI". JAMA 260, 1917, 1988*
3. *Najmafshar, A.wt al, 2012. "The Correlation between Overweight and Obesity with Plasma Levels of leptin, Insulin and sdLDL in People over 20 Years Old". Journal of Obesity & Weight Loss Therapy. 2 (8), 1-3*
4. *Mora. S, 2006. "LDL Particle Size: Does It Matter?". Harvard Medical School. Boston, MA*
5. *Liu, ML, 2002. "LDL Oxidation and LDL Particle Size in the Development of Atherosclerosis". Department of Medicine, University of Helsinki, Finland.*
6. *Leary. ET, 2016, "AACC Presentation by Pacific Biomarkers". AACC Annual Scientific Meeting & Clinical Lab Expo; July 25-27; Chicago, IL*
7. *Schaefer, J. E. et al., 2023. Atherosclerotic cardiovasvcular disease risk and small dense low-density lipoprotein cholesterol in men, women, African Americans and non-African Americans: The pooling project. Atherosclerosis, 367(1), pp. 15-23.*
8. *Rajman, I., et al. (1999) LDL particle size: an important drug target? [Online] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2014286/*
9. *Roth, A. G., Huffman, M. D., Moran, A. E., Feigin, V., Mensah, G. A., Naghavi, M and Murray C.J. L. (2015). Global and Regional Patterns in Cardiovascular Mortality from 1990 to 2013. Circulation. 132, 1667-1678.*
10. *World Health Organisation (2018). Cardiovascular Disease [Online] http://www.who.int/cardiovascular_diseases/en/*

RANDOX
REAGENTS

MDSAP 699499

LT144 FEB23

Copyright © 2019 Randox Laboratories Ltd. All rights Reserved. VAT number: GB 151682708. Product availability may vary from country to country. Some products may be for Research Use Only. For more information on product application and availability, please contact your local Randox Representative