

The entanglement of NASH and atherosclerosis

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Valorisation

Socio-economic relevance

Nowadays it is well recognized that diet, together with lifestyle and environment, are important risk factors for the development of non-alcoholic fatty liver disease (NAFLD). NAFLD is one of the important contributors for the continuous rise of chronic liver disease in the Western society. NAFLD can be divided into two sub-types: fatty liver disease and non-alcoholic steatohepatitis (NASH), which is a chronic inflammatory condition of the liver. The fatty liver is a benign condition and reversible condition, the presence of inflammation in a fatty liver is irreversible and only treatment is a liver transplantation (1, 2). Epidemiological studies reveal that about 20% of the Western population has NAFLD. Approximately 15-30% of these NAFLD patients will ultimately develop NASH. NASH is associated with liver-related death, with a mortality rate between 30-40% in NASH patients (3). The prevalence of steatosis is estimated to be ranging from 84% to 96% whereas in this population the prevalence of NASH is ranging from 25% to 55% (4).

In addition to the association between NAFLD and hepatic-related morbidity and mortality, NAFLD is also related to increased cardiovascular-associated mortality. In support of this, NAFLD has been associated with increased cardiovascular disease (CVD) risk in patients with obesity and diabetes (5, 6). Moreover, multiple clinical studies have found a link between NAFLD and atherosclerosis and shows that NAFLD may act as an important contributor to CVD. However, although NAFLD/NASH and CVD share a common etiology, the precise underlying pathophysiological mechanisms are not fully understood yet (7). This thesis provides for the first time novel insights in the pathophysiological mechanisms of NAFLD and CVD, focusing particular on macrophage responses related to the crosstalk between lipids and inflammation. Our findings reveal novel therapeutic strategies, related to the inhibition of the inflammasome and redistribution of intracellular cholesterol, that consequently inhibit the progression of NASH and atherosclerosis. As such, our findings could positively affect the economic burden related to NASH and atherosclerosis, as therapeutic strategies could target both NASH and atherosclerosis simultaneously. Hereby, healthcare costs can be reduced, for example hospitalisation costs and the development of affordable medicine.

Innovation and future directions

A clear association between NASH and atherosclerosis has been established, as they display features of a shared etiology. Yet, evidence for a shared mechanism related to the progression of NASH and atherosclerosis are lacking and remain unclear. In this aspect, this thesis is innovative as it provides novel insights in a macrophage-specific mechanism that contributes to the progression of NASH and atherosclerosis. One of the most important take-home messages is that NASH and atherosclerosis do not only display inflammatory mechanisms but also mutually influence each other's progression. Knowledge utilization with regard to this insight could lead to the discovery of novel therapeutic interventions or diagnostic tools, applicable to both diseases.

Intervention strategies:

Several targets are identified within this thesis that potentially could lead to novel therapeutic targets. The inflammasome was identified as an important activator for inflammation in NASH and atherosclerosis (Chapter 3). Strong collaboration with the pharmaceutical industry could lead to promising novel drug target therapies to inhibit the inflammasome activation thereby tackling the disease progression of both NASH and atherosclerosis. Furthermore, pneumococcal vaccination described in this thesis was used as a proof of concept to abolish oxidative cholesterol uptake within lysosomes (Chapter 2). The therapeutic properties of pneumococcal vaccinations with regard to NASH and atherosclerosis have been shown, but is limited with regard to clinical data in human patients (8, 9). Therefore, more investments should be made to investigate the added value of pneumococcal vaccinations to NASH and CVD in a clinical setting. In addition, this thesis provides evidence that the distribution of intracellular cholesterol content can be modulated, i.e. by redirecting cholesterol from the lysosome to the cytoplasm, consequently reducing inflammation. Therefore, novel therapeutic interventions should not only focus on reducing cholesterol levels alone but rather focus on modulating intracellular cholesterol content to reduce inflammation and should be investigated more thoroughly. As such, intracellular cholesterol modulation, for example via gene modulation of CYP27A1 (Chapter 5), is a good novel therapeutic intervention to redirect intracellular cholesterol and should be tested in NASH and atherosclerosis patients. Important to note is that throughout this thesis it is repeatedly mentioned that NASH and atherosclerosis display features of an acquired lysosomal storage defect, suggesting that similar mechanisms are applicable to lysosomal storage diseases. Besides targeting CYP27A1, the use of 27HC has been shown to improve hepatic inflammation (10) and should be tested in a clinical setting. However, it is important to note, as 27-HC may function as a selective estrogen receptor (ER) modulator, its proposed anti-inflammatory effects may differ between tissues (11). Therefore, future research is necessary to identify the function of 27-HC ER-mediated effects in macrophage-induced inflammation and in other tissues, before

27-HC could be used as therapeutic agent for lowering intracellular cholesterol. Finally, future research may be directed to lysosomal-induced inflammation. By using a NPC1 mutant mouse model, an innovative way can be established to investigate the inflammatory mechanism related to lysosomal function. More importantly, bone marrow transplantation of NPC1 mutant mice to hyperlipidemic *Ldlr*^{-/-} mice would be a great model to investigate lysosomal function under metabolic conditions.

Screening procedures for risk factors in CVD and NAFLD

As NASH and atherosclerosis mutually influence each other's progression, the findings in this thesis may be beneficial and could raise awareness among clinicians with regard to screening methods in patients with risk factors for NASH and CVD. Screening for cardiovascular risk factors in NAFLD patients would be highly beneficial to detect early development of CVD, and vice versa. This point of view is also supported by Francque et al., who proposed to screen for cardiovascular risk factors in NAFLD patients. Here it was stated that several questions should be addressed first; as what to screen for and which screening techniques should be used and which disease populations should be screened (5). To address these questions, a strong collaboration between hepatic and cardiovascular clinicians is needed. Currently used biomarkers to identify NASH are limited to the liver enzymes ALAT, ASAT and gamma-glutamyl transferase (GGT), but lack accuracy. As such, the golden standard for the detection of NASH is still a liver biopsy (3, 6). As such, the need for new biomarkers for NASH holds high potential to be investigated in the future. Several studies also address an association between these liver enzymes and cardiovascular outcome but appear to be not that strong (6). Our group has now identified cathepsin D as a new biomarker for hepatic inflammation (12). As such, future directions could be taken to show how cathepsin D could function as potential biomarker for cardiovascular risk factors in combination with hepatic manifestations, as cathepsins D is involved in endothelial permeability and is associated with coronary events (13, 14). This knowledge could be implemented into new screening protocols, whereby CVD patients should be screened for NASH and the other way around.

Potential target groups and activities

Scientific value

The findings described in this thesis will increase our knowledge about the macrophage-specific mechanisms which are contributing to the progression of NASH and atherosclerosis. Next to identifying new macrophage-specific mechanisms, the goal of these studies was to identify therapeutic targets which can benefit society in reducing NASH and atherosclerosis development. As such, besides its socioeconomic relevance, our results also have a high scientific value, as they are very appealing to clinicians, healthcare workers, pharmacological industry, patient groups, society and other scientific researchers. To get this knowledge to the scientific community and beyond, our findings are published in peer-reviewed scientific journals, such as PLoS One, FEBS Journal, Journal of Hepatology and Atherosclerosis. In addition, our findings are presented by means of an oral presentation at highly esteemed national (Dutch Liver Retreat, Spier, 2013-2015; Dutch Lipoprotein Club, Leiden the Netherlands, 2012-2013; 25th Genetics Retreat, Kerkrade, 2015; 5th Joint Diabetes and Metabolism Research Symposium, Maastricht, 2015) and international conferences (20th Annual Scandinavian Atherosclerosis Conferences, Humlebæk Denmark, 2014) and via poster presentations (Annual Symposium School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht, the Netherlands, 2014-2015 and British Atherosclerosis Society Autumn Meeting, Cambridge, United Kingdom, 2015). Knowledge transfer to the patients and the clinic

Before novel therapeutic strategies can be utilized in the clinic, strong valorisation procedures should be followed in collaboration with clinicians, patient groups and pharmacological industry. Additionally, collaborations between patient groups, foundations, clinicians and health care workers are needed to raise awareness for cardiovascular and hepatic risk factors in patients. For example, advances could be made by clinicians and foundations, to prepare one general brochure to make NAFLD and CVD patients aware that both diseases are closely linked to each other and as such, that potential risk factors should not be underestimated. In addition, the view that NAFLD and CVD are closely linked with each other could be quickly spread by presenting on national and international scientific conferences. These conferences should not be limited to scientists and clinicians, but also include people from hepatic and cardiovascular foundations. Sharing information between clinicians and fellow scientists to these foundations would improve collaborations between these groups, thereby foundations could better assess where funding is needed. The creation of new grants that focus on the hepatic-cardiovascular axis would be innovative and would improve the accessibility for scientists to invest specifically on this topic. These investments may not only benefit patient outcome, quality of life but also improve healthcare costs. As our findings focus on reducing systemic inflammation in general and contribute to intracellular cholesterol redistribution, these findings are not only

limited to NASH and atherosclerosis, but could also be implemented to other chronic inflammatory diseases, such as lysosomal storage diseases.

The need for a systemic research approach and treatment

Relevantly, one of the features of macrophages is that their response to inflammation is diverse and not limited to one specific tissue. As such, macrophage-specific mechanisms may not only influence the progression of NASH and atherosclerosis, but also other chronic inflammatory diseases. Therefore, it is important to determine whether these responses are seen in other tissues and not only in the liver and vessel wall. It would be useful to investigate the effects of macrophage-driven mechanisms during inflammation, such as inflammasome activation, distribution of intracellular cholesterol and the anti-inflammatory effects of 27-HC in other tissues, e.g. the adipose tissue, lung and brain.

To summarize, these findings described in this thesis have high socioeconomic and scientific relevance. Good collaborations between different agencies are necessary to utilize the findings of this thesis to the field. Additionally, future research should focus on the intervention strategies regarding inflammasome activation and intracellular cholesterol distribution. Furthermore, reforming screening protocols in NAFLD and CVD patients is essential in reducing the economic burden for both and NASH and atherosclerosis. Overall, future research should not only focus on lowering cholesterol levels in general but rather focus on the lowering intracellular cholesterol levels in macrophages. Additionally, future research can be expanded by investing more in fundamental research by investigating lysosomal-induced inflammation in chronic inflammatory diseases.

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