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# Size matters: HDL particle populations and the risk of infection

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Low plasma levels of HDL cholesterol are a risk factor for infection and hospitalization for infectious disease. Recent work suggests that inadequate levels of HDL particles of specific sizes — small and medium — account for this risk. In this Comment, we discuss the mechanistic implications of these observations and the methodologies used to quantify HDL size.

Many lines of evidence suggest that HDL has a role in the immune system. For example, HDL regulates signal transduction by Toll-like receptors, which have key roles in the activation of macrophages — central components of both innate and acquired immunity[1,2]. In addition, inflammation is a potent regulator of HDL composition and its proposed biological functions[1,2]. Moreover, clinical studies suggest that the plasma level of HDL cholesterol — which is an indirect measure of HDL size, concentration and function[3] — is associated with host defence mechanisms. For example, a study using Mendelian randomization analysis indicated a causal association between levels of HDL cholesterol and the immune response against infection[4]. The investigators used genetic and blood-lipid data from more than 400,000 individuals, who were followed up prospectively for 6 years. After adjustment for a wide variety of potential confounders, low levels of HDL cholesterol and LDL cholesterol, and high levels of triglycerides, were associated with an increased risk of hospitalization for infectious disease[4]. However, the increased risk associated with elevated triglyceride levels disappeared after adjusting for HDL-cholesterol level (a well-known confounder in epidemiological associations), strongly suggesting that HDL was the primary risk factor for the observed positive association with triglyceride levels.

To avoid confounding and reverse causation, the investigators constructed polygenic risk scores using 223 single-nucleotide variants associated with plasma levels of HDL cholesterol, LDL cholesterol or triglycerides, and determined the link between the scores and the risk of infection[4]. Only the polygenic score for HDL cholesterol was significantly (and inversely) associated with the risk of hospitalization for infection. This observation strongly suggests that low levels of HDL cholesterol are indeed a risk factor for infection. The investigators next used Mendelian randomization to determine whether the HDL-cholesterol level was causally linked to the risk of infection[4]. The association between a polygenic risk score for low levels of HDL cholesterol and an increased risk of infection and death remained, whereas no significant association was found for the LDL-cholesterol and triglyceride polygenic scores. Collectively, these observations provide strong evidence that low plasma levels of HDL cholesterol are causally linked with the risk of infection and death from infectious disease.

Low plasma levels of HDL cholesterol are also a well-established risk factor for atherosclerotic cardiovascular disease. However, two different classes of drugs that can elevate HDL-cholesterol levels did not reduce the risk of atherosclerotic cardiovascular disease compared with placebo in individuals who were already receiving statins, suggesting that higher levels of HDL cholesterol do not necessarily reflect the cardioprotective effects of HDL. In addition, HDL-cholesterol level is a poor indicator of HDL concentration and function because HDL is a complex mixture of nanoparticles that range from 7 nm to 12 nm in diameter, and the cholesterol content per HDL particle varies more than fourfold in this size range[3]. This variability in the relationship between HDL-cholesterol level and HDL concentration has led to intense interest in other quantitative measurements of HDL. One potentially useful metric is HDL particle number, which quantifies both the concentration and size of HDL subpopulations in plasma[3].

Whether HDL size is an important factor in the host defence against infection is an unresolved issue. A study published in 2022 tested the hypothesis that low plasma levels of HDL particles of specific sizes are associated with a higher risk of infectious disease[5]. The investigators used the Nightingale nuclear magnetic resonance (NMR) spectroscopy method[6] to quantify HDL particle number and size in the plasma from 30,195 individuals from the Copenhagen General Population study, and found that low levels of small and medium HDL particles — but not large and extra-large HDL particles — were associated with increased morbidity and mortality from infectious disease[5]. However, the association between HDL size and infection was not adjusted for low levels of HDL cholesterol, which several groups have shown to be associated with an increased risk of infectious diseases.

These observations are potentially exciting because cholesterol accumulation in macrophages and other immune cells promotes inflammatory responses, including augmentation of Toll-like receptor signalling and inflammasome activation[1,2]. HDL antagonizes inflammation by promoting cholesterol export from macrophages[1,2,7,8]. HDL size markedly affects its capacity to promote cholesterol efflux by specific pathways. Small HDLs are potent promoters of macrophage cholesterol efflux by ATP-binding cassette transporter A1 (ABCA1), the first key step in cholesterol export[7], whereas large HDLs promote cholesterol efflux by the ABCG1 pathway[8]. The identification of HDL size as a risk factor for infection might therefore point towards related underlying mechanisms (Fig. 1).

Another important issue is the accuracy of the method used to quantify HDL particle number[5,6]. In the study by Harsløf and colleagues[5], a calculation based on median concentration of apolipoprotein A-I — the major protein in HDL — and HDL size indicates that the stoichiometry of apolipoprotein A-I per HDL particle was about 7.7 mol/mol. A study evaluating the Nightingale method for assessing HDL particle number in a large study population calculated a value of about 6.7 apolipoprotein A-I molecules per HDL particle[6]. These values are inconsistent with numerous biochemical and biophysical studies

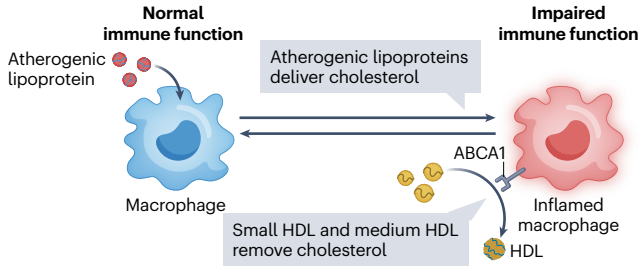


**Fig. 1 | HDL size and macrophage function.** Potential roles of small and medium HDL particles in regulating macrophage inflammatory response and host defence mechanisms. ABCA1, ATP-binding cassette transporter A1.

that demonstrate that the ratio of apolipoprotein A-I to HDL particle concentration ranges from 2 to 5 (refs. [3,9]). An examination of the distribution of HDL particle sizes in plasma, characterized by the Nightingale NMR method, showed that the abundance of HDL particles varied in a log-linear fashion with size[6]. The smallest HDL particles were the most abundant (more than 50% of total particles). However, analytical ultracentrifugation and 2D gel electrophoresis both indicate that medium-sized HDL particles are the most abundant subpopulation in plasma[3]. A study using LipoScience (another commercially available NMR method for quantifying HDL particle number) also demonstrated that small HDL particles are the most abundant subpopulation in plasma[10], suggesting that NMR approaches in general might over-represent smaller particles. Moreover, the LipoScience NMR method yielded a stoichiometry of about 1.5 apolipoprotein A-I molecules per HDL particle[10], in contrast to the Nightingale NMR-calculated value of 6.7–7.7 (refs. [5,6]).

NMR can be used to quantify a variety of analytes in a rapid and cost-effective manner[6]. For this reason, this technique is widely used in clinical studies to quantify HDL particle number. However, the observations from these studies raise important questions about whether NMR can accurately quantify total HDL particle number and specific HDL subpopulations. Going forward, it will be important to validate NMR approaches using independent methods. Nevertheless, absolute accuracy aside, relative comparisons between samples using the same methodology can be clinically useful. For example, multiple studies show that low HDL particle number in the plasma (as quantified by NMR) are associated with an increased risk of cardiovascular disease independently of plasma levels of HDL cholesterol.

Ion mobility analysis (IMA) is another approach to quantify HDL particle number. The strength of this method is the precise and accurate determination of HDL particle size, because it is based on the physics of charged particles moving in an electric field[10]. A limitation is its semiquantitative nature, because various factors can affect the detection of particles. To overcome this limitation, particles of known concentration can be used to calibrate the assay. Calibrated IMA empirically accounts for ionization efficiency and other sources of signal variation[10], permitting the conversion of IMA signal intensity (a relative measurement) to a metric of absolute concentration. Importantly, calibrated IMA was validated using known concentrations of monodisperse gold nanoparticles and reconstituted HDL particles.

The quantification of HDL particle number by calibrated IMA yields a distribution of HDL sizes that agrees with findings from analytical ultracentrifugation and 2D gel electrophoresis[4,10]. Moreover, the apolipoprotein A-I-to-HDL-particle stoichiometry of 3–4 is consistent with our current understanding of HDL structure[3,10]. It would be of great interest to use calibrated IMA to help to calibrate the algorithms used in NMR to quantify HDL size and concentration.

The relationships between the plasma level of HDL cholesterol, HDL-cholesterol polygenic risk score and HDL particle number and the risk of infection raise the exciting possibility that HDL has an important role in host defence mechanisms. In future studies, it will be important to confirm these observations in large cohorts with diverse genetic backgrounds and with the use of accurate methods to determine HDL particle number. It would also be of interest to determine whether low HDL-particle numbers in plasma are associated with both systemic inflammation and the risk of cardiovascular disease. One key question is whether HDL size, or an HDL component such as apolipoprotein A-I, is the prime mediator of resistance to infection, because HDL contains dozens of proteins that are known to be involved in host defence, and these proteins are distributed asymmetrically across the HDL size spectrum. Finally, it will also be crucial to explore potential mechanisms of HDL functions in the immune response, such as the capacity of HDL to inhibit inflammation and to regulate antimicrobial defence mechanisms of immune cells[1,2].

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## References

1. Tall, A. R. & Yvan-Charvet, L. Cholesterol, inflammation and innate immunity. *Nat. Rev. Immunol.* **15**, 104–116 (2015).
2. Sorci-Thomas, M. G. & Thomas, M. J. Microdomains, inflammation, and atherosclerosis. *Circ. Res.* **118**, 679–691 (2016).
3. Rosenson, R. S. et al. HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. *Clin. Chem.* **57**, 392–410 (2011).
4. Trinder, M., Walley, K. R., Boyd, J. H. & Brunham, L. R. Causal inference for genetically determined levels of high-density lipoprotein cholesterol and risk of infectious disease. *Arterioscler. Thromb. Vasc. Biol.* **40**, 267–278 (2020).
5. Harsløf, M., Pedersen, K. M., Afzal, S., Smith, G. D. & Nordestgaard, B. G. Lower levels of small HDL particles associated with increased infectious disease morbidity and mortality: a population-based cohort study of 30 195 individuals. *Cardiovasc. Res.* https://doi.org/10.1093/cvr/cvac194 (2022).
6. Ala-Korpela, M., Zhao, S., Järvelin, M. R., Mäkinen, V. P. & Ohukainen, P. Apt interpretation of comprehensive lipoprotein data in large-scale epidemiology: disclosure of fundamental structural and metabolic relationships. *Int. J. Epidemiol.* **51**, 996–1011 (2022).
7. He, Y. et al. Diabetes impairs cellular cholesterol efflux from ABCA1 to small HDL particles. *Circ. Res.* **127**, 1198–1210 (2020).
8. Rader, D. J. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J. Clin. Invest.* **116**, 3090–3100 (2006).
9. Huang, R. et al. Apolipoprotein A-I structural organization in high-density lipoproteins isolated from human plasma. *Nat. Struct. Mol. Biol.* **18**, 416–422 (2011).
10. Hutchins, P. M. et al. Quantification of HDL particle concentration by calibrated ion mobility analysis. *Clin. Chem.* **60**, 1393–1401 (2014).

## Competing interests

The authors declare no competing interests.