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N-Terminal Pro-B-type Natriuretic Peptide (NT-proBNP) in HIV-1 infected Individuals on HAART

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Abstract

Background: Studies suggest that highly active antiretroviral therapy (HAART) prolongs life in HIV infected individuals and that HIV infected individuals increasingly suffer from cardiovascular complications. NT-proBNP has been shown to represent an indicator of cardiac function.

Methods: 495 HIV infected individuals under HAART and 1980 blood donors (BD) were tested for N-terminal pro-B-type natriuretic peptide (NT-proBNP). NT-proBNP was performed by an automated electrochemiluminescence immunoassay (ECLIA) method.

Results: HIV infected individuals had significantly higher NT-proBNP levels than age matched blood donors (18-29 y: median: 33 pg/ml HIV vs. 5 pg/ml BD; $p = 0.0247$; 30-39 y: median: 25 pg/ml HIV vs. 5 pg/ml BD; $p = 0.0351$; 40-49 y: median: 35.5 pg/ml HIV vs. 5 pg/ml BD; $p < 0.0001$; 50-59 y: median: 42 pg/ml HIV vs. 36 pg/ml BD; $p = 0.3665$; 60-69 y: median: 82.5 pg/ml HIV vs. 46 pg/ml BD; $p = 0.0055$) the effect was consistently found in all age and both gender groups. HIV infected individuals differed from the blood donor control group with respect to cardiovascular risk factors (hypertension, cardiovascular (CV) medication, diabetes mellitus, smoking status). In HIV infected individuals NT-proBNP levels did not correlate to cardiovascular risk factors including GFR except for C-reactive protein (CRP) levels using multivariate analysis. There was also no evidence for cardiotoxic effects due to HAART or specific antiretroviral drugs. High NT-proBNP levels were found in Hepatitis C virus (HCV) infected individuals who had received alpha-interferon therapy.

Conclusions: HIV infected individuals had higher NT-proBNP levels than age matched blood donors possibly as a result of a higher prevalence of general cardiovascular risk factors and HIV associated risk factors, the finding is consistent with an increased incidence of cardiovascular events described in HIV infected individuals. Further studies on the relationship to cardiovascular outcome are warranted.

Key words: Human Immunodeficiency Virus, Hepatitis C Virus, highly active anti-retroviral therapy, N-terminal pro-B-type natriuretic peptide, natriuretic peptides, cardiovascular diseases, heart failure, cardiac dysfunction

1. Introduction

In 1986, when Centers of Disease Control and Prevention (CDC) presented their first definition of AIDS (Aquired Immunodeficiency Syndrome) the mean survival of individuals infected with the human immunodeficiency virus (HIV) was estimated ten years. In the year 1996 85 % of the HIV infected individuals survived more than 10 years and the frequency of opportunistic infections sharply decreased [1]. The prolonged survival of HIV infected individuals was largely attributable to the introduction of highly active antiretroviral therapy (HAART) which resulted in sustained reduction of HI viremia levels, frequently to undetectable levels by sensitive methods.

Prolonged survival of HIV infected individuals due to HAART resulted in a greater prevalence of HIV infected individuals above the age of 50 years [2], an age at which cardiovascular complications become apparent in the general population [3]. In addition HAART and preferentially protease inhibitors have been shown to be associated with lipid abnormalities and specifically lipodystrophy [4, 5], a clinical syndrome believed to be associated with an increased cardiovascular risk [6, 7]. In addition HIV infected individuals are considered to exhibit increased inflammation [8] which is known to be associated with increased cardiovascular risk in the general population.

NT-proBNP (N-terminal-pro-B-type natriuretic peptide) represents a novel marker of cardiac dysfunction known to be associated with cardiac events in a number of patients groups studied [9-11]. Natriuretic peptides specifically B and A type natriuretic peptides (BNP) and (ANP) have been shown to be released after wall stress and to exhibit a variety of biological functions including diuresis, vasodilatation, inhibition of the renin-aldosterone system and to increase vascular myocyte growth [12]. Increased natriuretic peptide levels have been documented in diastolic and systolic heart failure known to be associated with cardiovascular diseases but also with other cardiac conditions such as myocarditis and cardiomyopathy [13], conditions which have also been associated with HIV infection [14-16].

In this study we have compared NT-proBNP levels between HIV infected individuals and age-matched healthy controls.

2. Patients and Methods

A total of fourhundredandninetyfive (n = 495) HIV infected patients on HAART from a HIV outpatient center in Berlin, Germany were included into the study. Fourhundredandfourtynine (n = 447) were male and fourtyeight (n = 48) were female. Mean duration of known HIV infection was 4.8 years.

A total of onethousandninehundredandeighty (n = 1980 in total; n male: 1284; n female: 696) blood donors from the blood transfusion service in Mainz, Germany served as a control population [17, 18]. Blood donors and HIV infected individuals were extensively evaluated using medical history, physical examination and laboratory tests. HIV RNA and CD4 positive cells were determined in all HIV infected individuals.

Baseline characteristics for HIV infected individuals and blood donors are presented in Table 1.

All individuals gave their informed consent for participation in the study. The study was approved by a local ethical committee and was conducted according to the declaration of Helsinki.

Blood Sampling

Ten millilitre of blood at the time point of routine examination of the HIV infected patients and at blood donation of the blood donors was taken.

Blood was collected in EDTA tubes and was centrifuged for 15 minutes at 2500 g within 30 minutes after the blood was taken. The supernatant was removed and stored at -20 °C until analysis.

Methods

NT-proBNP assay

NT-proBNP was analysed using a newly developed immunoassay (ELECSYS® proBNP, Roche Diagnostics GmbH, Mannheim) using an ELECSYS®2010 instrument. The assay works according to the electrochemiluminescence sandwich immunoassay principle.

In a first step, the biotin-labeled IgG (1-21) capture antibody, the ruthenium-labeled F(ab')2 (39-50) signal antibody and 20 micro-liters of the blood sample are incubated at 37 °C for 9 minutes. Afterwards, streptavidin-coated magnetic micro-particles are added and the mixture is incubated for additional 9 minutes. After the second incubation the reaction mixture is transferred to the measuring cell of the ELECSYS®2010 system where the beads are magnetically captured onto the surface of an electrode. Unbound label is removed by washing the measuring cell with buffer. In the last step, voltage is applied to the electrode in the presence of a tri-propylamine containing buffer and the resulting electro-chemiluminescent signal is recorded by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

The detection limit of NT-proBNP is represented by the lowest measurable analyte level that can be distinguished from zero and was found to be < 5 pg/ml NT-proBNP. Measuring range is defined by the lower detection limit of NT-proBNP (< 5 pg/ml) and the maximum of the master calibration curve approx. 35000 pg/ml. The NT-proBNP assay on the ELECSYS®2010 instrument has an intra-assay precision between 1.2% and 1.5% and an inter-assay precision between 4.4% and 5.0%.

Glomerular Filtration Rate

Creatinine clearance was defined as glomerular filtration rate (GFR) and was calculated according to the MDRD equation for determining GFR (ml/min/1.73m2) using plasma creatinine concentration, age, gender recommended by www.nkdep.nih.gov.

Body Mass Index (BMI)

BMI was calculated using body weight [kg] divided by squared body length.

CD4 counts

CD4-positive and CD8-positive T cells were counted (FACScount; Becton Dickinson, San Jose,

CA, USA). HIV-1 RNA was assayed in peripheral-blood plasma by the Roche Amplicor HIV-1 Monitor Test, version 1.5 (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis

Regression analysis and Likelihood Ratio Test was performed according (log-) NT-proBNP and the covariates age, gender, creatinine, hemoglobin and GFR by using chi-square approximation in HIV patients under HAART compared to healthy blood donors respectively. Parametric unpaired t and non-parametric Wilcoxon/Kruskal-Wallis signed rank test for continuous and non-continuous variables was also performed. Multivariate correlations for all variables were calculated by non-parametric Spearman's Rho test. Based on Pearson Partial Correlation Coefficients, correlations were calculated for CV risk factors haemoglobin and C-reactive protein adjusted for age and body composition (body mass index). P values > 0.05 were considered as not-significant.

3. Results

Description of the Study Populations

Characteristics of the study populations are summarized in Table 1. Blood Donors differed from HIV infected individuals with respect to age, male/female ratio but not body mass index (BMI) significantly. In addition cardiovascular risk factors (hypertension, cardiovascular (CV) medication, smoking status) were more frequently documented in HIV infected individuals than in blood donors. HIV infected individuals had lowered haemoglobin levels when compared to blood donors. CRP levels were not available from blood donors.

NT-proBNP and Age

NT-proBNP values of HIV infected individuals and blood donors were matched to age, this is shown in Figure 1. Figure 1 presents a comparison between NT-

Table 1. Baseline characteristics of HIV infected individuals and blood donors.

	HIV infected Patients	Blood Donors	p-value Chi-Square (Wilcoxon)
N	495	1980	
Gender (N male/female)	447/48 (90%/10%)	1284/696 (46%/54%)	< 0.0001
Age [years]	40	38	< 0.0001
BMI [kg/m^2]	22.1	23.5	n.s.
Haemoglobin [g/dl]	14.3	14.8	< 0.0001
Creatinine [mg/dl]	0.90	0.79	< 0.0001
GFR [ml/min/1.73m^2]	97	105	< 0.2129
C-Reactive Protein [mg/l]	4.99	n.a.**	n.a.
N Smoker (%)	301 (61%)	865 (44%)	< 0.0001
N Hypertension* (%)	23 (4.6%)	2 (0.1%)	< 0.0001
N CV Medication	41 (8.3%)	none	< 0.0001
N Diabetes	5 (1%)	none	n.a.

n.s.: not-significant p > 0.05; n.a.: not available;
* Hypertension was defined as systolic blood pressure > 140 mm HG and diastolic blood pressure > 90 mm HG;
** see reference [29]: median: 0.98 mg/L.

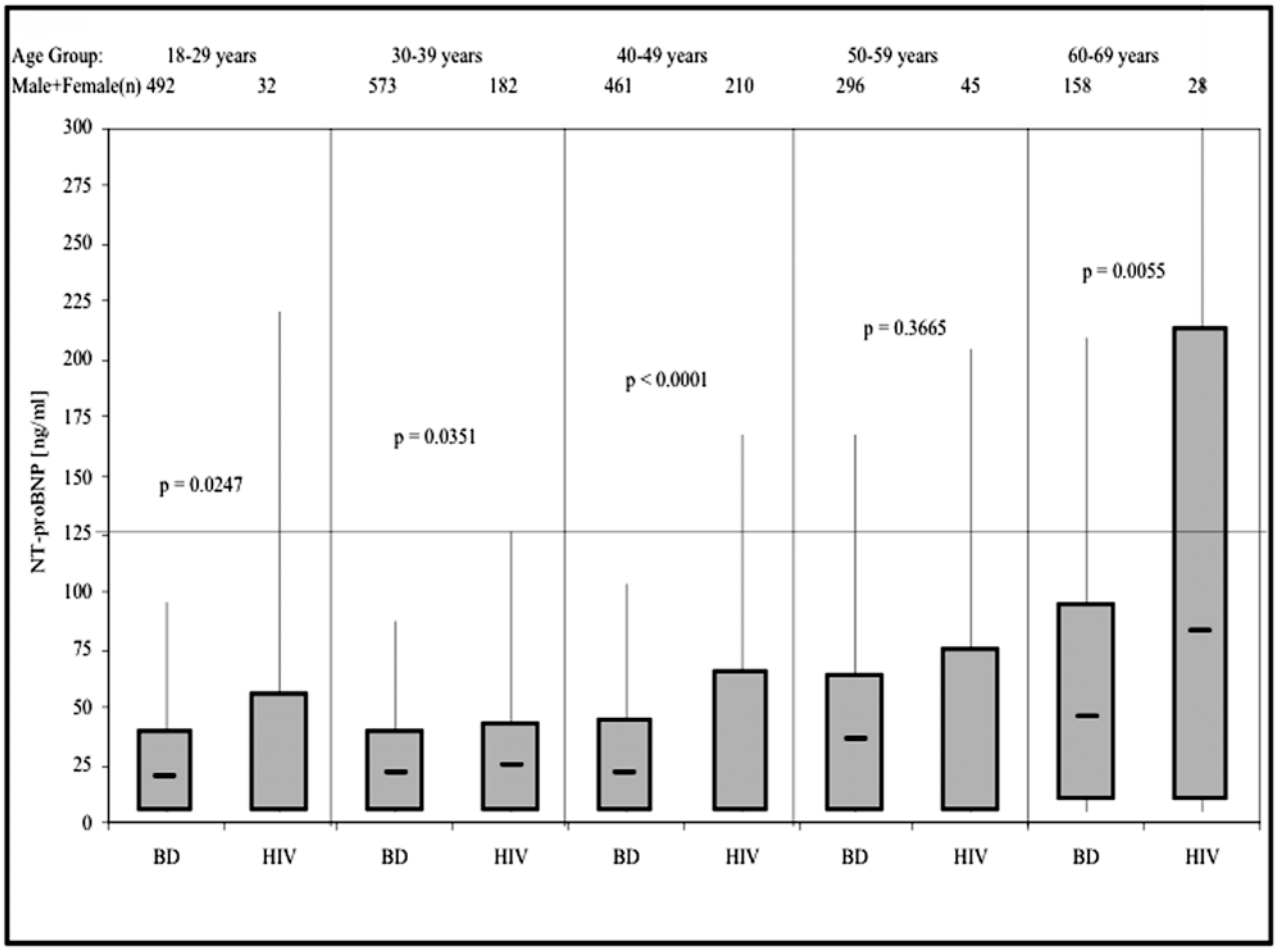

Fig. 1. Whisker Plots of NT-proBNP [pg/ml] values of HIV infected individuals (HIV: N=495) and blood donors (BD: N=1980) matched to different age groups (18-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years).

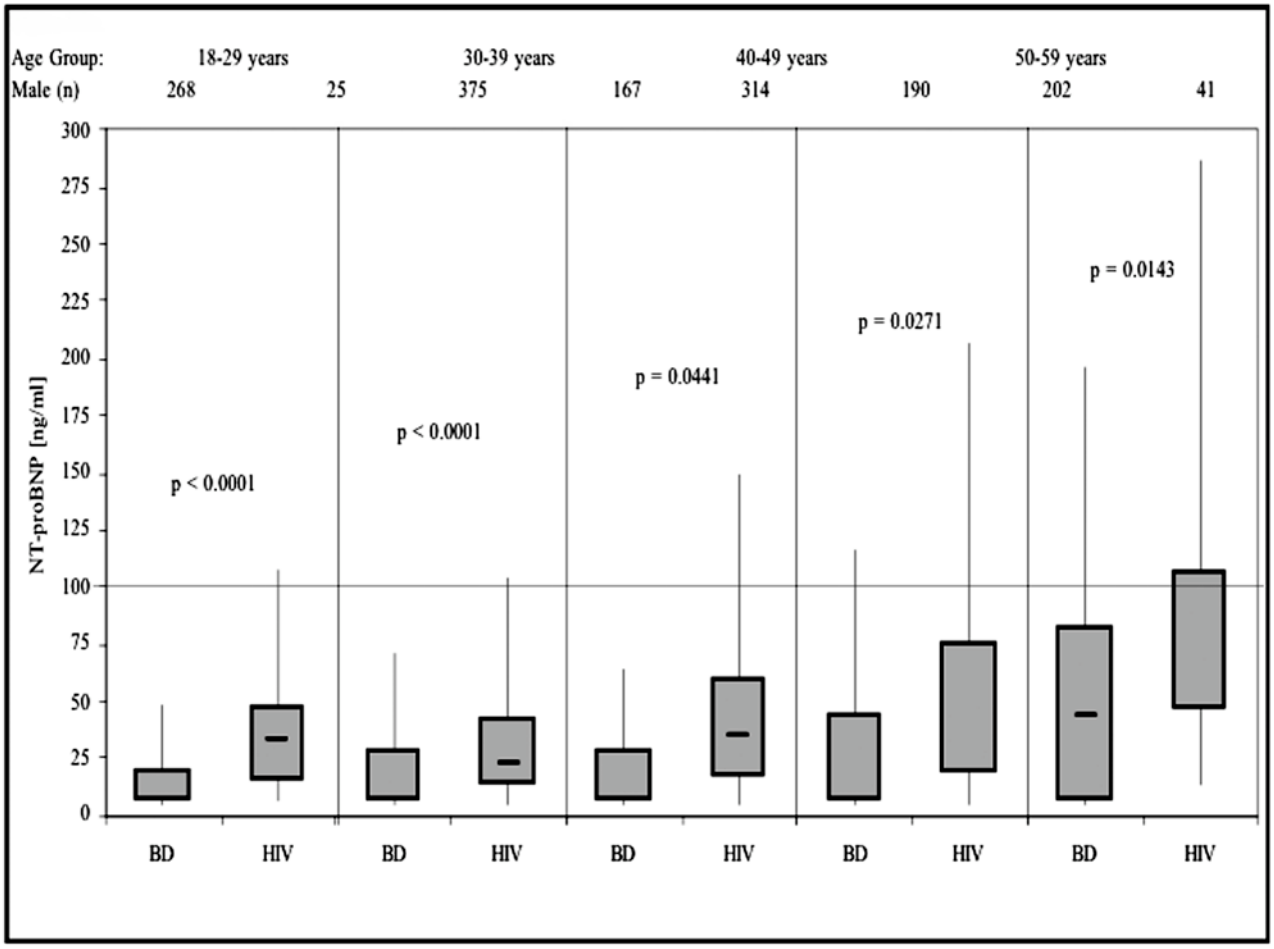

Fig. 2A. Whisker Plots of NT-proBNP [pg/ml] values of HIV infected males (males: N=447) and blood donors (males: N=1284) matched to age groups (18-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years).

proBNP levels of HIV infected individuals and blood donors. As can be seen HIV infected individuals had significantly higher NT-proBNP levels in comparison to individuals without HIV infection in most age groups.

As can also be seen from Figure 1 NT-proBNP levels increased with age in blood donors as well as in HIV infected individuals. In all age groups HIV infected individuals had higher NT-proBNP values than the blood donor control group. As healthy females are known to have higher NT-proBNP values when compared to age-matched males, both groups were reanalysed for males and females separately. The result is presented in Figures 2A and Figure 2B and revealed similar results.

NT-proBNP and GFR

In a subgroup of 890 blood donors GFR was calculated based on the age and gender specific MDRD equation. A GFR > 90 ml/min/1.73 m^2 chronic kidney disease (CKD) stage I was found in 710 (80%) blood donors and a GFR between 90-61 ml/min/1.73 m^2 (CKD stage II) in 180 (20%) individuals. A GFR below 60 ml/min/1.73 m^2 was not recorded. In contrast in a subgroup of 316 HIV infected individuals a GFR > 90 ml/min/1,73 m^2 (CKD stage I) was found in 190 (60%) individuals, a GFR 90-61 ml/min/1,73 m^2 (CKD stage II) in 114 (36%) individuals and a GFR 60-31 ml/min/1.73 m^2 (CKD stage III) in 11 (3.5%) individuals. Thus the portion of individuals with impaired kidney function based on GFR was higher in the HIV infected patient group than in the group of blood donors. Using regression analyses differences in NT-proBNP values found between blood donors and HIV infected individuals could not be attributed to differences in GFR found between both groups.

Factors Influencing NT-proBNP Levels in HIV Infected Individuals

In order to identify factors that might be associated with increased NT-proBNP levels the group of HIV infected individuals was separated into NT-proBNP quartiles and analysed using univariate analysis (Table 2A).

When routine laboratory tests regularly conducted during the monitoring of HAART treated patients were compared to the NT-proBNP quartiles, there was no association between liver, thyroid and kidney function tests and NT-proBNP levels. Similar results were obtained for homocysteine, white blood cell counts

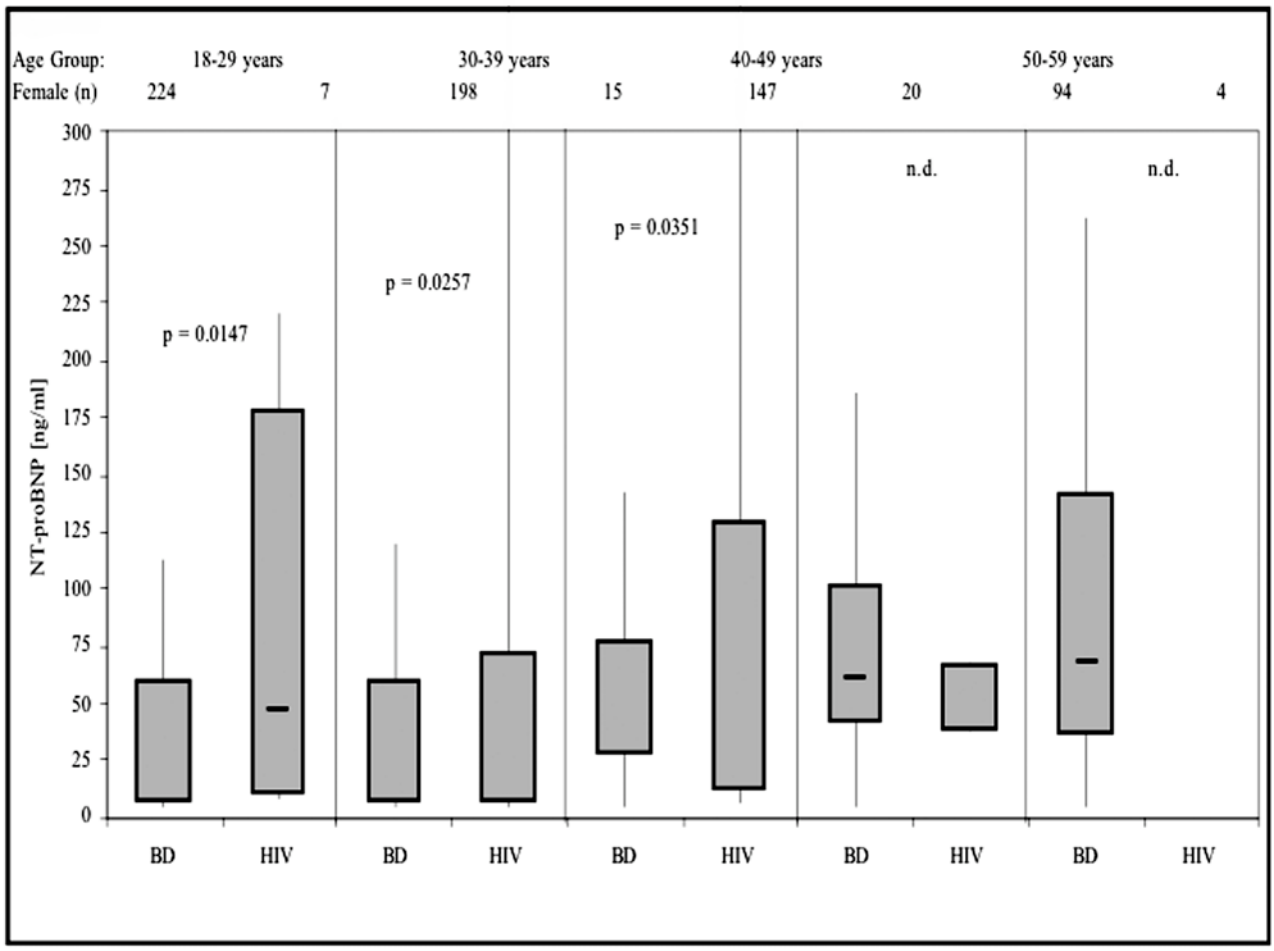

Fig. 2B. Whisker Plots of NT-proBNP [pg/ml] values of HIV infected females (females: N=48) and blood donors (females: N=696) matched to age groups (18-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years).

and lipid levels (Table 2A). There was a trend towards lower haemoglobin levels with higher NT-proBNP levels.

When markers for HIV disease (CD4 and CD8 counts, HIV-RNA level) were related to NT-proBNP, there was no apparent relationship.

Co-infections of HIV with active Hepatitis B virus (HBV) (HBsAg positive) and Hepatitis C virus (HCV) infections were rare, it was however apparent that HCV infected individuals clustered in the fourth quartile of NT-proBNP, all patients had received alpha-interferon therapy in the past.

Using multivariate analysis only CRP levels ($p = 0.0079$) and haemoglobin concentrations ($p = 0.0021$) controlled for age and body mass index and known CV risk factors were independently associated with NT-proBNP concentration.

NT-proBNP Levels and HAART:

All individuals were on HAART in average for 4.8 years. There was no association between duration on HAART and NT-proBNP levels. When the association of the current antiretroviral medication to NT-proBNP level was analysed, there was no clear relationship between specific treatment and NT-proBNP levels observed except for Efavirenz (EFV) (Table 2B). As the majority of patients were on Lamivudine, Abacavir, Zidovudine, lopinavir, Nevirapin and Tenofovir this conclusion can only be drawn for these antiviral drugs.

4. Discussion

The study clearly indicates that HIV positive patients on HAART have significantly higher NT-proBNP values than a control group of age-and gender-matched blood donors. Increased levels of B-type natriuretic peptides (BNP and/or NT-proBNP) have been shown to be associated with an increased risk of cardiovascular events namely myocardial infarction, heart failure and cardiac death in different populations [19-23] as well as with the presence of diastolic and systolic dysfunction as assessed by echocardiography [10, 11]. Thus our data are concordant with the observation that HIV infected patients on HAART are at increased risk of cardiovascular events when compared with a HIV negative control population [24-26].

Evidence for cardiovascular disease has been documented previously. Elevated levels for soluble endothelial intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), P-selectin and endothelin have been described earlier as in-

Table 2A. Factors influencing NT-proBNP levels in HIV infected individuals.

		Total	NT-proBNP				p-value Spearman's rho
			1. Quartile	2. Quartile	3. Quartile	4. Quartile	
N	N	495	129	124	119	123	
NT-proBNP, median		33	9	23	44	95	
(range)		(5 – 10326)	(5 – 16)	(17 – 33)	(34 – 61)	(62 – 10326)	
N NT-proBNP		54	0	0	0	54	
> c.o. 125 pg/ml		(11%)				(44%)	
Age, median		40	39	39	40	43	< 0.0001
N Gender, male/female		447/48	122/7	115/9	106/13	104/19	0.0017
BMI [kg/m^2]		22.1	22.3	21.9	22.3	21.9	n.s.
(range)		(17.2-31.1)	(18.9-28.5)	(18.3-27.8)	(18.7-27.1)	(17.9-30.2)	
N Smoker		301	62	78	78	83	n.s.
N Hypertension		23	4	5	6	8	< 0.0001
N CV Medication		41	8	10	6	17	< 0.0001
N HCV Coinfection.		14	1	1	1	11	< 0.0001
N Diabetes		5	2	1	1	1	n.s.
N HBV Coinfection		2	0	0	0	2	0.0351
Duration HIV infection		4.8 y	4.8 y	4.9 y	4.9 y	4.2 y	
	N			median			
HIV-RNA	279	0.05	0.049	0.155	0.150	0.049	n.s.
CD4	282	487.0	511.5	501.5	453.0	483.5	n.s.
CD8	282	1102	1076	1171.5	1038	1070	n.s.
CRP	279	4.99	5.00	4.99	4.99	5.00	n.s.
g-GT	282	33	39.5	26	35	33	n.s.
GOT	282	31	31	32.5	30	30	n.s.
GPT	275	25	27	27	24	24	n.s.
Haemoglobin	282	14.3	14.7	14.5	14.1	13.5	< 0.0001
Cholesterol	274	183	182.5	181	184	188.5	n.s.
Albumin	279	43	43	43.5	43	41.5	0.0018
Homocysteine	280	9	9	9	9	10	n.s.
Creatinine	281	0.9	0.9	0.9	0.9	0.9	n.s.
GFR [ml/min/1.73m^2]	281	97	95	99	100	94	n.s.
Leucocytes	281	5.4	5.25	5.7	5.35	5.8	n.s.
Triglycerides	274	167.5	182.5	163	162	177	n.s.
TSH	278	1.27	1.17	1.15	1.4	1.49	n.s.

n.s.: not-significant p > 0.05

dicators for endothelial dysfunction [27]. Also arterial stiffness has been reported in HIV infections and has been linked to HAART [28]. Thus overwhelming evidence suggests that HIV infected individuals specifically the heart undergo vascular processes that ultimately are associated with development and prognosis of cardiovascular diseases and its complications.

In our study HIV infected patients differed from blood donors in that they had significantly more frequently risk factors for cardiovascular diseases such as hypertension, CV medication, diabetes and smoking when compared to blood donors. Differences in NT-proBNP values between HIV infected individuals and blood donors can thus at least partly be explained by differences in general cardiovascular risk between both populations.

C- reactive protein (CRP) has only been measured in the HIV infected population and was found to be 4.99 mg/l (median) which is significantly higher than the CRP values reported in blood donors which was found to be 0.98 mg/l (median) in one study [29]. Whether the differences in CRP levels between blood donors and HIV infected individuals are only caused by HIV infection itself, concomitant infections related to HIV or HIV risk or both could not be clarified in this study.

Table 2B. NT-pro-BNP levels in HIV patients on HAART.

		Total	NT-proBNP				p-value Spearman's rho
			1. Quartile	2. Quartile	3. Quartile	4. Quartile	
N		495	129	124	119	123	
NT-proBNP, median (range)		33 (5 – 10326)	9 (5 – 16)	23 (17 – 33)	44 (34 – 61)	95 (62 – 10326)	
Duration HAART		4.8 y	4.8 y	4.9 y	4.9 y	4.2 y	n.s.
3TC (Lamivudin)	N	282	78	72	73	59	n.s.
ABC(Abacavir)	N	128	32	39	27	30	n.s.
APV(Amprenavir)	N	1	0	0	1	0	n.s.
ATV(Atazanavir)	N	36	13	5	11	7	n.s.
AZT(Zidovudin)	N	72	20	23	15	14	n.s.
d4T(Stavudin)	N	30	9	6	5	10	n.s.
DdC(Zalcitabin)	N	5	1	1	0	3	n.s.
Ddi(Didanosin)	N	20	4	5	4	7	n.s.
DLV(Delavirdin)	N	0	0	0	0	0	n.s.
EFV(Efavirenz)	N	57	21	12	14	10	0.0591
FTC(Emtricitabin)	N	7	2	3	0	2	n.s.
GW908(Fosamprenavir)	N	13	4	4	3	2	n.s.
IND(Indinavir)	N	6	1	2	0	3	n.s.
LPV(Lopinavir)	N	90	16	24	21	29	n.s.
NFV(Nelfinavir)	N	17	5	7	1	4	n.s.
NVP(Nevirapin)	N	64	17	15	20	12	n.s.
RTV(Ritonavir)	N	15	5	1	7	2	n.s.
SQV(Saquinavir)	N	16	5	2	7	2	n.s.
T20(Enfuvirtide)	N	7	1	1	2	3	n.s.
TDF(Tenofovir)	N	96	22	29	24	20	n.s.
ZDV(Zidovudin)	N	174	49	42	46	37	n.s.

n.s.: not-significant $p > 0.05$

CRP has been shown to be an independent predictor for cardiovascular events in the general population and in defined patient groups. In fact drug induced reduction of CRP levels has been shown to be associated with reduced cardiovascular risk in non-HIV infected populations [30]. Thus inflammation as documented by CRP is likely to be a significant contributor for cardiovascular complications in HIV patients [31]. This hypothesis is further supported by the fact that increased NT-proBNP levels were associated with increased CRP levels in the HIV population based on multivariate analysis.

Highly active antiretroviral therapy (HAART) is known to induce dyslipemia and systemic insulin resistance especially if protease inhibitors are used. Protease inhibitors have been shown to accumulate in adipocytes, and to alter mitochondrial function and to result in increases of tumor-necrosis-factor-alpha and interleukin-6 concentration associated with reduction in adiponectin levels which results in a pre-inflammatory state [32-36]. In fact a recent study suggests that patients on HAART do have higher CRP levels than those without antiviral treatment, this effect was unrelated to CD4 counts [31].

The complexity of the development of atherosclerosis in HIV infection has been reviewed recently and shown to be a complex mechanism involving HIV induced pro-inflammatory responses related to adiposic tissue and innate and adaptive immunity resulting in insulin resistance including CRP and triglycerides elevations. HIV infection has also been shown to induce coagulapathy and endothelial dysfunction [27]. Antiviral drugs and preferably protease inhibitors have been shown to promote inflammatory processes and to induce coagulapathy [37].

NT-proBNP is considered to be a late event in this process and may increase only after early signs of the metabolic syndrome or complications of atherosclerosis become apparent.

In contrast HIV or drug induced cardiomyopathy and pulmonary hypertension are complications of HIV infected individuals on HAART [38-41]. In this

case NT-proBNP is considered an early indicator of cardiac disease as has been demonstrated for cardiotoxic drugs [42-44].

In this study we could not find evidence for cardiomyopathy or cardiotoxicity related to HAART suggesting that such side effect are rare.

A further interesting aspect of the study was that patients with HCV infection who had been on alpha-interferon therapy had higher NT-proBNP values than the HCV negative group. These data are consistent with data indicating that NT-proBNP increases after alpha-interferon therapy and that NT-proBNP levels did not decrease to pre-treatment levels after therapy has been discontinued [45, 46], the interpretation of this finding is still open, however there is no evidence that this effect is due to altered clearance of NT-proBNP in the liver [47].

Limitations

Several mayor limitations of this study should be taken into account. First, HIV infected patients and blood donors differed with regard to general cardiovascular risk which did not allow distinguish general from HIV specific cardiovascular risk, second, echocardiography was not done which restricts the independent validation of NT-proBNP results. Third, this was a cross-sectional study which limits the interpretation of the study with respect to cardiovascular outcome.

Future directions

The result of the study calls for further research. Clearly outcome studies will shed further light into cardiovascular complications in HIV infected individuals as well as studies using echocardiography. In addition the use of biomarkers that reflect different pathophysiological mechanisms in HIV associated cardiovascular diseases will be helpful to differentiate the mechanisms involved in HIV related cardiovascular diseases. This will potentially give evidence for therapeutic directions.

In Summary

The data presented indicates that HIV infected patients have higher NT-proBNP values than a healthy control group of blood donors most likely due to differences in cardiovascular risk between the populations and a higher level of inflammation in the HIV infected group. We could find no evidence for HIV and HAART associated cardiomyopathy or cardiotoxicity in this study.

Future longitudinal and outcome studies accompanied by echocardiography need to prove whether NT-proBNP alone or in association with other diagnostic tests/procedures may be useful to predict cardiac and/or cardiovascular risk and to allow appropriate intervention.

Disclosure: Dietmar Zdunek and Georg Hess are employees of Roche Diagnostics GmbH, Mannheim, Germany. Thomas Berg has done the analysis of the HIV infected individuals. Jost Stalke, Stephan Dupke, Axel Baumgarten and Andreas Carganico have collected the clinical information of the HIV infected individuals. Dietmar Zdunek has analysed the blood donor population. Georg Hess and Thomas Berg have designed the study and Thomas Berg, Georg Hess and Dietmar Zdunek have analysed the data and have written the manuscript. All authors have reviewed the manuscript and their content.

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