1ST EUROPEAN SYMPOSIUM

Clinical Significance of Lipoprotein Disorders in HIV-positive Patients

MUNICH – NOVEMBER 21 – 22, 2003

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Chairmen:

G. Moyle (London, U. K.)
N. Brockmeyer (Essen, Germany)

Friday, November 21, 2003, 13.00 – 13.15 h

Introduction into the Problem

Werner O. Richter

Institute for Lipid Metabolism and Hemorheology, Blumenstrasse 6, D-86949 Windach, Germany

In the literature more than 100 publications on hypertriglyceridemia in HIV+ patients can be found. But carefully looking through all these publications gives no clear picture neither on the prevalence nor on the possible pathomechanisms. This is obviously due to the fact that several factors may determine lipoprotein metabolism in these patients:

- HIV infection
- Highly active antiretroviral therapy (HAART)
- Life style
- Pre-existing lipoprotein disorders and the
- Presence of lipodystrophy.

In addition, in the interpretation of lipid concentrations in HIV+ patients several well-known facts have to be considered:

• Hypertriglyceridemia is caused by an increased number or increased size of very-low-density lipoproteins (VLDL). The increased size is due to a higher content of triglycerides. But VLDL do not only contain triglycerides – in normolipidemic subjects around 55 % - but also 20 % of cholesterol. Therefore an increase of triglyceride concentration in plasma is accompanied by an increase in cholesterol. But it has to kept in mind, that this is not due to an increase in the atherogenic LDL-cholesterol but to an elevation of VLDL-cholesterol concentration. Therefore in the case of elevated triglycerides elevated cholesterol concentration has an other importance as in the case of hypercholesterolemia caused by LDL-cholesterol.

• Large, triglyceride-rich VLDL are a bad substrate for their catabolism by lipoprotein lipase to intermediate density lipoproteins and thereby to LDL. Therefore, in most cases of hypertriglyceridemia LDL-cholesterol is low. For example, the typical finding in patients with a metabolic syndrome are elevated triglycerides, low HDL-cholesterol but normal or low LDL-cholesterol. If LDL-cholesterol is elevated an additional lipoprotein disorder such as polygenic hypercholesterolemia is present. This is a very common disorder in clinical practice. More than 75 % of patients with hypercholesterolemia suffer from this disorder. The situation may be similar in HIV+ patients.

• But the situation regarding LDL-cholesterol is even more difficult. VLDL can be regarded as the precursors of LDL. When hypertriglyceridemia improves or normalizes an increased number of LDL is produced. The conversion of VLDL to LDL requires hours, the half-life of LDL in plasma is four days or longer. Therefore after improving triglycerides an increase of LDL-cholesterol occurs which is "secondary" to hypertriglyceridemia. Such elevations of LDL-cholesterol can also be observed after initiation of therapy with fibrates or n-3 fatty acids. This is one cause why it is impossible to get full information by one lipid determination only.

• The higher the triglyceride concentration the lower HDL-cholesterol. The production of VLDL requires the transfer of cholesterylesters from HDL to VLDL mediated by cholesterylestertransferprotein. Therefore, for increasing HDL-cholesterol the production rate of VLDL has to be lowered, that means that triglycerides have to be lowered mainly by dietary therapy.

These different points are not only important for the interpretation of the lipid findings but also for the therapeutic concept.

Friday, November 21, 2003, 13.15 – 15.45 h

I. Lipoprotein Disorders I

Principles of lipoprotein metabolism: the role of the SREBPs

André R. Miserez

Cardiovascular Risk Clinics, Department of Internal Medicine, Basel University Hospital Bruderholz, CH-4101 Bruderholz, Switzerland, Cardiovascular Genetics, Institute of Biochemistry and Genetics, University of Basel, Vesalgasse 1, CH-4051 Basel, Switzerland and Research Laboratories, diagene gmbh, Kaegenstrasse 17, CH-4153 Reinach, Switzerland

Sterol-regulatory element-binding proteins (SREBPs) are members of the basic helix-loop-helix leucine zipper transcription factor family and are regulators of crucial metabolic pathways. The two major forms of the SREBPs, SREBP-1 and SREBP-2, control and coordinate the lipid and glucose metabolism. In humans, a splice variant of SREBP-1, SREBP-1c, is a key regulator of fatty acid synthesis, peripheral lipolysis, glycolysis and other insulin-mediated processes. In addition, SREBP-1c controls the determination and differentiation of adipocytes and is therefore also called adipocyte determination and differentiation factor (ADD)-1. Based on the pattern of physiological processes regulated by SREBP-1c/ADD-1, the gene encoding SREBP-1c/ADD-1, being possibly involved in the pathogenesis of the metabolic syndrome, became an interesting candidate gene for us. To gain further insights into these mechanisms, a syndrome frequently associated with highly-active antiretroviral treatment (HAART) in HIV-1-infected subjects, was investigated. This HAART-associated syndrome is similar to the metabolic syndrome, thus characterized by a combination of hypertriglyceridemia, hypercholesterolemia, hyperinsulinemia/insulin-resistance, and lipodystrophy and served therefore as a model to study part of the underlying pathogenetic mechanisms.

We characterized the structures of the genes and the promoters of SREBP-2 and of the alternatively spliced forms of SREBP-1, SREBP-1a and SREBP-1c/ADD-1. We then screened the gene encoding SREBP-1c/ADD-1 for sequence variations such as single nucleotide polymorphism (SNPs) and identified an SREBP-1c/ADD-1-specific SNP (3322C/G). By analyzing a cohort of 67 HIV-1-infected subjects treated with HAART, we could demonstrate that this particular SNP is predictive of the HAART-associated syndrome.

To dissect the effects of HAART on the transcriptional activity of SREBP-1c/ADD-1, we measured the activation of SREBP-1c/ADD-1-dependent genes (lipoprotein lipase, fatty acid synthase) using dual-luciferase reporter gene assay systems. The protease inhibitor indinavir (as part of HAART) inhibited the SREBP-1c/ADD-1-dependent gene expression of the lipoprotein-lipase (103 nmol/L, -12.4%, P = 0.0051) and the fatty-acid synthase (103 nmol/L, -30.3%, P = 0.036) highly significantly and in a dose-dependent fashion.

In conclusion, an inhibition of the SREBP-1c/ADD-1 activity was identified as being responsible for the metabolic adverse reactions associated with HAART. A novel marker predictive of the HAART-associated syndrome, the SREBP-1c/ADD-1-3322C/G polymorphism, was identified. The mechanisms explaining the inhibition of the SREBP-1c/ADD-1-dependent pathways were further elucidated.

Risk of lipoprotein disorders

Klaus G. Parhofer

Medical Department II, Klinikum Grosshadern of the Ludwig-Maximilians-University Munich, Marchioninistrasse 15, D-81366 München, Germany

Changes in lipoprotein metabolism are important in many fields of medicine. Such changes are related to inflammatory processes, acute pancreatitis, neurodegenerative diseases (in particular Alzheimer`s disease) the development of insulin resistance, and above all to atherosclerosis. From a clinical and health-economic point of view the causal relationship between dyslipoproteinemia and atherosclerosis are of utmost importance. Epidemiological studies across different populations have shown that increases in total cholesterol, LDL-cholesterol, triglycerides as well as reduction of HDL-cholesterol are strong predictors of atherosclerosis, in particular coronary artery disease. The pathophysiological basis of LDL-cholesterol induced atherosclerosis is relatively well understood and it is beyond doubt that lowering LDL-cholesterol reduces atherosclerosis. Much less is known about the underlying mechanisms that link low HDL-cholesterol or elevated triglycerides with atherosclerosis. This is complicated by epidemiological data indicating that not all forms of hypertriglyceridemia carry an atherogenic potential. In patients with insulin resistance or diabetes mellitus hypertriglyceridemia carries a high atherogenic potential. Other forms of hypertriglyceridemia (for example in lipoprotein lipase deficiency) are associated with atherosclerosis to a much lesser degree. In accordance with these ambiguous epidemiological data intervention studies in hypertriglyceridemia are less clear-cut than intervention studies in LDL-hypercholesterolemia. In evaluating the necessity of triglyceride lowering treatment it is therefore important to consider the clinical situation (i.e. the presence or absence of insulin resistance) and the overall risk status. From a practical point of view dyslipoproteinemias should be classified as LDL-hypercholesterolemia, hypertriglyceridemia, combined hyperlipidemia or isolated low HDL-cholesterol. Previously used classifications (Fredrickson classification etc.) provide little additional information. Furthermore, treatment should be directed towards the predominant lipid abnormality. If life style modification fails, statins are first choice in LDL-hypercholesterolemia and combined hyperlipidemia, while fibrates are first choice in hypertriglyceridemia and low HLD-cholesterol. In addition to its role in atherosclerosis severe hypertriglyceridemia may also result in acute pancreatitis. In such patients dietary and drug therapy (fibrates) should be used to control hypertriglyceridemia. Other aspects of lipoprotein metabolism that are particularly important in HIV-positive patients relate to inflammatory processes and the observation that hypertriglyceridemia can induce/worsen insulin resistance, which may be crucial in HAART-treated patients.

Lipoprotein metabolism in HIV-positive patients

Bertrand Perret, Corinne Ferrand, Eric Bonnet, Francois Tercé, Xavier Collet

INSERM 563, Dpt LML, Hôpital PURPAN, Toulouse 31059-Cédex, France

Hyperlipidemia is a common metabolic disorder occurring in HIV-infected patients, conferring an atherogenic lipoprotein profile during long-term antiretroviral therapy. Those metabolic abnormalities may have several origins. The HIV-infection itself has been associated with increased plasma triglycerides (TG), low levels of HDL-cholesterol, and, in symptomatic patients, of LDL-cholesterol. Those alterations most likely result from the large production of pro-inflammatory cytokines, TNF-alpha, IL-1 and IL-6, by activated monocytes and macrophages. Certain cytokines induce a decreased expression and activity of Lipoprotein Lipase (LPL), the enzyme responsible for the first step in VLDL-TG clearance. Moreover, acute phase proteins can bind to HDL particles, promoting their uptake by macrophages and increasing their clearance rate. However, those alterations are not specific of a given pathogen but are observed in severe inflammatory syndromes. Introduction of active antiretroviral therapy has been accompanied by an increase in metabolic complications, including hyperlipidemia, occurrence of a lipodystrophy, glucose intolerance and insulin resistance. Those complications are more frequent upon treatment by protease inhibitors (PI). Various phenotypes of dyslipidemia can be observed, but most of them include patent hyperTG and markedly low HDL-cholesterol levels. Associated with the accumulation of small and dense LDL particles and with a context of insulin-resistance, those abnormalities resemble those typical of the “metabolic syndrome”.

Among the different hypotheses, hyper-TG might be related to the lipoatrophy component, with peripheral adipocytes undergoing apoptosis and releasing TG and fatty acids in the circulation. Due to their mitochondrial toxicity, RTNIs might contribute to adipocyte apoptosis. Experimental evidences have shown that PIs favour lipolysis from mature adipocytes and inhibit differentiation of pre-adipocytes. A proposed hypothesis was that PIs would interfere with the activation of the nuclear receptor RXR, the obligate partner of PPAR-gamma, which is a key regulator of adipocyte differentiation. Actually it has been recently demonstrated that PIs block the maturation of an upstream transcriptional factor, SREBP1, preventing its access to the nucleus and impairing PPAR-gamma expression. The facts that, in cultured cells, those metabolic alterations were reversed by glitazones which are PPAR-gamma activators, and that similar obser-

vations were made in peripheral adipocytes taken from patients, lend support to the hypothesis that a defect in PPAR activation would be a major determinant of the peripheral lipoatrophy. Moreover, alterations in VLDL synthesis and/or in their catabolic clearance upon PI treatment must play major roles in the pathogenesis of the observed dyslipidemia. Apo B synthesis and secretion are partly regulated by the balance between association to lipids, as mediated by intracellular lipid transfer proteins, and degradation in the proteasome. It has been demonstrated that PIs can block the apo B proteolytic degradation, thus promoting its secretion, bound to VLDL particles. Regarding VLDL catabolism, ritonavir administration to healthy control subjects did not induce any effect on LPL activity. In a cross-sectional study, we observed an accumulation of lipoparticles containing apo C-III and apo E, in association with apo B, in PI-treated patients, at levels 2 to 3-times above those seen in PI-naive or control subjects. Those complex particles would represent persistent, potentially atherogenic, cholesterol-rich remnant particles, derived from TG-rich lipoproteins. It has been suggested that PIs could impair the clearance of apo E-containing particles due to a partial homology between the HIV-1 protease and LRP, a receptor that binds apo E in the liver. However, excess apo C-III might be a major determinant of a slower catabolism of TG-rich lipoproteins. Indeed, apo C-III is a natural inhibitor of LPL and it also impairs the interaction of neighbour apo B and apo E with the LDL-receptor and LRP. Apo C-III levels were also found associated with the presence of a lipodystophy. Apo C-III is down regulated by PPAR-alpha, an isoform expressed in the liver. Any defect in PPAR-alpha activation could result in a lack of apo C-III regulation. The effectiveness of fenofibrate, a PPAR-alpha agonist, to reduce TG and the accumulation of complex lipoparticles gives some support to this hypothesis. Thus, the mechanisms underlying the pathogenesis of hyperlipidemia occurring in HIV-patients under long term AART are probably multifactorial. A better understanding will help to define therapeutic strategies in order to treat those metabolic adverse effects.

Switch of antiretroviral therapy to avoid lipoprotein disorders?

Juergen Rockstroh

Department of Medicine I, University of Bonn, D-53105 Bonn, Germany

Hyperlipidemia has emerged as an important complication of highly active antiretroviral therapy (HAART). The major concern is its potential for premature atherosclerosis and coronary artery disease in the upcoming years, although this has not been conclusively established so far. Statins are the mainstay of drug therapy for hyperlipidemia, but they have additional side effects and are characterized by drug-drug interactions, especially with protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) which are considered to be cornerstones of HAART therapy. More recently switch strategies have been developed to evaluate whether identification of the antiretroviral drug presumptively associated with hyperlipidemia and subsequent switch to a more lipid neutral antiretroviral agent may have an overall impact on the development of lipoprotein disorders in HIV-infected individuals receiving HAART therapy.

The increase of lipids has been associated more frequently with most PIs and stavudine. In a recently presented study (Gilead 903) tenofovir was compared to stavudine in combination with a backbone of lamivudine and efavirenz in antiretroviral naïve patients. Interestingly, the tenofovir arm showed a better total fasting lipid profile with significantly lower increases in triglycerides, total and LDL-cholesterol, and a greater increase in HDL-cholesterol at week 48 compared to the stavudine arm. Subsequent studies substituting tenofovir for stavudine in HIV patients with newly developed dyslipidemia were able to demonstrate that triglyceride levels (n = 94) dropped from a median of 385 mg/dL (range 206-2399) at baseline to a median of 243 mg/dL (range 84-812) at 12 weeks ($p < 0.001$). Twenty percent of these patients returned to normal values. Cholesterol levels (n = 70) dropped from a median of 255 mg/dL (range 208-527) at baseline to a median of 226 mg/dL (range 154-446) at 12 weeks ($p < 0.001$).

There is increasing experience with switching the PI component of a successful HAART with the objective of reducing toxicity, including hyperlipidemia. Most of these studies have substituted the PI with nevirapine, efavirenz or abacavir and have shown that dyslipidemia is at least partially reversible. Overall, abacavir seems to cause a greater decrease in plasma lipids than nevirapine or efavirenz. Unfortunately, the effect of either one of the three agents to ameliorate body-fat abnormalities has been less convincing. Although the clinical significance of HAART associated lipoprotein disorders remains unclear, switch strategies represent an adequate possibility to decrease or reverse corresponding HAART induced dyslipidemia.

Other cardiovascular risk factors in HIV-positive patients

Frank-D. Goebel

Medizinische Poliklinik of the Ludwig-Maximilians-University, Pettenkoferstrasse 8a, D-80336 München, Germany

While antiretroviral combination therapy (ART) has proven to strongly reduce morbidity and mortality of HIV-infected patients there is mounting concern in regard of increased cardiovascular events as long-term complications of this treatment. Hyperlipidemia is a well known and frequently observed adverse event in ART, but quite a few additional risk factors for CHD have been identified: about 50% of patients treated with protease inhibitors (PI, in particular with indinavir) develop insulin resistance (IR) with 5 – 10% frank diabetes mellitus type 2, but reduced insulin sensitivity can also be observed in patients only treated with NRTI. The pathogenesis of IR appears to be multifactorial with inhibition of GLUT-4, accumulation of intraabdominal fat (resembling the metabolic syndrome X) and reduced metabolism of adipocytes. It remains to be proven whether or not patients with HIV have an increased prevalence of hypertension. Recent French and Italian population based studies have revealed conflicting results. In addition to the well known risk factors for CVD smoking, hyperlipidemia, diabetes mellitus and hypertension several metabolic alteration affecting blood vessels have been observed in HIV patients. There is evidence of severe endothelial dysfunction, in-

creased levels of plasma-homocysteine and highly sensitive C-reactive protein. Also disorders in the coagulation system have been described, such as prolonged activated partial thromboplastin time due to lupus-like anti-coagulant, deficiency of protein S, heparin co-factor II, antiphospholipid antibodies, increased D-dimer and von Willebrand levels, and high levels of tissue plasminogen activator and plasminogen-activator inhibitor 1. Several cases of vasculitis have been reported, but it's prevalence remains obscure. Pathogenesis of many of these disorders appears to be interrelated and has not been studied adequately. Also it is not clear whether these alterations are of clearly increased prevalence in HIV and whether they are a consequence of the viral infection itself or the antivirals used and, if so, which drug or drug combination is most likely to be responsible.

With this rather difficult situation therapeutic interventions for the various disorders seem to be problematic. There is clearcut proof that the percentage of heavy smokers is significantly higher in HIV-infected patients. Giving up this habit would reduce the cardiovascular risk considerably, but this problem certainly is hard to come by.

Friday, November 21, 2003, 16.15 – 18.15 h

II. Prospective cohort trials on cardiovascular risk

Veterans Affairs Cohort

Sam A. Bozzette

San Diego Veterans Affairs Medical Center, San Diego, California, USA

Our investigation, which was an outcome study, clearly showed that antiretroviral therapy has a benefit that is enormous relative to the risk of cardiovascular or cerebrovascular disease. Some clinical studies have suggested that HAART has only a small effect or no effect on this risk, but these studies had a small number of events or short follow-up or failed to correct for selection or the amount of drug exposure. Our conclusions were not different for the approximately 1000 patients treated with protease inhibitors for 48 months or more. Ascertainment may have affected the point estimates but probably did not change over time in a way that would attenuate a putative drug effect. Furthermore, we obtained similar results from national death-index data. (Cited from S. A. Bozzette and T. A. Louis, N. Engl. J. Med. 349 (2003) 1870).

Prospective cohort trials on cardiovascular disease: HIV outpatient study (HOPS)

Scott D. Holmberg

Clinical Epidemiology Section, Division of HIV/AIDS Prevention, Centers for Disease Control, Atlanta, GA, USA

Background and Methods:
Use of protease inhibitor drugs (PIs) is associated with increased total cholesterol, triglycerides, low-density lipoproteins and diabetic diathesis in HIV-infected patients. Because these metabolic problems can engender cardiovascular disease (CVD), we examined data from a large cohort of HIV outpatients followed at 9 HIV clinics (7 cities) in the United States: 3,247 patients who had taken PI drugs for more than 6 months; and 2,425 patients who had not taken PI drugs. These patients were followed for 17,712 py of observation.

Results:
As published a year ago (Lancet 2002; 360:1747-8), we found that PI use was strongly correlated with hyperlipidemia (Spearman corr. coeff., $p < 0.005$) and with diabetes mellitus ($p < 0.012$). There were 19 documented myocardial infarctions (MIs) in those who had taken PIs, but only two in patients who had not taken these drugs (left-censored Cox proportional hazards ratio [HR]= 8.06 [95% CI 1.14, 56.8]; p= 0.036). An analysis that controlled for age, sex, cigarette smoking, hypertension, diabetes and hyperlipidemia slightly reduced but did not eliminate this association (Cox model, HR_{adj}= 6.51 [0.89, 47.8], p = 0.065).

In a subsequent analysis, that incorporated additional patients (N = 7,542), followed to July 2003, use of PIs for more than a year was strongly associated with any CVD in HIV-infected patients aged 35- 65 years (HR_{adj} =1.90 [95% CI 1.13, 3.20], p= 0.02) (U Iloeje et al, submitted to 11th Conf on Retro & OIs). Increasing exposure to PIs was associated with increasing risk of CVD. In another analysis of the original HOPS patients (reported in Lancet), we found that the rate of MIs may be somewhat decreasing now after 2000; this decrease in MIs was preceded by declining use of PIs and increasing use of statin and other lipid-lowering therapy (LLT)

Conclusions:
Several lines of evidence suggest that PI drug use may engender MIs and other CVDs: temporal association; biologic plausibility; statistical association; and dose-response data. CVD in HIV patients is still uncommon and should not seriously compromise the use of this valuable class of antiretroviral drugs. However, US clinicians are now using PIs less and statins and other LLT more in the care of HIV patients.

Hospitalizations for coronary heart disease and myocardial infarction among Northern California men with and without HIV-1 infection

D. Klein, L. Hurley, C. Quesenberry, and S. Sidney

Kaiser Permanente, Oakland, California, USA

Background:
Whether patients with HIV-1 infection are at increased risk for CHD/MI, regardless of treatment regimen, compared to HIV-negative persons from the same base population is still controversial. Furthermore, there is uncertainty regarding the mechanism(s) that might account for reported differences. Few settings have data on sufficient numbers of HIV-infected patients and also have data on the base populations that are needed to conduct such analyses. Further study is also needed to establish the extent to which the documented atherogenic lipid changes consequent to protease inhibitor

(PI) therapy for HIV-1 infection contribute to CHD/MI risk. To date, duration of PI-exposure has been relatively short, events have been few, and traditional risks, as well as the dyslipidemia associated with HIV infection itself may confound any direct or indirect effect of PIs on risk.

Methods:
For a number of years we have monitored and compared hospitalization rates for CHD (ICD9 410-414) and separately for MI (ICD9 410) among a cohort of HIV(+) males and among a random sample of presumed HIV(-) males. All persons studied were members of the Kaiser Permanente Northern California health plan, a large closed system HMO, and had no prior CHD events. Observational follow-up (FU) began in 1996 and now extends through 6/30/03. In addition, among HIV+'s, person-years (PY) of FU was assigned as either no/pre-PI exposure vs. during/post PI-exposure. A person could contribute PY to both exposure categories. Age-adjusted CHD and MI rates for ages 35-64 were calculated overall for HIV+'s and HIV-'s, and by PI exposure and duration of PI exposure for HIV+'s. Traditional risk factors were assessed from electronic medical records.

Results:
In the 7.5-year observation period, 4726 HIV+ patients contributed 20,589 PY of FU (median 4.5) and there were 111 CHD events (66 MIs). Over forty thousand HIV-'s contributed 256K PY of FU. Among HIV+'s exposed to PIs, median PI exposure was 4.0 years. Age-adjusted CHD and MI rates among HIV+'s continue to be roughly twice that of HIV-'s (CHD: 6.6 vs. 3.0 events/1000 PY, $p < .0001$; MI: 3.9 vs. 2.2, $p < .005$). Among HIV+'s, overall rates for never vs. ever PI exposure were not different (CHD: 5.6 vs. 7.4, $p = .21$; MI: 3.6 vs. 4.2, $p = .61$). However, the age-adjusted relative risk for CHD hospitalization per two years of PI exposure was 1.17 (95% CI: 1.03, 1.33, $p = .01$); relative risk for MI was 1.07 (0.9, 1.3, $p = .41$).

Conclusions:
Even in the absence of PIs, HIV(+) persons have twice the rate of hospital CHD and MI as their HIV(-) counterparts. Traditional risks, including the dyslipidemia associated with HIV infection (low HDL), as well as chronic inflammation may provide a likely basis for this observation. Furthermore, hospitalization for CHD, but not MI (possibly due to small numbers) was associated with duration of PI use. Longer follow-up is needed but risk reduction management is warranted in all patients with CHD risks. Any risks associated with PI use must be weighed against the known benefits.

Antiretroviral combination therapy and risk of myocardial infarction. Results from the DAD Study

N. Friis-Møller, for the DAD Study Group

Copenhagen HIV Programme, Section 044, Hvidovre Hospital, Copenhagen, Denmark

Background:
It remains controversial whether exposure to combination antiretroviral treatment increases the risk of myocardial infarction.

Methods:
The DAD Study is a prospective observational study of 23,468 patients from 11 cohorts, enrolled from 1999 to 2001 with follow-up until February 2002. Data on HIV disease, risk factors for and incidence of myocardial infarction were collected. Relative rate from Poisson regression models are reported. Combination antiretroviral therapy was defined as any combination antiretroviral drug regimen containing a protease inhibitor and/or a non-nucleoside reverse transcriptase inhibitor.

Results:
Over 36,199 person-years, 126 patients developed a myocardial infarction. The incidence of myocardial infarction increased with longer exposure to combination antiretroviral therapy (adjusted relative rate per year of exposure was 1.26 (95 percent confidence interval: 1.12 to 1.41, $P < 0.001$)). Other factors associated with myocardial infarction were: age (per 5 years increase: 1.38 (1.26 to 1.50)), current or ex-smoking (2.17 (1.30 to 3.62)), previous cardiovascular disease (5.84 (3.51 to 9.72)) and male gender (1.99 (1.04 to 3.79)), but not family history of cardiovascular disease (1.18 (0.64 to 2.17). Total serum cholesterol level (1.16 (1.04 to 1.30) per mmol/L higher), triglycerides (1.39 (1.12-1.73) per two-fold higher) and diabetes (2.38 (1.38 to 4.10)) were also associated with an increased rate.

Conclusions:
Combination antiretroviral therapy was independently associated with a 26 percent relative increase in rate of myocardial infarction per year of exposure over the first 4 to 6 years of use. Although of concern, the absolute risk of myocardial infarction was low and should be balanced against the marked benefit of antiretroviral treatment.

Saturday, November 22, 2003, 9.00 - 11.30 h

III. Lipoprotein Disorders II

Lipoprotein metabolism under HAART

M. Galli, A. L. Ridolfo, C. Gervasoni, M. Vaccarezza, P. Fedeli, M. Ortu, S. Trovati, M. Moroni

Institute of Infectious Diseases and Tropical Medicine, University of Milan, Italy

Several reports describe abnormalities of blood lipids among patients treated with HAART both in association and independently from lipodystrophy alterations. Most of the data demonstrate convincingly that protease inhibitor (PI) originate changes in the lipoprotein profile, consisting of elevations in triglyceride-rich lipoproteins (TG), total cholesterol (CHOL) and LDL cholesterol, with some differences related to the particular drug used. On the contrary the role of reverse transcriptase inhibitors in causing metabolic alterations is less defined.

We assessed the risk of developing hyperlipidaemia in 322 patients (46,6 % females) followed up for a median time of 1100 days (range 94-2952) while on treatment with only two NRTI. Subjects who developed TG levels >200 mg/dl were 102/309 (33,0%), including 51/184 patients on ZDV (27.7%) and 51/125 patients on d4T (40.8%). A total of 41 patients

(12,7%) had TG levels ≥ 400 mg/dl al least in one determination. Altered CHOL serum levels (>250 mg/dl) were found in 20/305 patients (6,6%). Abnormally high serum levels of TG were sustained in 19.1% of patients. Male gender and treatment with d4T were associated with an increased risk of presenting hyperTG in the multivariate analysis (respectively, RH 1.61, 95%CI 0.99-2.62, p = 0.05 and RH 2.44, 95%CI 0.45-4.00, p<0.001). Older age was associated, at borderline statistical significance (p = 0.06), to an increased risk of developing hyperCHOL. Patients treated with d4T + ddI showed a 4.5 higher risk of developing hyperTG compared to those treated with AZT + 3TC (95%CI 1.95-10.45, p<0.001); d4T+ddI combination was also associated with a higher, although not statistically significant, risk of developing hyperCHOL. In a logistic regression analysis, male gender (RH 2.52, 95%CI 0.17-5.46, p=0.019), treatment with d4T+ddI (RH 17.01, 95%CI 1.58-183.56, p = 0.02) and d4T+3TC (RH 2.91, 95%CI 1.33-6.36, p = 0.007), a more prolonged time on treatment (RH 1.02, 95%CI 1.00-1.04, p = 0.02), and having presented abnormal levels of plasma CHOL (RH 6.16, 95%CI 1.56-24.33, p = 0.009)., glucose (RH 2.71, 95%CI 1.42-5.16, p = 0.003). or lipohypertrophy (RH 3.46, 95%CI 1.35-8.89, p = 0.01) were significantly associated with a higher risk of presenting hyperTG.

In another prospective study (LIPOICONA Study) we evaluated the role of metabolic changes in predicting the first lipodystrophy manifestation. The study included 873 ART-naïve patients. In a Cox model including age, gender, CD4 nadir, baseline body weight and vRNA, most recent change in CD4, HCV serology, most recent TG, CHOL and glucose values, and duration of exposure to each specific antiviral, the risk of lipoatrophy was higher in patients that acquired HIV by routes other than iv drug use (RH 3.08, 95%CI 1.12-8.43, p = 0.03) and in those with higher levels of the most recent TG (per 100mg/dl higher: RH 1.26, 95%CI 1.04-1.51, p = 0.02). In contrast, lipohypertrophy was associated with female gender (RH 2.03, 95%CI 1.12-3.67, p = 0.02), CD4 increase (per 100 cells/μl higher: RH 1.10, 95%CI 1.01-1.20, p = 0.04), use of 3TC (per year exposure: RH 1.03, 95%CI 1.00-1.05, p = 0.03).

In conclusion, hyperTG is a frequent finding in patients treated with NRTI. The role of NRTI in causing and maintaining metabolic alterations deserves further investigation. TG levels seem to predict early lipoatrophy manifestations in first-line treated patients while specific antivirals appear to play a minor role. Factors associated with lipohypertrophy are confirmed to be different from factors predicting lipoatrophy.

Diagnosis of lipoprotein disorders in HIV-positive patients

Stefan Mauss

Center for HIV and Hepatogastroenterology, Duesseldorf, Germany

Lipoprotein disorders in HIV-seropositive patients may have multiple origins. As a practical approach different causes for dyslipidemia can identified: 1. inherited lipoprotein disorders, 2. lifestyle associated dyslipidaemias, 3. dyslipidaemia due to HIV infection, 4. dyslipidaemia associated with antiretroviral treatment, 5. dyslipidaemia as a result of concomitant diseases or medication.

The first hint of an inherited lipoprotein disorder with a clinical relevance may come from the family history showing premature cardiovascular deaths in close relatives. This may justify a more thorough clinical assessment including apolipoprotein B, lipoprotein (a) and homocysteine. A more frequent reason for dyslipidaemia is the metabolic syndrome characterised by obesity, hypertriglyceridaemia, low HDL cholesterol, hyperglycaemia and hypertension. The diagnosis in treatment naïve HIV-seropositive patients, except for the low HDL cholesterol, can be made as in HIV-seronegative patients. However lipoatrophy or treatment with some antiretrovirals may partially mimic metabolic syndrome and complicate the diagnosis in cases where pretreatment data are missing. Dyslipidaemia due to the natural course of the HIV-infection is characterized by a decrease in total cholesterol and HDL-cholesterol, appearance of smaller LDL particles and in late stage HIV-infection increased triglycerides. Antiretroviral drugs may rise lipids partially by immune restoration and partially by drug specific effects with a distinct pattern. In addition concomitant diseases like nephrotic syndrome or hypothyroidism and the effect of drugs such as anabolic steroids in high doses should be excluded.

For clinical use the metabolic profile of a patient can be assessed by taking a fasting blood sample in the morning analysing total cholesterol, HDL- and LDL-cholesterol, triglycerides, apolipoprotein B, and blood glucose. High triglycerides without impaired glucose tolerance suggest dyslipidaemia due to familial hypertriglyceridaemia or some antiretroviral drugs. In combination with hyperglycaemia the cardiovascular risk may be more pronounced due to metabolic syndrome or metabolic changes because of antiretrovirals mimicking this syndrome. Apolipoprotein B adds information on the number and size of LDL-, IDL and VLDL-particles in total not obtainable by the cholesterol analysis. Small particles and high apolipoprotein B levels are associated with a high cardiovascular risk, whereas large particles and low apolipoprotein B levels may be less atherogenic.

Longitudinal information on the metabolic profile of a patient particularly before the start of antiretroviral treatment, during antiretroviral treatment interruptions or before switching to another antiretroviral regimen is of great value to differentiate between the different aetiologies of dyslipidaemia in HIV infected patients.

Treatment of hypertriglyceridemia: dietary factors

Elizabeth J. Parks

University of Minnesota, Minneapolis/St. Paul, USA

Blood triglyceride (TAG) concentration becomes significantly elevated in HIV through mechanisms inherent to the illness itself and as a result of pharmacological therapy. The relationship of fat redistribution syndrome to elevated blood TAG concentrations in HIV may be similar to that shown in healthy, obese populations. Blood TAGs may rise through a mechanism of increased release of TAG-rich li-

poproteins by the liver or as a result of reduced clearance of lipid from the plasma. Many lifestyle factors affect the rate of turnover of TAG in the plasma. Aerobic exercise, weight loss, and the addition of n-3 fatty acids to the diet can reduce blood TAG concentration, while increased alcohol intake, lack of physical activity (sedentariness), dehydration can increase blood TAG, as can the content of the meal consumed the day before the TAG measurement occurred. The process by which dietary carbohydrate is transformed into fat in the human body is termed de novo lipogenesis and the consumption of dietary carbohydrate that is primarily monosaccharide in structure, can stimulate lipogenesis. New methods for the measurement of this process in humans are available and have been used to investigate the role of carbohydrate form (fed as a liquid or solid), the level of processing of carbohydrate in foods, and the role of lipogenesis in the control of liver triglycerol secretion. Of interest is the relationship between the glycemic index of a food (or indicators of a food's glycemic index) and that food's ability to stimulate lipogenesis in humans. Given the many therapeutic strategies that can potentially restrain hypertriglycerolemia, efforts should be made to clarify which of these lifestyle strategies will be efficacious counteracting insulin resistance in the HIV-positive population. The present paper will discuss factors that alter blood TAG concentration in healthy individuals and how these factors impact hypertriglyerolemia in HIV and AIDS.

Lifestyle management for the treatment of lipoprotein disorders in HIV-infected patients: What do we know? What can we do? What could be expected?

Gilles J. Thöni

Laboratorium of Cardiovascular Adaptations to Exercise, Faculty of Sciences, Avignon; Lab. "Motor efficiency and deficiency", University of Sports Sciences, Montpellier; Dpt. of Clinical Physiology, Lapeyronie University Hospital, Montpellier, France

Changes in the blood lipid profile have been described in HIV- infected patients for many years before the widespread use of Highly Active Antiretroviral Therapies (HAART).

Nowadays, the progressive hypertriglyceridemia – common with others severe chronic infections – remains associated with an advanced stage of the disease. In such situations, modifications in the patients' lifestyle and prevention against cardiovascular disorders are not a major concern yet. On the opposite, the high prevalence of patients under antiretroviral multitherapies, with isolated hypercholesterolemia, hypertriglyceridemia or both, leads clinicians to be especially attentive. To date, the means likely to protect HIV-positive patients against cardiovascular risks are now a matter of concern. The numerous reports about cardiac events and vascular dysfunction in this population strongly urge clinicians on developing efficient management strategies.

Historically, and because HIV-related dyslipidemia is strongly associated with protease inhibitors, switching for a non-containing PI regimen was first considered in the treatment of dyslipidemia. But the obtained results are pretty inconstant and the corrections of the lipid parameters are described over a long term. The second pharmacological strategy consists in using lipid-lowering therapies (fibrates or statins) which is in fact the second stage of intervention, according to the recommendations from the National Cholesterol Education Program (NCEP).

Because of the relative youth of the problem and its rapid evolution, clinicians started to provide advises based on the classical recommendations for the general population (regular biochemical follow-up, research and correction of associated risk factors...). Patients were then often asked to modify their lifestyle (reduction of cigarette and alcohol consuming, change in diet habits and physical activity). Dieticians working in infectious disease departments modified their clinical intervention. And some departments, specialised either in smoking cessation or in cardiovascular risk evaluation were recently associated in the management of HIV-infected patients. But their interventions were most often not objectively evaluated. Besides, programs aiming to modify usual physical activity in this population are almost non-existent. As a consequence, the literature about the effects of lifestyle modifications (the first intervention measures as defined by the NECP) on patients' lipoprotein disorders remains dramatically poor. Even though a meta-analysis of the data is not possible yet, many strong epidemiological, physiopathological and economical arguments sustain the major role of lifestyle interventions in this context.

The preliminary experimental results seem promising: if the effects of smoking cessation on the blood lipid profile need to be evaluated, the first investigational "low fat diet" studies have shown interesting decrease in both cholesterol and triglycerides. Similar results were obtained after several supervised physical training programs. All the patients should benefit from these prevention and therapeutic measures. Nevertheless, ageing patients, women and children may be considered as special and privileged targets. All this data – and the recent publication of specific guidelines for intervention against HIV- and HAART related metabolic disorders – emphasize many perspectives and will surely favour the development of a new, wide and necessary field of research. We hope that this will specify the extent of lifestyle management effects on HIV-positive individuals' dyslipidemia.

Treatment of lipoprotein disorders in HIV-positive patients – Drug therapy

Leonardo Calza, Roberto Manfredi, Francesco Chiodo

Department of Clinical and Experimental Medicine, Section of Infectious Diseases, University of Bologna "Alma Mater Studiorum", S. Orsola Hospital, Bologna, Italy

Despite the profound impact of the protease inhibitor (PI)-based antretroviral treatment (highly active antiretroviral therapy or HAART) on the natural history of human immunodeficiency virus-type 1 (HIV-1) infection, leading to a remarkable decrease of its morbidity and mortality, this pharmacological association has frequently been associated with a wide range of clinical and metabolic complications. The fat redistribution syndrome or lipodystrophy (charac-

terized by either localized fat loss in the face or limbs, or fat accumulation in the abdomen, breasts or dorso-cervical region), hyperlipidaemia, insulin resistance, and hyperglycaemia have been extensively reported in subjects treated with PIs and nucleoside reverse transcriptase inhibitors (NRTIs). Event though therapy with zidovudine, lamivudine, stavudine, or non-nucleoside reverse transcriptase inhibitors (-NNRTIs) has been sometimes associated with the occurrence of dyslipidaemia, abnormalities of plasma lipid levels appear to be a prevalent condition among patients receiving a PI-based regimen. The scenario of concurrent and/or related lipid metabolism alterations includes hypertriglyceridaemia, hypercholesterolaemia (or rather increased total and LDL cholesterol levels), and decreased serum HDL cholesterol levels, besides other metabolic or clinical abnormalities, such as insulin resistance with hyperinsulinaemia, raised C-peptide concentrations, diabetes mellitus, and the lipodystrophy syndrome. In patients receiving a PI-containing antiretroviral regimen, the prevalence of hyperlipidaemia ranges from 28 to 80%, and it includes hypertriglyceridaemia in the majority of cases (40-80%), followed by hypercholesterolaemia (10-50%), and hyperglycaemia-hyperinsulinaemia (5-30%), with prevalence of fat redistribution syndrome ranges from 10 to 80% in different studies. The so-called lipodystrophy syndrome is frequently (but not always) associated with dyslipidaemia: although metabolic alterations are more common among patients with lipodystrophy, they are also present in those without these morphologic changes. It has been observed that metabolic abnormalities usually precede the body fat redistribution. Particularly, fat depletion or lipoatrophy syndrome is often related to the hypertriglyceridaemia, while fat accumulation or lipohypertrophy syndrome is accompanied by dyslipidaemia, peripheral insulin resistance, raised C-peptide levels, and diabetes mellitus in an elevated number of cases. Epidemiological, clinical and laboratory risk factors for the PI-related hyperlipidaemia are still today controversial when comparing different published researches. Even though elevations in serum triglyceride and cholesterol levels have been associated with all the available PIs, hypertriglyceridaemia seems more frequent in patients receiving a ritonavir, ritonavir-saquinavir, or ritonavir-lopinavir combination therapy, and may sometimes be extreme, reaching a triglyceride plasma concentration >1000 mg/dL in subjects on ritonavir therapy. At the same time, a mild to moderate increase of cholesterol levels seems more frequent among patients treated with ritonavir and probably nelfinavir, as opposed to indinavir. Since the introduction of HAART into clinical practice has remarkably changed the natural history of HIV disease and led to a notable extension of life expectancy, prolonged metabolic alterations could significantly act on the long-term prognosis and outcome of HIV-infected persons. Therefore, these metabolic imbalances are gaining an increasing concern, particularly about the elevated risk of cardiovascular complications. A pharmacologic hypolipidaemic therapy becomes necessary when the HAART-related hyperlipidaemia is remarkable or persists for a long time, and if dietary changes, physical exercise and switching treatment are ineffective or not applicable. Drug therapy for dyslipidaemia in HIV-infected persons receiving HAART is problematic, because of potential drug interactions, toxicity, intolerance, and decreased patient adherence to multiple pharmacologic regimens. Simvastatin, lovastatin, and atorvastatin are extensively metabolized by CYP 3A4, leading to a remarkable risk of drug interactions with PIs and NNRTIs. On the other hand, fluvastatin is metabolized by CYP 2C9 and pravastatin is not significantly metabolized by the CYP enzyme system, with a very low risk of drug interactions. Consequently, it is reasonable to recommend the use of pravastatin as first-line treatment for hypercholesterolaemia in PI-treated patients, and the use of fluvastatin (characterized by a slightly lower efficacy), as second-line regimen. On the other hand, fibrates represent the cornerstone of drug therapy for hypertriglyceridaemia and mixed hyperlipidaemia. However, concomitant use of both fibrates and statins can increase the risk of skeletal muscle toxicity and should be avoided. With regard to other hypolipidaemic agents, niacin should be avoided as first-line therapy in subjects treated with PIs, because it often causes cutaneous rash, pruritus, and insulin resistance. Bile sequestering resins should be discouraged because they may produce elevated triglyceride levels and bind several co-administered medications, with a reduction in their oral bioavailability. Fish oils (omega-3 fatty acid supplements) show a variable effect on plasma triglyceride concentrations, and their efficacy has not been fully demonstrated. In a study including 106 HIV-positive subjects on HAART with hyperlipidaemia, a hypolipidaemic therapy was started with bezafibrate, gemfibrozil, fenofibrate, pravastatin, or fluvastatin. At the close of 1-year follow-up, fibrates led to a reduction of 40.7% and 21.9% versus baseline triglyceridaemia and cholesterolaemia, respectively ($p<0.001$), and statins led to a reduction of 32.9% and 24.7% versus baseline triglyceride and total cholesterol levels, respectively ($p<0.001$), without significant differences according to different administered drugs. During these 12 months, both fibrates and statins showed a favourable tolerability profile, plasma HIV viral load did not present any variation, and the mean CD4+ lymphocyte count increased as expected.

Saturday, November 22, 2003, 12.00 – 13.15 h

IV. Free communications

Risk of metabolic abnormalities in HIV-infected patients receiving lopinavir-ritonavir containing HAART

Esteban Martinez[1] Pere Domingo[2], Maria J. Galindo[3], Ana Milinkovic[1], Juan A. Arroyo[2], F. Baldovi[3], Maria Larrousse[1], Agat León[1], Elisa de Lazzari[1], José M. Gatell[1]

[1]Hospital-IDIBAPS, Barcelona, [2]Hospital de la Santa Creu i Sant Pau, Barcelona, [3]Hospital Clinico Universitario, Valencia, Spain

Background:
Metabolic abnormalities particularly hyperlipidaemia, are one of major adverse effects associated with lopinavir/ritonavir therapy, although its incidence and risk factors associated with them are poorly studied by now.

Methods:
Consecutive HIV-infected patients receiving antiretroviral therapy containing lopinavir-ritonavir were included in the study and followed for 6 months or until lopinavir-ritonavir discontinuation whichever came first. Clinical and fasting laboratory dates were collected at baseline, 3 and 6 months.

Antidiabetic and lipid-lowering drugs in the moment of the entry at the study were maintained but new prescriptions of these drugs were not allowed during the study. Primary objectives were the evolution of metabolic parameters, the proportion of patients reaching plasma glucose >124 mg/dL, triglycerides >400mg/dL or total cholesterol >240 mg/dL at 6 months and the risk factors associated with the development of each one of those metabolic abnormalities after starting lopinavir/ritonavir.

Results:
Of 353 patients included, 302(86%) received lopinavir/ritonavir for at least 6 months. In this cohort, 50% of the patients had clinical evidence of lipodystrophy and 80% had previously received protease inhibitors. The evolution of fasting metabolic parameters and the factors associated with developing glucose >126 mg/dL, triglycerides >400 mg/dL, or total cholesterol >240 mg/dl at 6 months were assessed. Although glucose did not change, triglycerides and cholesterol ($P<0.0001$ each) as well as the proportion of patients with triglycerides >400 mg/dL (13% at baseline, 20% at 6 months; $P = 0.016$), and cholesterol >240 mg/dL (18% at baseline, 28% at 6 months; $P = 0.002$) significantly increased. Baseline glucose >126 mg/dL (OR 12.2, 95%CI 1.9-79.5, $P=0.009$), triglycerides >400 mg/dL (OR 4.5, 95%CI 2.1-10.7, $P<0.0001$), and cholesterol >240 mg/dL (OR 8.4, 95%CI 3.9-18.0, $P<0.0001$) were identified as independent predicting factors for developing clinically significant metabolic abnormalities at 6 months.

Conclusion:
The risk of developing diabetes, hypertriglyceridemia or hypercholesterolaemia warranting therapeutic intervention with lopinavir/ritonavir-containing HAART depends on the baseline values of the metabolic parameters. These findings may have clinical implications when the therapeutic option of lopinavir-ritonavir is considered.

ApoC3 variants are associated with dyslipidemia and lipodystrophy in HIV-patients treated with protease inhibitors

E. Bonnet[1,2], J. Fauvel[2,3], J. Bernard[1], M. Obadia[1], P. Massip[1] and B. Perret[2,3]

[1]Service des Maladies Infectieuses, Hôpital Purpan, Toulouse, [2]Unité INSERM 563, Hôpital Purpan, Toulouse, [3]Laboratoire de Biochimie III, Hôpital La Grave, Toulouse, France

Background:
Dyslipidemia and lipodystrophy are two major complications of PI-including-HAART. Although some NRTI have been implicated in the development of lipodystrophy, lipidic disturbances are mostly due to PI. High interindividual variability for dyslipidemia has been observed among patients on PI-containing-regimen. Some genetic profiles could be predisposing factors for the occurrence of lipodystrophy and dyslipidemia. In transversal studies high levels of apolipoprotein C3 have been shown to be highly correlated with lipodystrophy. ApoC3 is located on VLDLs and are normally repressed by insulin. Polymorphisms on insulin response elements are frequent and may play a role in the appearence of dyslipidemia when insulin resistance occurs.

Aim of the study:
To evaluate the impact of ApoC3 polymorphisms on the severity of dyslipidemia and on the anomalies of body fat/thin mass repartition measured by DEXA.

Patients and methods:
We focussed our study on HIV-male who have been treated with PI for at least one year and d4T for at least 2 years. Two polymorphisms have been selected in this study : one concerning the insulin reponse element and one in the 3'-untranslated region which is often associated with hypertriglyceridemia. For all patients we measured the levels of total cholesterol (TC), HDL and LDL-cholesterol, triglyceridemia (tgc), ApoA1, ApoB, ApoC3, ApoE, LpA1, LpB:C3, LpB:E, Lp(a), glycemia and insulinemia, and we measured the percentage of body fat mass (%BFM), ratio of trunk fat mass/lower limbs fat mass (TFM/LLFM) and the ratio of the percentage of TFM/percentage of LLFM (%TFM/%LLFM).

Results:
31 patients have been included in this study. -455C variant is associated with more severe dyslipidemia and lipodystrophy although the difference between the groups does not reach a significant threshold for all variables probably because of the small size or our population.

ApoC3 polymorphisms ->	-455 T/T	-455C	p
tgc	2.14 (± 0.40)	2.90 (± 0.54)	0.14
ApoC3	30.81 (±5.41)	34.67 (±3.3)	0.28
ApoE	68.98 (±13.36)	105.26 (±10.12)	0.03
LpB:C3	16.97 (±5.96)	23.05 (±0.21)	0.21
LpB:E	53.26 (±15.24)	95.06 (±11.53)	0.03
%BFM	19.54 (±2.56)	13.89 (±1.29)	0.03
TFM/LLFM	3.23 (±0.33)	3.73 (±0.28)	0.13
%TFM/%LLFM	1.74 (±0.17)	2.01 ± (0.11)	0.1

Similar trends for lipids parameters and body mass composition were observed with SstI (3'-untranslated region) polymorphisms.

Conclusion:
These preliminary data show that ApoC3 polymorphisms seem to be associated not only with the severity of dyslipidemia but also with the anomalies of body mass composition, objectively measured by DEXA.

Mid-term follow-up after coronary angioplasty in HIV-infected patients. A multicenter case control study

F. Boccara[1], S. Hernandez[2], E. Teiger[2], E. di Angelantonio[1], A. Cohen[1], G. Barbaro[3], on behalf of the French-Italian Study Group on coronary artery disease in AIDS patients (FRISCA-I)

[1]Saint Antoine University Hospital, Assistance Publique-Hôpitaux de Paris and Université Paris VI, France, [2]Federation de Cardiologie, Henri Mondor University Hospital, Assistance Publique-Hôpitaux de Paris and Université Paris XII, Creteil, France, [3]Department of Medical Pathophysiology, University La Sapienza, Rome, Italy

Background:
Acute coronary syndromes (ACS) are an emerging complication in HIV-infected patients under highly active antiret-

roviral treatment. Prognosis of coronary angioplasty after ACS remains unknown in this population.

Methods:
Using a case-control study design, we compared baseline characteristics and mid-term outcome at 12 months (MACE [Major Adverse Cardiac Event] defined as cardiac death, non fatal myocardial infarction (MI), or target lesion +/- vessel revascularization (TLR, TVR) in 43 HIV-infected patients (HIV+) and 43 controls (HIV-) matched for age and gender who underwent coronary angioplasty.

Results:
ST segment elevation MI (STEMI) was more frequent in the HIV- group compared with the HIV+ group (55.8 % vs. 18.6 %, p < 0.001) whereas NSTEMI was more frequent in HIV+ group (55.8 % vs. 25.6 %, p = 0.004). Late diagnosed MI (< 1 month) was observed in 23.3 % and 7.0 % (p = 0.035), respectively. Multivessel coronary artery disease (≥ 2 vessels) was present in 58.1 % and 48.8 % (p = 0.378), respectively, with predominant LAD lesion (81.4 % and 69.8 %, p = 0.209). LVEF was lower (51.5 ± 9.7 vs. 55.8 ± 6.8, p = 0.03). Procedural success rate was achieved in 98 % and in-hospital course was uneventful in the two groups. Rate of occurrence of first MACE is summarized in the table. In a multivariate model, MI < 1 month was the only predictor of MACE at 12 months follow-up (OR 4.54, 95 % CI: 1.25 – 16.38, p = 0.02). No patients were under a statin before coronary angioplasty.

Conclusion:
Coronary angioplasty is feasible and safe in HIV-infected patients. Mid-term follow-up demonstrates no difference in MACE between HIV+ and HIV- patients.

Table:

Risk factors	HIV+ n = 43	HIV- n = 43	P
Mean age, years	43 ± 7	44 ± 3	0.582
Male sex (%)	38 (88.4)	37 (86.0)	0.747
Hypertension (%)	4 (9.3)	10 (23.3)	0.080
Diabetes mellitus (%)	-	5 (11.6)	0.055
Total cholesterol < 210 mg/dl	34 (79.1)	28 (65.1)	0.149
Smoking (%)	37 (86.0)	41 (95.3)	0.138
Outcome (first event reported)			
MACE-12 months (%)	10 (24.4)	6 (14.3)	0.243
Death (%)	-	-	
MI (%)	4 (10.0)	-	0.052
TLR (%)	7 (17.5)	3 (7.1)	0.152
TVR (%)	-	4 (9.5)	0.116

Coronary artery bypass graft in HIV-infected patients. A multicenter case control study

F. Boccara[1], G. Odi[1], E. di Angelantonio[1], A. Cohen[1], G. Barbaro[2], on behalf of the French-Italian Study Group on coronary artery disease in AIDS patients (FRISCA-II)

[1]Saint Antoine University Hospital, Assistance Publique-Hôpitaux de Paris and Université Paris VI, France, [2]Department of Medical Pathophysiology, University La Sapienza, Rome, Italy

Background:
Coronary artery disease is an emerging complication in HIV-infected patients under antiretroviral therapy. Prognosis of coronary revascularization remains unknown in this population.

Methods:
Using a case-control study design, we compared clinical characteristics, angiographic findings and 30-day outcome (mortality or non-fatal myocardial infarction or reoperation) of 17 HIV-infected patients (HIV+, cases) and 34 non-HIV infected patients (HIV-, controls) who underwent coronary bypass graft (CABG) [1995 – 2003]. The control group was matched for gender and was under 60 years old.

Results:
In the HIV+ group, mean preoperative CD was 461 ± 165 mm3 compared with 408 ± 161 mm^3 postoperative (p = 0,004). Late diagnosed MI (< 1 month) was observed in 58.8 % (HIV+) and 17.6 (HIV-) (p = 0.028). Non ST segment elevation MI was similar in the two groups (35.3 % vs. 50 %, p = 0.319). Stable angina was more frequent in HIV- compared with HIV+ (32.3 % vs. 5.9 %, p = 0.035). Coronary multivessel disease (2-vessel disease) was present in 17 (100 %) HIV+ and 33 (97.1 %) HIV- (p = 0.475). LVEF and mean number of grafts was similar in the two groups (55 ± 10 % vs. 51 ± 12 %, p = 0.278; 2.7 ± 0.6 vs. 2.8 ± 1.0, p = 0.675). Rate of occurrence of 30-day outcome is summarized in the table. There were neither death nor postoperative stroke during hospitalisation and after 30 days in the two groups.

Conclusion:
CABG is a feasible and safe revascularization procedure in HIV-infected patients. Postoperative outcome demonstrates no difference between HIV+ and HIV- patients.

Table:

	HIV+ n = 17	HIV- n = 34	p
Mean age, years	48 ± 11	54 ± 7	0.009
Hypertension (%)	8 (47.1)	14 (41.2)	0.698
Diabetes mellitus (%)	2 (11.8)	11 (32.4)	0.112
Total cholesterol >210 mg/dl (%)	16 (94.1)	27 (79.4)	0.173
Smoking (%)	15 (88.2)	27 (79.4)	0.436
Prior MI (%)	4 (23.5)	7 (20.6)	0.810
Postoperative MI < 30 days (%)	1 (5.9)	0	0.347
Reoperation <30 days (%)	0	2 (6.3)	0.537

Effect of growth hormone (GH) on dyslipidemia in HIV patients with excess VAT

Christine Wanke[1], Carl Grunfeld[2], Donald P. Kotler[3], Deana Bock[4], Joseph Gertner[4], Gregg Simons[5], Norma Muurahainen[4], on behalf of the Serostim® in the Treatment of Adipose Redistribution Syndrome (STARS) Trial Investigators

[1]Tufts-NEMC, Tufts University School of Medicine, Boston, MA [2]UCSF-VA Med Center, San Francisco CA, [3]St Luke's-Roosevelt Hospital Center, NY, NY, [4]Serono Laboratories Inc, Rockland MA, [5]Quality Clinical Research, Hull, MA and the STARS Trial investigators

Introduction:
In HIV patients (pts) with visceral adiposity, GH was found to reduce visceral adipose tissue (VAT) and non-HDL cholesterol (Kotler 2002) while improving body image and qual-

ity of life (Thompson, 2003). GH effects on lipoprotein parameters are detailed in this analysis.

Methods:
In a double-blind, placebo-controlled, multi-center trial, 245 HIV patients with excess VAT (13.3% female) on combination antiretroviral (ARV) therapy were blindly randomized to placebo (P), GH 4 mg daily (DD), and GH 4 mg on alternate days (AD) for 12 weeks (wks). For Weeks 13-24, DD patients were re-randomized to P or AD (DD-P, DD-AD). Patients on AD continued receiving AD (AD-AD). P switched to DD (P-DD). Fasting lipid levels were measured at baseline, Week 12, and Week 24.

Results:
At baseline, pts had low HDL cholesterol (0.96 mmol/mL) and elevated total cholesterol (5.6 mmol/L), LDL-C (3.23 mmol/L), non-HDL C (4.6 mmol/L) and triglycerides (3.7 mmol/L), and 30% were receiving lipid-lowering agents (statins, fibrates, or both). Between treatment groups, there were no baseline differences in mean lipoprotein levels or percentages of patients receiving lipid-lowering, PI, NRTI, or NNRTI therapies. At Week 12 compared to baseline, total cholesterol and non-HDL-C decreased while HDL-C increased in DD (-5.1%, -8.1%, +11.3%) and AD (-4.5%, -6.4%, and +7.4%), all $p<0.05$. Triglycerides decreased (-9.6%, $p<0.05$) from baseline in AD but not in DD or P. From baseline to Week 24, significant ($p<0.05$) reductions in non-HDL-C and increases in HDL-C were retained in DD-AD and AD-AD, and significant decreases from baseline in triglycerides were observed in AD-AD, DD-P (-9%, -15%) but not in DD-AD or P-DD.

Conclusion:
This study suggests that a regimen of r-hGH dosed 4 mg daily for 12 weeks improves the cholesterol profile in HIV-infected patients on ARV therapy with excess VAT. The optimal regimen to sustain VAT and lipid benefits awaits determination.

Saturday, November 22, 2003, 13.15 – 14.15 h

V. Posterlunch

Relation between lopinavir plasma-levels and lipoprotein disorders in HIV-positive patients on a stable HAART

A. Benesic, R. Winzer, M. Zilly, H. Klinker, P. Langmann

Schwerpunkt Hepatologie/Infektiologie, Medizinische Poliklinik of the University of Wuerzburg, Bau 18, Josef-Schneider-Strasse 2, D-97080 Wuerzburg, Germany

Background:
Disturbance of lipid-metabolism in HIV-positive patients receiving highly active antiretroviral therapy (HAART) is becoming a major topic, since its meaning for the development of cardiovascular disease is still discussed controversially. Therapeutic drug monitoring is useful to evaluate relations between side effects of antiretroviral agents, especially PI´s and NNRTI´s and their plasma-levels. There is evidence for a dose-dependent PI-induced dyslipoproteinemia in patients receiving salvage therapy containing lopinavir/r. In this study HIV-positive patients on a stable HAART containing LPV/r were observed for a dose-dependent effect of lopinavir on lipid-levels.

Materials and Methods:
In this prospective, non-randomized study 38 HIV-positive patients (12 females, 1 afro-american, 2 asian, 9 caucasian and 26 males, all caucasian) receiving a LPV/r containing HAART were followed over a median period of 56 weeks. All patients received a median lopinavir-dose of 10.5 mg/kg body weight. Median body-mass-index was 23.8 kg/m^2. Use of lipid-lowering drugs led to exclusion from the study. Total cholesterol, LDL- and HDL-levels and serum triglycerides were determined at regular intervals and fasting state. All patients had a dietary education and were advised to regular exercise. Lopinavir plasma levels were analyzed by HPLC.

Results:
In the observed patient collective there was no significant difference in lipid-levels between female and male patients as well as untimed and trough lopinavir plasma-levels (analysis with Kruskal-Wallis-Test, $p>0.4$). Total cholesterol was 204.6 ± 37.6 mg/dl, LDL-cholesterol 109.5 ± 33.5 mg/dl, HDL-cholesterol 48.3 ± 13.3 mg/dl, triglycerides 283.9 ± 246.5 mg/dl.

The mean lopinavir trough-level was 5747 ± 2238 ng/ml, whereas untimed levels had a mean of 6141 ± 2341 ng/ml. In this patient collective, no significant correlation between the elevation lipid-levels and lopinavir plasma-levels, determined as trough levels and untimed, could be observed ($R^2<0.14$). Of the 12 female patients, 2 showed combined hyperlipidemia, 1 hypercholesterolemia and 6 hypertriglyceridemia. In the 26 male patients, 8 showed combined hyperlipidemia, 1 hypercholesterolemia and 10 hypertriglyceridemia.

Conclusions:
1) Under antiretroviral therapy containing LPV/r 28 of 38 patients showed hyperlipidemia (16 of 38 hypertriglyceridemia, 2 hypercholesterolemia and 10 combined hyperlipidemia). 2) No correlation between lopinavir plasma-levels (-trough and untimed) could be shown in this patient collective. 3) In this study no significant difference in lipid-disorders under HAART could be observed between the female and male population. 4) A lopinavir-dose of 10.5 mg/kg bodyweight results in comparable trough levels in women and men in the therapeutic range of >4500 ng/ml.

Oral fat challenge in HIV patients with and without HAART – a pilot study

V. Wolf, G. Friese, J. Lohmeyer, H. U. Kloer

Medical Department II and III, Justus-Liebig-University, D-35392 Giessen, Germany

Introduction:
Several studies have been conducted to study hyperlipoproteinemia in HIV patients under HAART (Highly Active Antiretroviral Therapy). Most studies are retrospective and

focus on fasting lipid levels. We aimed to look at the postprandial lipid metabolism in order to show possible differences in the metabolism.

Methods:
9 patients with confirmed HIV disease participated in this study. All patients had a double protease inhibitor therapy (ritonavir and indinavir) and a NRTI (nucleoside analogue reverse transcriptase inhibitor) and/or NNRTI (non-NRT) for more than 3 months. Patients underwent an oral standardized fat challenge and consecutively blood samples were drawn after 12 hour fasting and in defined timeframes. The same test was performed without therapy for at least 3 months in the same patient. Levels of cholesterol and triglycerides were measured in serum and in the lipoprotein fraction.

Results:
At all measured time points mean triglyceride and cholesterol levels were higher when patients took PI. Without PI the postprandial slope of the curve of the mean triglyceride levels in serum was comparable to the expected physiological reaction. Four hours after an oral fat challenge it is expected that triglyceride levels in serum of healthy individuals increase due to chylomicronemia. This was not seen in patients with HIV taking PI.

Discussion:
The observed small postprandial differences, at the time where patients took PI, may reflect the already high triglyceride levels after 12 hour fasting. Our findings support the hypothesis that the amount of VLDL particles in plasma could be increased by reduced clearance from the circulation due to PI mediated inhibition of LDL receptor related protein LRP. Further studies are necessary since the individual results show severe differences and the number of patients was limited.

Safety profile of the two available non-nucleoside reverse transcriptase inhibitors. Focus on serum lipid abnormalities

Roberto Manfredi, Leonardo Calza, Francesco Chiodo

Department of Clinical and Experimental Medicine, Division of Infectious Diseases, University of Bologna "Alma Mater Studiorum", S. Orsola Hospital, Via Massarenti 11, I-40128 Bologna, Italy

Aim of our study is to assess the tolerability profile of the two available non-nucleoside reverse transcriptase inhibitors (NNRTI): efavirenz and nevirapine, with special attention deserved to dysmetabolism. A cross-sectional study performed on 988 HIV-infected patients treated with antiretroviral compounds during at least 12 months and naïve to NNRTI, allowed us to identify 212 patients given nevirapine, and 234 subjects who introduced efavirenz. After excluding 47 patients because of adherence levels below 90%, 191 patients who received nevirapine were compared with 208 ones treated with efavirenz on the basis of a multivariate analysis which focussed on adverse events, toxicity, and related treatment interruptions. Both study groups were comparable as to demographic and epidemiological features, HIV disease stage, mean HIV viremia and mean CD4+ lymphocyte count, rate of HCV and/or HBV co-infection, antiretroviral therapy background, and pre-existing metabolic disturbances and/or lipodistrophy syndrome (in pre-treated subjects). When examining the 121 patients naïve to antiretrovirals, the tolerability index measured during the first three months of treatment did not differ between the two NNRTI, but clinical features were substantially different, with predominant hypersensitivity reactions for nevirapine, and central nervous system disturbances for efavirenz ($p < .0001$). When considering patients with previous experience of antiretrovirals, and those on salvage regimens, a grade 1-3 liver toxicity occurred in over one fifth of nevirapine-treated individuals, compared with three patients only in the efavirenz group ($p < .0001$). When NNRTI substituted a protease inhibitor due to prior dysmetabolic abnormalities, a drop of serum lipid levels (triglyceridemia and/or cholesterolemia) of at least 30% versus time of NNRTI introduction, occurred in over two thirds of patients who switched towards nevirapine, compared with less than 30% of those who introduced efavirenz ($p < .0001$), while in ten patients a frank dyslipidemia appeared only after efavirenz use. The two available NNRTI have a comparable activity and resistance profile, but the remarkably different pattern of potential adverse events has to be taken into careful consideration, due to the broad spectrum of short- and long-term toxicity, and the significant differences noticed between efavirenz and nevirapine, in terms of incidence and clinical presentation of untoward events. A long-term observation of patients pre-treated with other antiretroviral regimens seems to show a tendency towards a cumulative liver toxicity for nevirapine, and stable or worsening metabolic (and lipid) abnormalities for efavirenz. The investigation of the potential pathogenetic pathways of the profoundly different toxicity patterns of these two compounds belonging to the same therapeutic class of NNRTI, warrants further studies.

Lipomatosis during HIV disease treated with antiretroviral therapy. A novel, emerging untoward effect?

Roberto Manfredi, Leonardo Calza, Francesco Chiodo

Department of Clinical and Experimental Medicine, Division of Infectious Diseases, University of Bologna "Alma Mater Studiorum", S. Orsola Hospital, Via Massarenti 11, I-40128 Bologna, Italy

The fat redistribution syndrome (so-called lipodystrophy syndrome) with its different features, together with multiple and variably associated metabolic abnormalities, emerged during recent years as a consequence of the administration of potent antiretroviral combinations. Local fat accumulation may present as central adipositiy, increased breast dimension, gynecomastia, lipomastia, and the so-called "buffalo hump". As to our knowledge, lipomas and other benign tumors of fatty tissue have not been reported with increased incidence during HIV disease, even during the highly active antiretroviral therapy (HAART) era. Eight patients with HIV disease out of around 1,000 HIV-infected patients referring to our tertiary outpatient centre, experienced the occurrence of multiple lipomas since the year 2000. All patients suffered from ultrasonography-confirmed multiple lipomatous lesions (three to over 20), predominantly localized at limbs, thorax, and anterior abdomen, associated with limit-

ed local discomfort, and in absence of pain. Six subjects were male and two were females, their age ranged from 36 to 58 years, the duration of known seropositivity varied between 38 and 116 months, and none of them had a previous diagnosis of full-blown AIDS. Risk factors for HIV disease included i.v. drug addiction and heterosexual contacts in three cases each, and homo-bisexual transmission in the two remaining cases. At the time of onset of lipomas, all patients were given a protease inhibitor-based HAART regimen since 17-56 months (mean 24.6 ± 13.8 months). Our patients experienced 4 to 9 different anti-HIV therapeutic lines: almost all available protease inhibitors and nucleoside analogue reverse transcriptase inhibitors had been used previously or at the time of occurrence of lipomas, while this patient group never resorted to non-nucleoside reverse transcriptase inhibitors. While clinical and laboratory markers of HIV disease proved satisfactory at the time of onset of lipomas (with a mean viral load of 3.1 ± 0.6 Log_{10} HIV-RNA copies/mL, and a mean CD4+ lymphocyte count of 432.2 ± 145.9 cells/μL), some features of the lipodystrophy syndrome and concurrent dysmetabolism were detected. In particular, peripheral lipoatrophy was present in six patients out of eight, associated with central adiposity in four cases, while no localized fat accumulation was present (i.e. breast enlargement or "buffalo hump"). From a laboratory point of view, hypertriglyceridemia, hypercholesterolemia, and hyperglycemia were detected at the time of occurrence of lipomas in five, three, and one patient respectively (with some correlation with the onset of signs of the fat redistribution syndrome). The subsequent follow-up (13 to 28 months), allowed us to identify the appearance of further lesions in four patients, and a substantially stable disease in the remaining four cases, while spontaneous regression never occurred. Clinical monitoring is still ongoing, and surgery never became necessary to date. The relationship between lipomas, HIV infection, and HAART is still poorly defined, but the frequent association with other clinical and metabolic disturbances possibly related to antiretroviral associations should prompt further epidemiological, pathogenetic, and clinical studies. The possible pathogenetic role carried out by antiretroviral classes or drugs, and the concomitant occurrence of lipodystrophy and dysmetabolism, need further investigation. Malignant degeneration (observed in very few cases of HIV-associated breast lipomastia), should be a rare event, but careful surveillance seems recommendable in this setting.

The Study OF HAART's implication in accumulation of defective proviral genomes in peripheral blood mononuclear cells of HIV infected patients

Marina N. Papuashvili, Tatyana I. Shcherbakova, Vladimir Morozov

Advanced Medical Researches. Moscow, Russia

The purpose of our research was to perform the comparative analysis between 2 groups, (30 HIV infected patients with Highly Active Anti-Retroviral Therapy (HAART) and 30 HIV+ naïve patients) to clarify the role of HAART in accumulation of defective genomes in PMBCs. Patients, aged from 21 to 48 years with subtypes of HIV A, B, C and G were included. A syndrome of peripheral fat wasting, central adiposity and dyslipaemia has been identified among 9 HIV patients receiving HAART.

Materials and methods:
Genomic DNA of HIV from PMBCs was amplified for five different regions: 1,784 Kb 5'LTR-gag region (HXB2 542-2324), 0,406 Kb (HXB2 942-1307), 0,301 Kb(HXB2 1758-2018), 0,749 Kb (HXB2 2548-3256), 0,359 Kb(HXB2 7338-6978) by short-distance- nested- PCR (SD-nPCR). To reveal defective variants proviral DNA we performed amplification of HIV sequences by SD-nPCR with long-distance primers (HXB2 542-7781, 542-7947). The bands of interest were excised from an agarose gel and DNA was column purified (Sigma, Inc.).The purified DNA was sequenced by BigDyeTerminator v3.0 on ABI Prism 377 automatic sequencers (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's protocol. Sequences data analysis was performed manually using Vector NTI Suite 6.0., ClustalX(1.5b), MEGA(version 2.1)programs.

Results:
Defective genomes were detected in 29 patients. Inside this group, 17 were with HAART (virus loading from 893 up to 54000 RNA copy/ml), and 12 HIV positive naïve patients (virus loading from 37962 up to 183 728 RNA copy/ml). The length of defective genomes from different patients varied from 0.4 Kb up to 2.0 Kb and showed different type of sequences. Defective genomes were not revealing during the cocultivation of infected PMBCs in the presence of interleukin-2.

The summary:
Comparative analysis of the defective genomes of HIV from naïve and treated with HAART patients revealed, that patients with dyslipaemia, treated by HAART, have shown defective genomes of a constant size and sequence over the time. Accumulation of defective genomes in PMBCs as we suppose, grows out as a consequence of multiple passages of HIV during the long unproductive period of life of the infected cell. However, intensity of accumulation can essentially vary on a background of HAART, what probably one can prove after realization of quantitative calculation the defective genomes of HIV in dynamics.

Gynecomastia and its metabolic correlates during antiretroviral treatment

Roberto Manfredi, Leonardo Calza, Francesco Chiodo

Department of Clinical and Experimental Medicine, Division of Infectious Diseases, University of Bologna "Alma Mater Studiorum", S. Orsola Hospital, Via Massarenti 11, I-40128 Bologna, Italy

Aim of our study is to identify all episodes of gynecomastia occurring during HIV infection and antiretroviral therapy in a single-centre cohort including around 1,000 patients, and to investigate all potential correlations between this emerging disturbance and metabolic abnormalities, as well as demographic and epidemiological variables, clinical and laboratory markers of HIV disease progression, prior and underlying anti-HIV therapy, and subsequent follow-up. A cross-sectional survey of 988 subjects treated with antiretrovirals for at least 12 months (661 males, leading to 66.9% of cases), allowed us to retrieve all patients with ultrasonography-con-

firmed, true gynecomastia, considered after exclusion of all other concomitant conditions potentially involved in the pathogenesis of this disorder. Particular attention was warranted for eventual concurrent metabolic alterations, including the lipodystrophy syndrome, dyslipidemia, and hyperglycemia, and administered antiretroviral drugs (and their combination). Fifteen patients of the 513 evaluable HIV-infected males (2.92%) developed gynecomastia when aged 12 to 58 years. The duration of known seropositivity and the time elapsed from start of antiretroviral therapy (and HAART) varied significantly in our patient group, and no correlation was found with clinical and laboratory markers of HIV disease. But five patients out of 15 (33.3%) never received protease inhibitors, while an efavirenz-based treatment apparently prompted gynecomastia in four patients who were naïve for protease inhibitors, and worsened this sign in other three patients who switched from a protease inhibitor-based HAART towards this last non-nucleoside reverse transcriptase inhibitor. One patient developed gynecomastia while on prolonged, isolated dual nucleoside analogue therapy in absence of both protease inhibitors and non-nucleoside reverse transcriptase inhibitors. A complete hormonal workout failed in detecting significant abnormalities in all patients but one, and hyperprolactinemia was never found. A concurrent form of fat redistribution syndrome (peripheral lipoatrophy, central adiposity, combined lipodystrophy) was present in all patients who developed gynecomastia, while hypertriglyceridemia, hypercholesterolemia, and hyperglycemia were found at the time of diagnosis of gynecomastia in 11, six, and three patients, respectively. Among nucleoside analogues, stavudine (followed by lamivudine), represented the compound administered more frequently and for a more prolonged time in the whole patient group interested by gynecomastia. During the subsequent follow-up (seven to 23 months), no significant clinical amelioration of gynecomastia was observed, despite eventual therapeutic changes (determined by regimen failure and/or toxicity), but plastic surgery never proved necessary. In conclusion, gynecomastia, as an emerging untoward event of HIV infection treated with antiretroviral combinations, warrants further investigation, from an epidemiological, clinical, and especially pathogenetic point of view. The apparently frequent association with other metabolic anomalies suggests some common ethiologic pathway with other HIV- and antiretroviral-associated disturbances, so that special attention should be paid on concurrent anti-HIV treatment, and the role of single antiretroviral classes and compounds.

Occurrence of gynecomastia in a male adolescent treated for congenital HIV infection since birth

Roberto Manfredi, Leonardo Calza, Francesco Chiodo

Department of Clinical and Experimental Medicine, Division of Infectious Diseases, University of Bologna "Alma Mater Studiorum", S. Orsola Hospital, Via Massarenti 11, I-40128 Bologna, Italy

Gynecomastia is an emerging untoward event during HIV infection treated with antiretroviral compounds. Although first related to protease inhibitors and eventual associated dysmetabolism, it has been recently reported also in subjects who never received protease inhibitors, as well as in patients who abandoned protease inhibitor-based HAART. At the age of 13.5 years, a boy with congenital HIV infection developed a true bilateral gynecomastia, confirmed by a sonographic examination, and associated with mild local paresthesia. At the time of occurrence of gynecomastia, height and weight developmental milestones and pubertal stage were within normal limits, according to patient's age (G_3 P3, Tanner staging). An endocrinological workout did not detect any gonadal, hypophyseal, and thyroid abnormality; serum prolactin levels tested also normal. Chronic kidney and liver disease, and administration of drugs potentially related to gynecomastia, were carefully excluded. At the age of five our patient, notwithstanding the absence of HIV-related disorders, had a rise in viremia and started antiretroviral therapy, continued until now. During the 8.5-year follow-up, five different therapeutic lines were administered, including HAART regimens based on ritonavir, nelfinavir, and lopinavir-ritonavir. When considering nucleoside analogues, the compound administered for a more prolonged time was stavudine (61 months). At the time of onset of gynecomastia (six months ago), a negligible viremia was retrieved (280 HIV-RNA copies/mL), while CD4+ lymphocyte count was very favourable (848 cells/μL). Our patient never presented clinical alterations included in the definition of the lipodystrophy syndrome, and no abnormality of serum lipid and glucose metabolism was detected through the entire follow-up. Gynecomastia did not show significant modifications in its clinical features during the last six months of monitoring. Our case report is exceptional, since among the around 70 literature cases of gynecomastia described until now among HIV-infected patients, none emerged in pediatric or adolescental age. Both pathogenesis and evolution of gynecomastia are increasingly investigated, and special attention is deserved to eventual correlations with dysmetabolic alterations and the fat redistribution syndrome (all absent in our case report), as well as single antiretroviral agents and their associations. In the described boy, nucleoside analogues were administered during 8.5 years, and protease inhibitors during around 7 years, while non-nucleoside reverse transcriptase inhibitors were never used. While all available protease inhibitors were involved in the literature-reported cases of gynecomastia, among nucleoside analogues stavudine seems to represent the most frequent molecule, although biases due to the very frequent resort to this last compounds in many HAART combinations, cannot be excluded. Our patient, who represents the first case of true gynecomastia reported during pediatric HIV disease with absence of dysmetabolic and lipodystrophy alterations makes even more uncertain every possible pathogenetic hypothesis, and even contradict many potential correlations postulated or claimed until now.

Myocardial infarction in HIV+ patients under HAART

Miguel San Martin[1], Francesco Blanco[1], Teresa Garcia-Benayas[1], Angel Garcia[2]

[1]Services of Cardiology and [2]Infectious Diseases, Hospital Carlos III, Instituto de Salud Carlos III, Madrid, Spain

Background:
A higher rate of coronary events in HIV+ patients receiving HAART has been reported. Possible mechanisms of premature atherosclerosis and endothelial dysfunction in HIV+

persons undergoing potent antiretroviral therapy are increasingly being evaluated.

Methods:
Overall, out of 2100 HIV+ individuals on regular follow-up at our outclinic (1640 of them on antiretroviral therapy) we reviewed ten patients diagnosed of acute myocardial infarction (AMI) within the period from January 2001 to September 2003. Patients´ demographics, time from diagnosis of HIV, immunological and virological status, time of exposure to antiretroviral treatment, risk factors for atherosclerosis, and lipid values at the onset of the AMI are evaluated. Clinical presentation, vessels affected and left ventricular eyection fraction are also reported.

Results:
Nine of them were male. Mean age was 46.7 years (70% of patients were younger than 50 years). Six patients referred previous intravenous using. The interval between the diagnosis of HIV infection and the occurrence of AMI was 9.6 ± 5.3 years. CD4+ cell count was 413 ± 256 cells/mL at the onset of AMI. The viral load was 3.1 log10 cop/ml (1.7-5.55) [median (range)]. Mean period of exposure to HAART was 55.3 months, and three of them never had been treated with protease inhibitors (PI). Regarding cardiovascular risk factors, a family history of coronary artery disease was present in five patients, eight subjects were smokers, two had hypertension and one was diabetic. A lipodystrophy syndrome was observed in six patients. The serum mean lipid values were: cholesterol 198 ± 42 mg/dL, low-density lipoprotein cholesterol 122 ± 38 mg/dL, high-density lipoprotein cholesterol 34 ± 10 mg/dL, and triglycerides 242 ± 270 mg/dL. Two patients followed lipid lowering therapy with statins, other two with fibrates and one patient with both. Chest pain was the first manifestation of the AMI in six individuals, one subject referred atypical chest pain, another one abdominal pain and in two patients it was asymptomatic. Four patients had multivessel coronary artery disease. Their mean left ventricular ejection fraction was 55% and in three patients it was 50% or lower.

Conclusions:
An aggressive atherosclerosis and hypercoagulability status induced by the high prevalence of risk factors in patients under HAART, and HIV itself may explain the AMI observed in these patients. Control of HIV infection and metabolic alterations induced by antiretroviral drugs, as well as the correct management of other modifiable risk factors, are decisive in order to prevent cardiovascular morbidity and mortality in the future.

Saturday, November 22, 2003, 14.15 – 16.45 h

VI. Lipodystrophy

Risk factors of lipodystrophy

Enos Bernasconi

Infectious Disease Unit, Department of Medicine, Regional Hospital, Lugano, Switzerland

Identification and characterization of drug toxicity, i.e. clinical presentation, spectrum of severity, and risk factors, has become a priority as prolonged utilization of antiretroviral drugs makes long-term side effects a critical issue for the management of HIV-infected patients. Metabolic disorders (hyperlipidemia and insulin resistance) and abnormalities in body fat distribution (lipodystrophy) have raised to a major drawback in the treatment of HIV infection. Since lipodystrophy can evolve to severely disfiguring features, there is a urgent need to better understand the risk factors for the development of abnormalities of body fat distribution. In the last few years a large amount of experimental and clinical data have helped us to better characterize drug related and non-treatment related risk factors of lipodystrophy.

The role of nucleoside reverse transcriptase inhibitors (NRTI), particularly stavudine, in the development of lipodystrophy in HIV infected persons has been supported by findings of many published studies. NRTI have been shown to inhibit the mitochondrial DNA polymerase gamma, resulting in impaired synthesis of mitochondrial enzymes that generate ATP. This leads to a broad spectrum of tissue damage, particularly to impaired adipocyte ultra structure with prominent mitochondrial abnormalities. The use of protease inhibitors has also been shown to correlate with lipodystrophy, more often with central fat accumulation. However, some studies came to the conclusion that duration of exposure to ART more than then any individual drug is an independent risk factor for lipodystrophy.

In a study published last year, we assessed the prevalence of fat distribution abnormalities in the Swiss HIV cohort study, a large observational cohort of HIV infected patients, which so far has enrolled more than 12'000 HIV-infected persons aged 16 years or older. We found that current use of stavudine was associated with the presence of lipodystrophy. Among non-treatment related risk factors, we were able to confirm the contributory role of increasing age, but not of other variables independently associated with fat loss in other studies, such as presence and time since AIDS diagnosis, severity of HIV infection, low nadir CD4+ cell count. In addition, we found an independent association of lipodystrophy with elevated lactate. Since lactate elevation can reflect altered mitochondrial function, the association between lactate elevation and lipodystrophy is a further argument in favor of the mitochondrial toxicity theory. The increase of lipodystrophy prevalence in the older age group could be related to mitochondrial aging.

Pathogenesis of metabolic disorders and lipodystrophy

R. E. Schmidt, K. Weber, M. Stoll, G. Behrens

Klinische Immunologie, Medizinische Hochschule Hannover, 30625 Hannover, Germany

Metabolic abnormalities and morphologic changes in HIV patients under highly active antiretroviral therapy (HAART) had become an important focus during the recent years. The recent D:A:D study demonstrates an increase of myocardial infarction by exposure to combinations of antiretroviral therapy (CART).

Dyslipidemia is characterized by hypercholesterolemia, hypertriglyceridemia or both. Peripheral insulin resistance, im-

paired glucose tolerance, and diabetes mellitus have been described in a significant number of patients. Frequently, these metabolic alterations precede or accompany body habitus changes like central adiposity and/or peripheral fat loss.

Until now very little is known about possible defects in insulin-induced skeletal muscle glucose uptake, the mechanisms or specific tissues involved in insulin resistance under HAART. In this study we aimed to investigate the metabolism using positron-emission tomography (PET).

Whole body glucose disposal and oxidation were determined by combination of the euglycemic-hyperinsulinemic clamp technique and indirect calorimety in six patients on HAART and six untreated HIV patients. Body composition analysis was performed by anthropometric measurements as well as dual energy X-ray absorptiometry and body impedance analysis. Muscle glucose uptake of the thighs was measured simultaneously by dynamic F-18-fluorodeoxyglucose PET.

Whole body glucose disposal was significantly reduced in patients on HAART compared to untreated patients. Analysis of kinetic constants using a three-compartment model indicated reduced skeletal glucose uptake caused by significantly impaired glucose phosphorylation in treated patients but normal transmembrane glucose transport. Skeletal muscle glucose uptake was reduced by 79% in treated patients and explained 32% or 94% of whole body glucose disposal in patients on HAART and therapy-naive patients, respectively. In addition, insulin-stimulated whole body oxidative and non-oxidative glucose disposal was significantly lower in the treated group. Suppressive insulin action on lipolysis was also impaired. Patients receiving HAART had signs of lipodystrophy.

This is the first report providing in vivo evidence that in HIV patients receiving HAART impaired glucose phosphorylation in the skeletal muscle contributes significantly to reduced insulin-mediated glucose uptake.

Lipodystrophy and adipocytokines

Jean-Philippe Bastard

Biochemistry Department, Tenon Hospital and
INSERM U.402, Paris, France

A high proportion of HIV-infected patients on antiretroviral therapy develop lipodystrophy, characterized by peripheral fat wasting, visceral fat redistribution and metabolic alterations with dyslipidemia and insulin resistance. Although the lipodystrophy syndrome is multifactorial, protease inhibitors (PI) and nucleoside reverse transcriptase inhibitors (NRTI) have been clearly implicated in cohort studies and in vitro experiments. NRTI have been implicated in peripheral lipoatrophy and their combination with PI results in an increased incidence and severity of this phenotype. However, the mechanisms involved in lipodystrophy are not currently clearly elucidated. The mRNA expression of C/EBP-alpha and SREBP1c, two key transcription factors involved in adipocyte differentiation and insulin sensitivity, is markedly lower in fat from patients with HIV-related lipoatrophy than in controls, and inversely correlated with apoptosis and increased blood vessel density. Altered cytokine expression in adipose tissue could represent a possible link between differentiation and apoptosis. In vitro and animal studies have shown that TNF-alpha which mainly acts through autocrine/paracrine mechanisms can promote adipocyte dedifferentiation, resistance to insulin, and apoptosis. While TNF-alpha production by white adipose tissue is low in physiological conditions, it can be markedly increased in animal models of obesity. A strong set of arguments suggests the involvement of TNF-alpha in the adipose tissue changes associated with HIV-related lipodystrophy. Higher mRNA concentrations of TNF-alpha have been found in fat from HIV lipodystrophic patients compared to non-lipodystrophic HIV infected patients or non-HIV controls. Likewise, higher circulating levels of TNF-alpha and its soluble receptors have been reported in lipodystrophic patients as compared to controls. Interestingly, we found that TNF-alpha expression correlated negatively with the expression of adipogenic factors and positively with apoptosis. Together with other published data, this suggests that TNF-alpha could be involved in adipocyte dedifferentiation and in apoptosis in fat of lipodystrophic patients. IL-6 mRNA expression is increased in fat from lipodystrophic patients and negatively correlated with C/EBP-alpha and SREBP-1c expression while positively related to apoptosis. This suggests that IL-6 could also play a major role at the local level, through paracrine/autocrine mechanisms as it was observed in type 2 diabetic patients. Antiretroviral drugs could be responsible for the increased expression and secretion of these cytokines. A recent study indicates that some PI can induce the expression and secretion of TNF-alpha and IL-6 by cultured 3T3F442A adipocytes, while some NRTI increase TNF-alpha expression. It is therefore conceivable that PI and NRTI can increase the expression of TNF-alpha and IL-6 in adipocytes, resulting in decreased differentiation and increased apoptosis. The adipose tissue of patients contains an increased number of macrophages, which surround adipocytes in lipogranuloma-like structures. These macrophages are probably activated, expressing IL-6 and TNF-alpha. Therefore, the increased IL-6 and TNF-alpha expression observed in patients' fat could also derive partly from macrophages. As IL-6 and TNF-alpha act through autocrine and paracrine mechanisms, these cytokines could be responsible for adipocyte dysfunction, whatever their cellular origin. The role of leptin and adiponectin in insulin sensitivity has been reported several times. Serum adiponectin and leptin levels are decreased in serum from HIV lipodystrophic patients. A decreased mRNA concentration of adiponectin in fat from patients with HIV-related lipodystrophy was recently described, and was found to correlate with insulin resistance. This data argue for a role of adipocytokines in insulin resistance in this setting. The decreased expression of adiponectin could result from drug toxicity since PI and NRTI were both found to decrease adiponectin mRNA expression in 3T3F442A adipocytes. In conclusion, adipose tissue from lipodystrophic HIV-infected patients presents some morphological and molecular alterations that are inter-related. The increase in TNF-alpha and IL-6 expression in adipose tissue could result from PI and NRTI exposure and result in altered adipocyte differentiation and insulin sensitivity, as well as increased apoptosis, ultimately leading to lipoatrophy. Finally, a drug-induced decrease in adiponectin secretion, together with an increase in free fatty acid release by

insulin-resistant adipose tissue, could also be involved in insulin resistance in muscle and liver and metabolic disorders.

Cellular mechanisms of lipodystrophy

Ulrich A. Walker

Medizinische Universitätsklinik, Abt. Rheumatologie und Klin. Immunologie, Hugstetterstrasse 55, D-79106 Freiburg, Germany

The term "lipodystrophy" is commonly used to describe certain alterations of body physique in HIV-infected individuals receiving long-term antiretroviral treatment. Patients are most troubled by a vanishing subcutaneous adipose tissue in the face, buttocks and limbs, but an abnormal fat accumulation may simultaneously occur in the central abdomen or the dorsocervical region. Moreover "lipodystrophy" is also used to denote features of a disturbed metabolism, such as insulin resistance and dyslipidemia. A case definition of "lipodystrophy" has been developed, but several studies suggest that the common lipoatrophic features have a different aetiology than the lipohypertrophic features. Therefore the different syndromes of lipodystrophy should not be lumped together. This abstract will analyze mainly lipoatrophy.

There is increasing in vitro and in vivo evidence that the irreversible fat wasting is related to the use of nucleoside-analogue reverse transcriptase inhibitors (NRTIs). Several randomized and prospective studies demonstrate that the long-term use of stavudine, a particular NRTI represents the most important risk factor for lipoatrophy, whereas the evidence for a contribution of antiretroviral protease inhibitors (PIs) is much less well documented. A recent longitudinal trial has shown that it takes at least 72 weeks of continuous NRTI-exposure until the onset of objective peripheral fat loss.

NRTIs inhibit polymerase-gamma and thus the replication of mitochondrial DNA (mtDNA). Such mitochondrial toxicity probably represents the principal cellular mechanism of NRTI-related mitochondrial and probably also of adipocyte toxicity, although AZT might have additional effects on mitochondria. Clinically relevant mitochondrial toxicity is suggested by the demonstration of ultrastructural abnormalities in adipocytes of lipoatrophic individuals, such as marked variation of mitochondria in size and shape, a disturbed architecture of the cristae and the presence of inclusion bodies. Moreover, a quantitative deficiency of mtDNA copy number (mtDNA-depletion) and of mtDNA-encoded respiratory chain subunits has been detected in lipoatrophic regions and associated with the use of stavudine. MtDNA-depletion was shown to improve upon switching away from stavudine, along with a slow increase of subcutaneous fat. Cessation of the incriminated NRTI also improved adipocyte apoptotic indices, whereas cytokine mRNAs in adipose tissue remained unaffected.

The use of PIs has also been incriminated in the onset of lipoatrophy. Adipocyte cultures exposed to PIs demonstrated an altered nuclear morphology; the nuclear translocation of SREBP, a transcription factor necessary for adipocyte differentiation was inhibited, along with the expression of PPAR-gamma and C/EBP-alpha. Although these effects were demonstrated for only some PIs and a concentration adjustment for the strong protein binding of PIs has not been performed in the in vitro studies, reduced amounts of SREBP and other downstream adipocyte transcription factors have been also demonstrated in the adipose tissue of lipoatrophic subjects. Despite these in vitro findings, switching away from PIs did not improve lipoatrophy by objective measurements, nor did it improve adipocyte apoptosis.

Recently it has been suggested that the use of NucleomaxX®, a dietary supplement rich in uridine can prevent and treat NRTI-related mtDNA-depletion and abrogate all its deleterious consequences. The mechanism is thought to involve the correction of an intracellular depletion of pyrimidines, which occurs secondary to the inhibition of respiratory chain dependent dehydroorotate dehydrogenase. Such pyrimidine deficiency sensitizes polymerase-gamma to the inhibitory action of pyrimidine analogue antiretroviral NRTIs. A clinical trial that has just been approved will analyze the efficacy of NucleomaxX in HIV-related lipoatrophy.

Management of Lipid Abnormalities in HIV

Graeme Moyle

Associate Director of HIV Research, Chelsea and Westminster Hospital, London SW10 9NH, UK

Dyslipidaemia with raised total cholesterol, low HDL cholesterol and raised triglycerides with increased lipid cycling and insulin resistance, with hyperglycaemia in susceptible individuals, are commonly observed during antiretroviral therapy. These may be accompanied by morphological changes. Fat accumulation in the abdomen or viscera is also commonly accompanied by insulin resistance, glucose intolerance and dyslipidaemia. This is now commonly called 'the metabolic syndrome' or 'syndrome X', a syndrome that is increasingly diagnosed in the setting of general medicine.

Dyslipidaemia and insulin resistance generally precedes morphological changes. It is presently not known if the development of lipid elevations or insulin resistance is predictive of the development of fat loss or fat accumulation. Thus, lipid and insulin sensitivity abnormalities may be considered separately from morphological change. Studies in HIV negative volunteers indicate that insulin resistance can be induced with a single dose of indinavir and that modest shifts in cholesterol and triglycerides are seen with some protease inhibitors in particular ritonavir. Studies using radiolabelled lipid, glucose, and using in vitro systems have indicated that some protease inhibitors interfere with glucose uptake receptors (GLUT 4 located on adipocytes and myocytes, GLUT 2 located on pancreatic islet cells), hepatic output of VLDL and peripheral lipoprotein lipase activity. Nucleoside analogue drugs have been associated with reduced peripheral lipid trapping and may disrupt metabolism in multiple tissues through mitochondrial toxicity.

The metabolic syndrome, once established, may be a vicious cycle and a cycle which is hard to break. Lipid accumulation in liver, pancreas, skeletal muscle and visceral sites due to abnormal peripheral storage adds to insulin resistance, fur-

ther worsening peripheral lipid storage. Thus, avoidance of establishing the syndrome may be particularly relevant.

The type of lipid profile that accompanies HIV infection and antiretroviral therapy often includes a low HDL, elevations in total and LDL cholesterol (in particular the high risk LDL particles) and elevations in triglycerides. This lipid profile is a typical high risk of profile for future cardiovascular disease. Additionally, insulin resistance has been evidenced to be a risk factor for cardiovascular disease and diabetes mellitus is considered in NCEP guidelines to be a cardiovascular disease risk equivalent. Thus, the metabolic disturbances that company HIV and antiretroviral therapy are likely to have important consequences for future cardiovascular health. The DAD study has reported that cumulative years on combination antiretroviral therapy is independently associated with risk of a cardiovascular event in persons with HIV infection. These data support the possibility that people with HIV on therapy may be at greater risk of cardiovascular disease and thus are appropriate targets for interventions against modifiable cardiovascular disease risk factors.

In general, lipid abnormalities should be managed along NCEP guidelines. However, physician and patients are generally more concerned about preventing HIV disease progression and limiting risk of immediate drug related adverse events (such as rash and hypersensitivity, hepatotoxicity, diarrhoea, anaemia, CNS/psychiatric effects, etc) and medium term risks (especially lipoatrophy/lipodystrophy) rather than managing modest longer term increases in cardiovascular risk. All cause morbidity rather than risk of an individual morbidity is where risk management with therapy stands or falls. The more effective, well tolerated regimens we have available, the more the impact each of these regimens have on cardiovascular risk will need to be considered.

Clearly, the best way to limit the need for additional interventions is to endeavour to choose antiretroviral combinations which do the job at controlling the virus but that have the lowest risk of triggering metabolic disturbances. In individuals established on therapy, the primary approach to management, wherever possible, is to manipulate an individual therapy to reduce the contribution of their antiretroviral regimen to the metabolic abnormalities before pursuing intervention with additional agents.

This short review will focus on some of the differences observed between drugs within class and briefly discuss which additional agents are preferred.

Management

Once established, the management of lipid abnormalities falls into 3 categories:

- Lifestyle such as diet, possibly dietary supplementation and exercise
- Additional therapies, generally focussing on managing individual manifestations
- Modifying the treatment regimen

The risk versus benefit of these approaches has not been comprehensively tested, although the risk of diet and exercise can be considered minimal. Details of dietary and exercise changes have been both extensively reviewed.

Individuals switching therapy must consider that they may risk their long-term HIV management in exchange for an uncertain outcome with regard to their lipids or lipodystrophy. The majority of switch studies that have reported data have focused on switching away from PIs, in general modest improvements in lipids and insulin resistance have been observed with switching to nevirapine, efavirenz or abacavir. Lipid changes in studies which have examined NRTI switching vary and are not invariably beneficial.

Adding new agents in to the regimen risks interactions with other antiretrovirals, new side effects and toxicities. Interest exits in the use of glitazones (for insulin resistance with weight loss), metformin (for insulin resistance with weight gain) and growth hormone (GH, for lipoaccumulation) although at present reliable data are only available for metformin and GH. Metformin may benefit fat accumulations, insulin resistance and may improve some lipid and coaguability factors. Benefits of growth hormone appear similar but may only last for the duration of therapy. Studies with glitazones are now underway in the US, Australia and the UK. The safety and potential for pharmacokinetic interactions with these drugs in people with HIV requires clarification before their use can be considered or recommended in persons with HIV, especially those with a hepatitis co-infection.

The use of statins and fibrates is appropriate for the management of dyslipidemia but no benefits have been described with regards to morphologic changes. The benefits of these agents appear similar to improvements in cholesterol or triglycerides described in endogenous dyslipidemia, hence are like to be associated with reduction in cardiovascular disease risk. Pravastatin is the most well studied agent due to the low likelihood of drug interactions. Lovastatin may also be suitable. Simvastatin is contraindicated due to substantial risk of drug interactions with PIs and atorvastatin levels may be increased about 2-fold suggesting caution should be used when using this agent. Studies with rosuvastatin are required. As this agent has greater effects on triglycerides than other statins it is of particular interest fro the mixed dyslipidemia often seen in the HIV treatment setting. Interactions between statins and NNRTIs have not been described. Interactions with fibrates and PIs or NNRTIs are not expected to be clinically important. Advice from a lipidologist should be sought before combining fibrates and statins.