Atherosclerosis in the HIV and non-HIV setting: detecting and modifying cardiovascular risk
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Atherosclerosis is a chronic disease of the arteries, which occurs at the level of the vessel wall. Cholesterol leakage in the vessel wall results in inflammatory activation of endothelial cells (endothelial dysfunction), resulting in increased expression of adhesion molecules (especially VCAM-1) on endothelial cells, which is followed by mobilization of monocytes to the site of endothelial dysfunction. The monocytes transmigrate to the subendothelial space where they acquire the properties of tissue macrophages. Transformation of these macrophages to foam cells occurs by inclusion of subendothelially located oxidized lipid particles. The local vessel wall has now become a site of inflammation with macrophages and T-lymphocytes as key players, which are a continuous source of chemo-attractants and pro-inflammatory cytokines. The subsequent gradual increase of inflammatory cells, debris and cholesterol leads to a progressive narrowing of a blood vessel, the atherosclerotic plaque. Proliferating smooth muscle cells in the plaque, producing abundant extracellular matrix, may render the plaque stable. However, the fibrous capsule covering the plaque can also rupture under the influence of matrix metalloproteinases, produced by the activated macrophages. Rupture of the plaque is associated with the release of tissue factor and leads to acute coagulation of the blood. The ensuing thrombus formation may occlude the blood vessel. Prolonged occlusion of the blood vessel may lead to an acute myocardial infarction (when the coronary arteries are involved) or cerebral infarction (when the cerebral arteries are involved). Reduction in LDL-C through drug treatment with statins may further inhibit growth of a plaque and very aggressive reduction of LDL-C can sometimes even lead to regression of atherosclerotic plaques. Statins have also shown their value in primary prevention for cardiovascular disease.

Part I of this thesis deals with the various imaging techniques, which are now available to visualize both clinical and subclinical forms of atherosclerosis. In chapter 1, we performed a literature study looking at the best methods to visualize atherosclerotic plaques in an early stadium both invasively and non-invasively. Here we compare non-invasive methods such as carotid intima-media thickness (IMT), Flow Mediated Dilation (FMD), multi-detector CT / electron beam CT and cardiac magnetic resonance imaging (MRI) with invasive methods such as quantitative coronary angiography (QCA) and intravascular ultrasound (IVUS). With regard to the non-invasive modalities, we show that IMT is the preferred method given the broadly validated technique, the simple applicability, the high degree of reproducibility and the ability to visualize both regression and progression of atherosclerosis. Among the invasive modalities, coronary angiography has been the gold standard for decades despite the fact that visualization thereof is limited to the arterial lumen and early stages of atherosclerosis can be missed easily. In our view, IVUS has the most potential to changes in atherosclerotic coronary arteries to visualize but the predictive value for future cardiovascular events for this modality should still be demonstrated. Yet, emerging techniques, including
320 multislice CT as well as MRI combined with functional measurements (either PET and/or molecular imaging techniques using selective ligands) are likely to enter the competition for optimal imaging technique. Currently, these techniques need further validation.

In **chapter 2** we pay closer attention to the role of IMT as a surrogate marker for atherosclerosis. To that end, the main results of several key studies are discussed. First, results of observational studies show that the presence of cardiovascular disease (CVD) is positively associated with IMT. Another observational study of 4476 patients showed that this positive association between IMT and CVD also applies to asymptomatic patients. Furthermore, results from key statin intervention trials performed in various patient populations show that IMT enables evaluation of the effects of these drugs on the vessel wall. Some comments on the use of IMT are also made. First, the comparative value of IMT between different studies is limited due to lack of standardization of imaging protocols. More specifically, IMT has been shown to be especially valuable in patients with high IMT progression rate, in whom the change in LDL-C is linearly related to change in IMT: higher LDL decrease, less IMT progression. However, in patients already receiving optimal LDL lowering therapy and hence low IMT progression, the limited resolution of IMT may preclude to observe differential IMT progression in these patients (Enhance study).

In **Part II** of this thesis we shift our attention to LDL cholesterol, and its role in the process of atherosclerosis. In **chapter 3**, we start off with a discussion of the genetic backgrounds of a condition named Familial Hypobetalipoproteinemie (FHBL). This is a rare disorder, which is characterized by extremely low levels (less than the 5th percentile for age and gender) of apolipoprotein B (apoB) and plasma LDL-C. The condition results from mutations in the gene that encodes for apoB leading to truncated forms of apoB. New mutations are described for people who meet the above mentioned lipid criteria. The hypothesis is postulated that the extremely low LDL-C values would lead to less atherosclerosis and thus confer protection against the development of CVD. In **chapter 4** the latter hypothesis was tested by measurement of the carotid IMT in 41 patients with FHBL and comparing these with measurements performed in 41 healthy control subjects. The observed slight difference in IMT in favor of the FHBL group lost significance after adjusting for traditional risk factors such as age, sex, smoking and body mass index. The small groups studied and the fact that the comparative arm consisted of healthy controls may partly explain this outcome in our opinion. It has recently become clear that the IMT technique is less suitable to detect less IMT progression compared to healthy controls. IN fact, this most likely reflects the limited resolution of the IMT technique as discussed above. So, to demonstrate a decreased risk of CVD by means of a thinner IMT in FHBL subjects, as compared to control subjects, would require inclusion of a large number of subjects (> 750 patients). However, additional analysis using a cumulative risk score based on age, systolic blood pressure and smoking, did show significantly lower arterial wall stiffness in the FHBL group as compared to the healthy controls. Previous studies have shown that arterial wall stiffness is an
important predictor for future cardiovascular events. Our findings confirm that life-long exposition of FHBL subjects to extremely low levels of LDL-C reduces the risk of developing atherosclerosis. In chapter 5 we show an overview of the accumulated clinical experience with a new cholesterol lowering drug called Inegy™. Inegy™ is a combination preparation of ezetimibe and simvastatin. Simvastatin belongs to the class of statins, which inhibit the production of cholesterol in the liver by inhibiting a key enzyme, the hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. Ezetimibe belongs to a whole new class of cholesterol absorption inhibitors and inhibits the absorption of cholesterol in the intestine without affecting the uptake of triglycerides and fat-soluble vitamins. With Inegy™ it is possible to achieve LDL-C reductions up to 61% from baseline, which exceeds reductions achieved with statin monotherapy. Moreover, the data shows that ezetimibe is well tolerated by patients with little side effects reported by patients.

As briefly discussed above, the recent results of several clinical studies with Inegy™ have evoked much debate about the ‘simple’ concept of LDL reduction. In 2008 the annual congress of the American College of Cardiology called for a broad change in prescription behavior to the detriment of Ezetimibe-containing combination preparations. This was based on the ‘negative’ findings of Ezetimibe combination therapy on IMT in the ENHANCE study and also on the lack of additional benefit in the SEAS trial, which showed a disappointing reduction of cardiovascular risk in patients with aortic stenosis. Does this mean that Section 5 should be rewritten? In the personal view of this author, this is not the case. First of all, it is not surprising that in the ENHANCE trial, no further reduction in IMT could be demonstrated in Familial Hypercholesterolemia (FH) patients treated with simvastatin-combination therapy as compared to FH patients treated with simvastatin monotherapy. The study shows that, in contrast to previous studies such as the ASAP trial, FH patients already exhibited ‘thin’ IMT at baseline of the study. Secondly, the progression of IMT under the ‘standard’ treatment, simvastatin monotherapy, had already been halted. Consequently, it is theoretically impossible to inhibit progression better than stagnation. The main reason for this study outcome seems to be due to the inclusion of particularly well-treated FH patients with low disease load. In the SEAS study, patients with aortic valve stenosis were treated with simvastatin-ezetimibe versus placebo. The primary endpoint, a composite of major cardiovascular events, showed no significant difference. However, it is unknown whether lowering of LDL-C can actually influence this process. The relatively low reduction in ischemic cardiovascular events in treated patients is difficult to interpret since the study was not powered for this particular endpoint. In conclusion, LDL-C in patients should be reduced optimally, primarily by properly dosing statin therapy. If this strategy fails for whatever reason, adding Ezetimibe to the medication should be considered. In 2012, results of the IMPROVE IT trial will enable us to establish whether the combination of simvastatin-ezetimibe vs. simvastatin alone is actually better in reducing cardiovascular events as compared to treatment with statin monotherapy.
In *Part III* of the thesis we focus on another patient group that increasingly has to deal with atherosclerosis, the patients who are infected with the human immunodeficiency virus type 1 (HIV-1). The risks for CVD are discussed as well as the harmful but also potentially beneficial effects of antiretroviral therapy. Therapeutic options to combat CVD in this particular group of patients are also discussed.

In *chapter 6* we start with an overview of the literature in which we indicate how infection with HIV-1 relates to CVD. Dyslipidemia in untreated HIV-1 infection is characterized by reduced high-density lipoprotein cholesterol (HDL-C) content. The inverse relationship between the level of HDL-C and the risk of CVD in the general population has been well validated in large epidemiological and clinical studies. HIV-1 infection is currently effectively suppressed by combining 3 or more antiretroviral drugs, often from different drug classes. This is called combination antiretroviral therapy or CART. We show that CART and, in particular HIV protease inhibitors (PI) containing CART, is associated with an increased risk of CVD. This is due to the metabolic complications of this therapy, such as dyslipidemia, insulin resistance and changes in body fat distribution (lipoatrophy). Non-nucleoside reverse transcriptase inhibitors (NNRTIs) show exactly the opposite with increases in HDL-C and do not contribute to this risk of CVD. HIV-1 infection itself also seems to contribute to the increased risk of CVD, as shown for instance in autopsy studies of the pre-CART era in which extensive atherosclerotic lesions were found in the coronary arteries of young deceased patients. These abnormalities could not be explained by traditional risk factors for CVD. Furthermore, HIV-1 appears to intervene directly on one of the main functions of HDL-C, the reverse cholesterol transport. Reverse cholesterol transport is the process by which excess cholesterol from peripheral tissues is transported to HDL particles, which are subsequently delivered to the liver where they are taken up and excreted via bile acid and through the bile. The first step in this journey is the ABCA1-mediated cholesterol efflux from tissue macrophages to the HDL particles. In vitro studies show that HIV-1 has been able to interfere with this ABCA1-mediated efflux by down-regulation of ABCA1, which will lead to the accumulation of cholesterol in the macrophages and could promote accelerated development of atherosclerosis. In addition to these direct effects, it is shown that HIV-1 promotes the atherosclerosis process by means of chronic immune activation. Various coagulating factors are influenced leading to a hypercoagulable status and it was shown that immune modulating therapy of HIV might limit the atherosclerotic plaques in animal studies. The accelerated atherosclerosis in HIV-1 patients is therefore based on both direct and indirect influences of the virus on the vessel wall, lipid profiles and clotting factors.

*Chapter 7* outlines the various ways in which anti-retroviral drugs can affect plasma lipids. Using a unique population of HIV-1 negative neonates who were born from HIV-1 positive mothers and received prevention of HIV-1 transmission through breast milk for at least 3 months by administration of the NNRTI nevirapine (NVP) or the nucleoside reverse transcriptase inhibitor lamivudine (3TC). Previous studies using NVP in HIV-1 positive adults have already shown the
strong HDL-enhancing properties of this drug. In this study it became clear that plasma levels of HDL and apoA-I concentrations were increased to a greater extent in the NVP group as compared with the 3TC group. Results from this study show that the observed effects on HDL-C are not merely a return of HDL-C levels to “pre-infection values” but that intrinsic properties of NVP may be concerned. Indeed, the current study was conducted in HIV-1 negative neonates. The question remains whether the observed increase in plasma HDL-C levels is potentially beneficial in terms of the risk of CVD.

**Chapter 8.** To answer the preceding question we performed carotid IMT measurements in 62 HIV-1 patients who were treated with PI and compared this with the results of 68 HIV-1 patients who were treated with NVP or efavirenz, another NNRTI. Indeed, significantly higher HDL-C levels were observed in the group of patients treated with NNRTIs. The carotid IMT measurements were also lower (and thus more favorable) in the group treated with NNRTIs, which is expected to translate into a lower risk of CVD. Unfortunately, HDL-C levels were found not to be independent predictors of carotid IMT in the multivariate analysis. Apparently, factors other than HDL-C may also play a role.

In **chapter 9** we tried to gain more insight into the mechanism behind the previously mentioned HDL-C increase with the use of the non-nucleoside reverse transcriptase inhibitor NVP. Within a 24 weeks study period 3 apoA-I kinetic tests were performed in 14 HIV-1 infected patients who were treated for at least 6 months with AZT/3TC/abacavir. Patients had to have an undetectable viral load (HIV-1 RNA <50 copies / ml) in the 6 months preceding inclusion. Key HDL-C modulating enzymes were also measured. We show that the addition of NVP to the treatment with AZT/3TC/abacavir leads to significant increases in plasma levels of HDL-C and apoA-I. This appears to be the effect of an increased production of apoA-I while catabolism of this protein remains unaffected. The significant increase in LCAT activity is low in absolute terms (9%) and therefore we believe it not to be an adequate explanation for the increase found in HDL-C/apoA-I levels. Recent results of the DAD study, a large multicenter prospective cohort study with clinical endpoints, show that the increased risk of CVD with HIV-1 patients is mainly related to the use of PI-containing CART and not to that of NNRTI-containing CART. In the SMART study therapy interruption of CART in HIV-1 patients was accompanied by a decrease in HDL-C levels, which was associated with a 2 times increased risk of developing CVD. The greatest decreases in HDL-C were observed in the patients treated with NNRTIs at the time of the interruption. These results suggest that higher HDL-C concentrations protect against the occurrence of CVD. The current study results may contribute to the development of future selective HDL-potentiators, not only for HIV-1 patients but also for patients from the general population. In the absence of potent and safe HDL-C raising drugs, the present therapy of PI-induced dyslipidemia in HIV-1 patients still focuses on the reduction of LDL-C. However, most statins are contraindicated because PI, including lopinavir/ritonavir, interferes with the metabolism
of statins by inhibition of the cytochrome P450. In chapter 10 we report the results from a pilot study comparing the efficacy and safety of rosuvastatin in HIV-1 patients with dyslipidemia caused by the PI lopinavir/ritonavir. Since rosuvastatin undergoes minimal metabolism via the cytochrome P450 enzyme it was thought that this statin would present a safe alternative for HIV-1 patients. As expected, the use of rosuvastatin was associated with strong reductions in plasma LDL-C levels. Contrary to expectations, increments in rosuvastatin plasma levels were observed for all dosages of rosuvastin. Based on this study we can conclude that cholesterol lowering with rosuvastatin is certainly possible in HIV-1 patients treated with PI-based CART. However, given the interaction between these drug classes, it seems wise to start with relatively low dosages, perform frequent checks of biochemical lab, and carefully inform patients about possible complications such as skeletal muscle toxicity (rhabdomyolysis). The current results are in line with the outcomes of previous studies investigating the interaction between PI and statins (other than rosuvastatin). Taken together, these results pose a powerful warning against liberal use of high dose statin therapy in this select group of patients.

**Future Prospects**

Techniques and methods for detection and modification of cardiovascular risk in patients with dyslipidemias, whether drug-induced or not, are continuously evolving on the basis of new scientific insights. The studies presented in this thesis argue for a high-throughput screening for cardiovascular disease using non-invasive imaging techniques. In particular, the one-stop-shop approach using MRI, in which structure, plaque composition and inflammation, as well as function (shear stress) can be visualized, is expected to present a revolutionary change in the risk assessment of individual patients.

With regard to LDL-C reduction, given the fact that most high-risk patients are already receiving aggressive LDL-lowering treatment it will become increasingly difficult to demonstrate further gains of novel interventions using the current surrogate markers. However, this notion should by no means be used as evidence of lack of effect in the assessment of investigational drugs, as this may lead to disqualification of potential favorable drugs. Clearly, further improvement of surrogate markers for CVD is desperately needed to assess the efficacy of novel therapeutics. Unlike the situation with respect to LDL lowering, HDL-enhancing strategies are still in their infancy. Moreover, HDL oriented research has received a heavy setback with the recent negative publicity concerning the CETP-inhibitor torcetrapib in patients at high cardiovascular risk. The increased morbidity and mortality in the torcetrapib arm appeared to be due to off-target toxicity of this compound. New modalities for selectively increasing HDL are essential to achieve auxiliary cardiovascular risk reduction in at risk patients. Our finding that NNRTIs may promote HDL production may contribute to further insights in the development of apoA-I production.
enhancers. Finally, we foresee that the contributory role of chronic inflammatory diseases in increasing the risk of CVD will become widely accepted within the next couple of years. With an ever-increasing incidence of CVD in the back of one's mind this added insight should guide doctors towards a policy change. This policy change would comprise improved screening for the presence of cardiovascular risk factors in patients suffering from inflammatory diseases such as HIV, rheumatoid arthritis, Crohn's disease and systemic lupus erythematosus. Presence of cardiovascular risk factors should prompt initiation of targeted risk-lowering therapy whenever possible.