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# Improvement of the cardiac marker N-terminal-pro brain natriuretic peptide through adjustment for renal function: a stratified multicenter trial

**Andreas Luchner[1,*], Alexander Weidemann[2], Roland Willenbrock[3], Sebastian Philipp[4], Norbert Heinicke[1], Matthias Rambausek[5], Uta Mehdorn[6], Burkhard Frankenberger[7], Iris M. Heid[8], Kai-Uwe Eckardt[2] and Stephan R. Holmer[1]**

[1] Klinik und Poliklinik für Innere Medizin II, Klinikum der Universität Regensburg, Regensburg, Germany
[2] Medizinische Klinik 4 mit Schwerpunkt Nephrologie und Hypertensiologie, Universitätsklinikum Erlangen, Erlangen, Germany
[3] Medizinische Klinik, St. Elisabeth Krankenhaus, Halle/Saale, Germany
[4] Klinik für Kardiologie, Westdeutsches Herzzentrum der Universität Duisburg-Essen, Essen, Germany
[5] Dialysezentrum Heilbronn, Heilbronn, Germany
[6] Dr. Köhler GmbH, Freiburg, Germany
[7] Gesellschaft zur Durchführung klinischer Studien – GDS mbH, Wuppertal, Germany
[8] Institut für Epidemiologie, Universitaet Regensburg, Regensburg, Germany

## Abstract

**Background:** N-terminal-pro brain natriuretic peptide (NT-proBNP) is a useful cardiac marker that is also influenced by renal dysfunction. It was our objective to assess the relationship between NT-proBNP concentrations in plasma and worsening renal function, and to attempt adjustment of NT-proBNP for renal dysfunction in a prospective, stratified multi-center study.
**Methods:** We stratified 203 male patients according to their cardiac status and the estimated glomerular filtration rate (eGFR). Cardiac disease was assessed by medical history, physical examination and standardized echocardiography. Patients were stratified according to the following: absence of cardiac history and abnormalities (control, CTRL, n = 66), cardiac history without left ventricular hypertrophy (LVH) or left ventricular systolic dysfunction (LVD) (history, n = 30), LVH without systolic dysfunction (LVH, n = 68), and LVD [ejection fraction (EF) < 40%, LVD, n = 39]. Renal disease was stratified according to the eGFR: 15–30 mL/min (n = 52), 31–75 mL/min (n = 99), and > 75 mL/min (n = 52).
**Results:** NT-proBNP was correlated with eGFR in the entire study population and for all levels of cardiac disease (all $p < 0.01$). Regression analysis allowed adjustment of NT-proBNP for eGFR in a continuous manner, and this adjustment significantly improved the predictive value (receiver operating characteristic curve for symptomatic LVD from 0.80 to 0.86, $p < 0.01$; sensitivity from 74% to 83% and specificity from 68% to 79%).
**Conclusions:** NT-proBNP correlates inversely and significantly with eGFR throughout all levels of cardiac strata. We propose for the first time a continuous adjustment algorithm which markedly improves the predictive values of NT-proBNP in male patients with impaired renal function.
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**Keywords:** echocardiography; glomerular filtration; heart; kidney; natriuretic peptide.

## Introduction

N-terminal-pro brain natriuretic peptide (NT-proBNP) is a useful cardiac marker that may facilitate the early diagnosis of heart failure and stratification of cardiac risk. Specifically, NT-proBNP has been shown to be useful for the diagnosis of left ventricular (LV) systolic (1–6) as well as diastolic dysfunction (7, 8), and acutely decompensated heart failure (9–13). While NT-proBNP plasma concentrations are primarily thought to indicate the severity of LV dysfunction, recent studies have shown that concentrations also correlate inversely with the glomerular filtration rate (GFR) and may be highly increased in patients on dialysis (14–17).

The influence of renal dysfunction on NT-proBNP is highly relevant for the clinical application of this marker since cardiac disease is frequently associated with renal disease (14, 18–20). Likewise, patients with chronic kidney disease (CKD) are at increased risk of suffering from cardiovascular complications, such as myocardial infarction (MI) and heart failure (21–23). Therefore, patients with known or suspected cardiac disease as well as patients with renal disease are potential candidates for NT-proBNP testing.

Although cardiac markers are utilized more and more frequently in these patient groups and concomitant renal dysfunction may distort the correct interpretation of individual test results, no algorithm for adjustment of measured NT-

*Corresponding author: Prof. Dr. Andreas Luchner, FESC, Klinik und Poliklinik für Innere Medizin II – Kardiologie, Klinikum der Universität, 93042 Regensburg, Germany
Phone: +49 941 944-7211, Fax: +49 941 944-7213,
E-mail: andreas.luchner@klinik.uni-regensburg.de
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proBNP concentrations for renal dysfunction has been established to date. Indeed, patients with more than mild renal dysfunction are often excluded from clinical studies (9, 10).

Therefore, our objective was to assess the effect of renal function on NT-proBNP concentrations, and to establish for the first time an algorithm that allows adjustment of measured NT-proBNP concentrations with respect to renal dysfunction. We hypothesized that NT-proBNP would correlate inversely with estimated glomerular filtration rate (eGFR), and that adjustment of NT-proBNP according to the severity of renal disease would increase the predictive power of this marker. To test our hypothesis, we conducted a cross-sectional study of male individuals with prospective stratification according to the degree of cardiac disease and the degree of kidney disease. All study subjects were well characterized by careful physical examination, medical history, and standardized echocardiography.

## Materials and methods

### Patients

In this cross-sectional study, a total of 204 clinically stable male patients between 18 and 75 years of age with an eGFR $\geq$15 mL/min (not on dialysis) were enrolled in four centers in Germany. Patients were stratified into the following groups according to their cardiac condition: 1) absence of LV hypertrophy (LVH) and absence of LV systolic dysfunction (LVD) during echocardiography (n=96), 2) LVH with preserved LV systolic function (LVH, n=68) or 3) presence of LVD [ejection fraction (EF)<40%, LVD, n=39]. One patient could not be stratified and was excluded from further analyses. Among the 96 patients without LVH and without LVD, 30 patients had a reported previous history of cardiac disease including MI or >2nd degree valvular disease and constituted the ''History'' group. In this group, quantitative echocardiography was available in all but one patient (n=29). The remaining 66 patients without LVH and LVD had no previous history of any cardiac disease and constituted the control group (CTRL). eGFR was calculated using the Cockroft-Gault formula (24) and patient counts were n=52 for eGFR 15–30 mL/min, n=99 for eGFR 31–75 mL/min and n=52 for eGFR >75 mL/min.

During a visit to one of the four study centers either as outpatients or during an in-hospital stay, all patients underwent a standardized interview, physical examination including blood and urine collection and standardized echocardiography. Patients with unstable angina pectoris, MI, or decompensated heart failure within 4 weeks, or patients with severe pulmonary hypertension (estimated systolic pulmonary artery pressure $\geq$50 mm Hg by echocardiography) were also excluded from the study. All study procedures were performed in accordance with GCP/ICH guidelines and the study protocol was accepted by local Ethics Committees. All patients gave written informed consent.

### Biochemical analyses

Serum creatinine, creatine kinase (CK), erythrocyte count, hematocrit and hemoglobin and urine protein and creatinine were measured using standard clinical laboratory procedures. Several formulas have been developed to estimate renal function from measurements of serum creatinine, the two most frequently used being the Cockcroft-Gault formula and the Modification of Diet in Renal Disease (MDRD) four variable equation. While the latter is more widely used for automated reporting eGFR values since it does not require body weight, we used the Cockcroft-Gault formula which includes body weight to provide estimates of creatinine clearance rates. A recent comparison of measured GFR using continuous inulin infusion with values derived by the Cockcroft-Gault and the MDRD formulas revealed similar accuracies for both (25).

NT-proBNP concentrations were measured by a central laboratory using the Elecsys®-system from Roche Diagnostics (Mannheim, Germany). The reference interval for normal NT-proBNP used in this study was derived from an age and gender stratified reference sample of 2948 healthy blood donors (data at Roche Diagnostics). In a male subgroup of this reference sample with a median age of 56.3 years (n=432), the 97.5th percentile for NT-proBNP was 193.5 pg/mL. Since our male study population was of similar age (mean 56.1 years, median 59.0 years) we chose this cut-off threshold as the upper limit of normal for the present study (reference cut-off).

### Echocardiography

Echocardiography (ECG) was performed according to criteria of the American Society of Echocardiography (26) following a predefined protocol by a single experienced investigator at each site. Videotapes were recorded for each patient and a core laboratory performed all quantitative measurements, including EF. Standard measurements included end diastolic thickness of septum and LV posterior wall as well as end diastolic and end systolic LV diameter. Left ventricular mass (LVM) was calculated as $LVM[g]=0.8(1.04)((IVS+LVID+LVPW)^3-LVID^3)+0.6$ according to Devereux et al. (27). LVM was indexed to body surface area and LVH was defined as LVM index >130 g/m$^2$. Left ventricular end diastolic volume (LVEDV) and end systolic volume (LVESV) were determined by the area-length method (28) from an ECG-triggered 2D-image in the 4-chamber view, and EF was calculated as $EF=(LVEDV-LVESV)\times 100/LVEDV$. Systolic LV dysfunction was defined as an EF$\leq$40%. Systolic pulmonary artery pressure was estimated in the presence of tricuspid regurgitation as central venous pressure + systolic pressure gradient between the right ventricle and right atrium.

### Statistics

Comparison of baseline characteristics between study groups was performed using ANOVA for continuous variables and the $\chi^2$-test for categorical variables. Since the distribution of NT-proBNP concentration is skewed, logarithmically transformed values were used for subsequent analyses. To determine the effect of eGFR on NT-proBNP, regression analyses were performed with NT-proBNP as the dependent variable and eGFR, as well as a number of other covariates as independent variables. The final model included eGFR, LVEF, LV end diastolic volume index (LVEDVI), New York Heart Association (NYHA) classification and the presence of rales, and was used to adjust measured NT-proBNP concentrations for eGFR. The resulting adjustment algorithm was: $NT\text{-}proBNP_{adjusted}=NT\text{-}proBNP/e^{1.892-0.025*eGFR}$. The predictive values of both crude and adjusted NT-proBNP were calculated for the detection of LVD and symptomatic LVD both for the reference cut-off (193.5 pg/mL) which was derived from the 97.5th percentile of a large healthy reference population (see above), as well as by receiver operator characteristic (ROC) analysis without pre-specified cut-points. Calculations were performed using SAS 8.2 and SPSS 12.0 statistical software (SPSS Inc., Chicago, IL, USA).

## Results

### Patient characteristics

Stratification according to cardiovascular comorbidities revealed a high prevalence of arterial hypertension in all groups, particularly in those with LVH (Table 1). Patients with systolic LVD also showed a high degree of LVH and were older. Mean EF in this group was 32% as compared to a mean EF of over 60% in all other groups. This group also showed significant LV dilatation and a high previous history of heart disease. Due to stratification, the severity of CKD was almost evenly distributed among the groups with or without cardiac abnormalities.

### Correlation of NT-proBNP with eGFR

Within cardiac disease groups, there was a wide scatter of NT-proBNP concentrations with a minimum value of 5 and a maximum value of 24,171 pg/mL. Significant negative correlation was found for NT-proBNP and eGFR in the entire study population ($R^2 = 0.236$; $p < 0.0001$), and in each predefined group stratified by cardiac status (Figure 1). Accordingly, although statistically significant differences between the cardiac groups were observed, a wide overlap of NT-proBNP concentrations was present (Figure 2, left).

Testing the interaction between cardiac disease and eGFR showed that the correlation of both, cardiac disease and eGFR with NT-proBNP was independent from each other. This indicates that eGFR greatly affects measured NT-proBNP concentrations regardless of the presence of cardiac disease. Multivariate regression models with NT-proBNP as dependent variable were evaluated. These models included eGFR, EF, LVMI and LVEDVI, as well as a number of clinical and anamnestic variables. The best prediction of NT-proBNP was found in a model that included eGFR, EF, LVEDVI (continuous variables), as well as NYHA-class (I–IV) and rales (yes or no) (Table 2). In this model, eGFR had a strong influence on NT-proBNP-concentrations which was not abolished by the other covariates. Using the regression coefficient determined for the correlation between eGFR and NT-proBNP from this model, NT-proBNP values were calculated for each individual that were adjusted for eGFR. The resulting adjustment algorithm was:

$$\text{NT-proBNP}_{\text{adjusted}} = \text{NT-proBNP}/e^{1.892-0.025\times\text{eGFR}}.$$

A comparison of the lower adjusted with the higher crude NT-proBNP concentrations demonstrates markedly less overlap between the subgroups and particularly better distinction of LVD (Figure 2, right).

### Abundance of reclassification and characteristics of reclassified patients

In several patients with increased measured NT-proBNP concentrations above the reference cut-point (193.5 pg/mL, derived from the reference population), mathematical adjustment decreased the NT-proBNP to below the reference cut-point and into the normal range. The frequency of reclassification from elevated to normal was 21% for all subjects with an eGFR of 31–75 mL/min, and 23% of all subjects with an eGFR $<30$ mL/min. These reclassified patients showed disease characteristics that were similar to individuals with low unadjusted NT-proBNP concentrations (Table 3). Specifically, there was no indication for a significant excess of cardiac disease in the group with normal NT-proBNP after adjustment (better EF, only slightly higher LVMI), however, eGFR was much lower in this group. This finding indicates that in these patients, renal dysfunction was indeed the underlying cause for measured NT-proBNP concentrations above the cut-off threshold.

Following adjustment of NT-proBNP for eGFR, a total of 81 patients with an LVEF $>40\%$ still exhibited increased

**Table 1** Demographics of patients stratified according to cardiac disease.

| | CTRL | History | LVH | LVD |
|---|---|---|---|---|
| n | 66 | 30 | 68 | 39 |
| Age, years | 49.3±1.7[a] | 57.7±2.2 | 57.7±1.5 | 63.8±1.4 |
| BMI, kg/m$^2$ | 25.9±0.5 | 26.7±0.6 | 26.6±0.5 | 28.5±0.6 |
| eGFR, mL/min | 57.7±4.3 | 60.9±5.4 | 44.6±3.0[a] | 60.5±4.7 |
| History of heart disease | 0 (0%)[a] | 30 (100%) | 22 (32.4%) | 36 (92.3%) |
| History of MI | 0 (0%)[a] | 20 (20.8%) | 15 (22.1%) | 24 (61.5%) |
| NYHA class | 1.2±0.07[a] | 1.8±0.12 | 1.4±0.07 | 2.2±0.1 |
| Presence of rales | 4 (6.1%) | 3 (10.0%) | 2 (2.9%) | 6 (15.4%) |
| Ejection fraction, % | 61.6±1.4 | 62.0±2.0 | 61.1±1.2 | 32.1±1.0[a] |
| LVEDVI, mL/m$^2$ | 58.1±1.6 | 59.5±1 | 69.1±2 | 103.2±5[a] |
| LVMI, g/m$^2$ | 103.6±2.0 | 104.1±2.4 | 157.9±2.5[a] | 154.6±7.0[a] |
| Renal transplant | 29 (43.9%)[a] | 7 (23.3%) | 28 (41.2%)[a] | 1 (2.6%) |
| Diabetes mellitus | 13 (19.7%) | 10 (33.3%) | 14 (20.6%) | 19 (48.7%)[a] |
| Arterial hypertension | 59 (89.9%) | 23 (76.7%) | 62 (91.2%) | 25 (64.1%) |
| Hemoglobin, g/dL | 12.8±0.3 | 13.4±0.3 | 12.2±0.3 | 13.7±0.3 |

CTRL, absence of cardiac history and LVH and LVD; history, presence of cardiac history but without LVH or LVD; LVH, LVH only (i.e. absence of LVD); LVD; LVD (EF $\leq 40\%$). Demographic data are given as mean ± standard error for continuous variables or n (%) for categorized variables. [a]Significant differences vs. other strata by ANOVA.

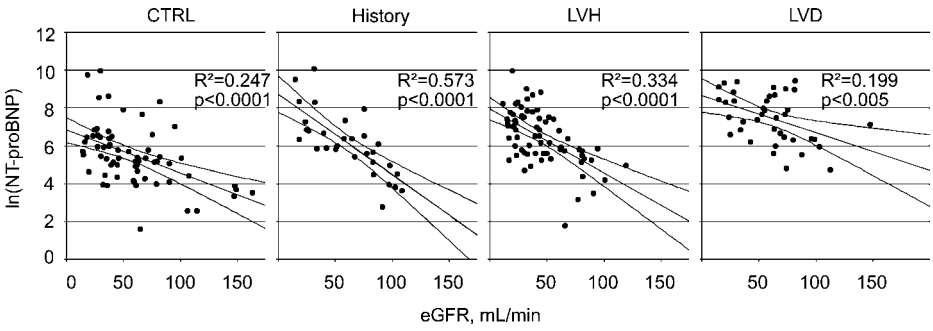


**Figure 1** Univariate correlations between eGFR and ln-transformed NT-proBNP in patient groups stratified according to cardiac disease status.
CTRL denotes absence of cardiac history and LVH and LVD; history, presence of cardiac history but absence of LVH or LVD; LVH, LV hypertrophy only (i.e., absence of LV dysfunction); LVD, LV dysfunction (EF ≤ 40%).

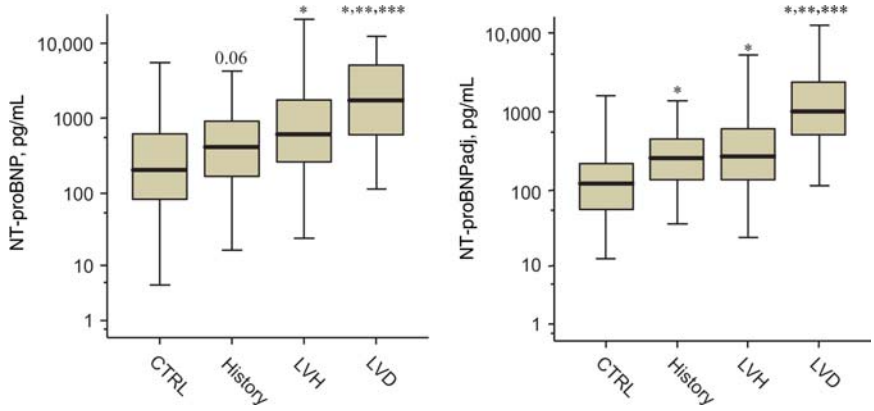


**Figure 2** Box and whiskers plot of measured (left) and adjusted (right) log-transformed NT-proBNP concentrations in patient groups stratified according to cardiac disease status.
CTRL denotes absence of cardiac history and LVH and LVD; history, presence of cardiac history but absence of LVH or LVD; LVH, LV hypertrophy only (i.e., absence of LV dysfunction); LVD, LV dysfunction (EF ≤ 40%). *p < 0.01 vs. CTRL, **p < 0.01 vs. history, ***: p < 0.01 vs. LVH.

NT-proBNP concentrations. Since increased NT-proBNP has been shown to be associated with diseases other than systolic LVD or overt heart failure, we assessed which comorbidities were present in these patients. Among these patients, 41.3% showed LVH, 21.3% had long standing arterial hypertension, 20.0% had a moderately impaired LV systolic function (40% < EF ≤ 50%), 10% had a previous MI with preserved systolic function, and 3.7% had pulmonary disease. Thus, in addition to significant systolic LVD, moderate LVD, LVH, previous MI with preserved LV function and arterial hypertension with no apparent cardiac abnormality are frequently associated with increased NT-proBNP in patients with decreased renal function.

**Table 2** Determinants of NT-proBNP as assessed by adjusted regression analysis.

| Covariate | β-Coefficient | SE | p-Value |
|---|---|---|---|
| eGFR, mL/min | –0.025 | 0.00287 | <0.0001 |
| LVEF, % | –0.022 | 0.00861 | 0.0117 |
| LVEDVI, mL/m² | 0.012 | 0.00432 | 0.0063 |
| NYHA class (I–IV) | 0.462 | 0.16299 | 0.0051 |
| Rales (yes vs. no) | 0.908 | 0.34911 | 0.0101 |
| Intercept | 7.15 | 0.8136 | <0.0001 |
| | | $R^2 = 0.53$ | F = 36.4 |

Results from adjusted regression analysis with log(NT-proBNP) as the dependent variable; SE, standard error.

## Predictive values of crude and adjusted NT-proBNP

Adjusted NT-proBNP concentrations were used in parallel to unadjusted NT-proBNP concentrations to predict systolic LVD and symptomatic LVD. The adjusted NT-proBNP concentrations were associated with a marked improvement in predictive values compared to the actual measured values. Specifically, the ROC areas for the detection of LVD, as well as for symptomatic LVD, increased significantly (Table 4).

When the predictive values were compared to each other at the reference cut-point (193.5 pg/mL), adjustment did not

**Table 3** Characteristics of patients with normal measured NT-proBNP as compared with patients with high measured NT-proBNP concentrations, but normal adjusted NT-proBNP.

| | NT-proBNP normal | Increased measured NT-proBNP, but normal adjusted NT-proBNP |
|---|---|---|
| n | 52 | 33 |
| Age, years | 48.7 ± 1.7 | 55.1 ± 2.7[a] |
| eGFR, mL/min | 77.9 ± 4.7 | 35.6 ± 2.6[a] |
| EF, % | 59.6 ± 1.4 | 65.2 ± 1.9[a] |
| LVMI, g/m$^2$ | 112.3 ± 3.2 | 126.8 ± 6.9[a] |
| LVEDVI, mL/m$^2$ | 58.8 ± 1.9 | 61.1 ± 2.8 |
| LVH | 14 (26.9%) | 12 (36.4%) |
| Heart rate, bmp | 72.5 ± 1.2 | 73.9 ± 1.5 |
| Previous MI | 11 (21.2%) | 5 (15.2%) |
| Anamnestic other heart disease | 9 (17.3%) | 7 (21.2%) |
| Anamnestic heart failure | 10 (19.2%) | 3 (9.1%) |
| Pulmonary hypertension | 0 | 0 |
| Valvular disease | 0 | 0 |
| Arterial hypertension | 45 (86.5%) | 29 (87.9%) |

[a]$p < 0.05$ vs. normal crude NT-proBNP. Anamnestic other heart disease includes coronary artery disease, dilated cardiomyopathy, valve replacement, arterial hypertension, atrial fibrillation.

diminish the sensitivity of the test, which was already at 95% for the detection of LVD and 100% for the detection of symptomatic LVD. However, specificity increased by ~20% without any loss of sensitivity because the rate of false positives was markedly decreased (Table 4, compare lines 1 and 3 for LVD and 5 and 7 for symptomatic LVD, respectively).

When optimal sensitivity, specificity and cut-point were computed using ROC analysis independently from the reference cut-point, adjustment of NT-proBNP was associated with a marked increase in sensitivity and specificity (each ~5%) as compared to actual measured values (Table 4, compare lines 2 and 4 for LVD and 6 and 8 for symptomatic LVD, respectively).

## Discussion

The current study addresses the effect of decreased renal function on NT-proBNP as a marker of LVD in prospectively stratified male patients. It demonstrates that in addition to the severity of cardiac disease, renal impairment is a major contributor to NT-proBNP concentrations. To improve the predictive properties of NT-proBNP for cardiac disease, a correction factor is proposed that allows adjustment of measured NT-proBNP concentrations according to the eGFR. This adjustment improves the predictive values of the marker to detect LVD and can help decrease false positive results.

### Correlation between NT-proBNP and renal function

NT-proBNP was correlated with eGFR in all groups of male patients with and without cardiac disease. This finding corroborates some very recent observational studies that indicated a correlation between NT-proBNP and eGFR in pre-dialysis patients (17), patients with acute dyspnea (9, 29), stable ischemic heart disease (3, 14, 16), heart failure (30) or peripheral artery disease (31), and in those with various

**Table 4** Effect of adjustment for renal impairment on NT-proBNP predictive value.

| Condition | NT-proBNP | Cases/cohort | ROC-area (95% CI) | Sensitivity/ specificity, % | PPV/ NPV, % | Cut-off, pg/mL |
|---|---|---|---|---|---|---|
| LVD | Crude | 39/202 | 0.77 (0.69–0.84) | 95/30 | 26/96 | 193.5 (reference) |
| | | | | 72/68 | 35/91 | 731[a] |
| | Adjusted | dto. | 0.82 (0.76–0.89)[b] | 95/51 | 32/98 | 193.5 (reference) |
| | | | | 77/77 | 45/93 | 473[a] |
| LVD-Sympt | Crude | 35/202 | 0.80 (0.73–0.87) | 100/30 | 25/100 | 193.5 (reference) |
| | | | | 74/68 | 33/93 | 731[a] |
| | Adjusted | dto. | 0.86 (0.80–0.91)[b] | 100/49 | 30/100 | 193.5 (reference) |
| | | | | 83/79 | 45/96 | 538[a] |

LVD, EF < 40% by echocardiography; LVD-Sympt.; symptomatic LVD; ROC, receiver operator characteristic; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; reference, pre-specified cut-point corresponding to 97.5th percentile of healthy reference population. [a]Predictive values and corresponding cut-point derived from ROC analysis for highest pair of sensitivity and specificity. [b]$p < 0.01$ for comparison of ROC-areas.

stages of renal function (32), and is in contrast to pooled data which suggested that there is no interdependence between renal impairment and increased NT-proBNP concentrations (33). However, unlike previous studies, our study was specifically designed to resolve the relationship between eGFR and NT-proBNP concentrations by using careful prospective patient stratification. The stratification procedure allowed us to correlate data over a broad and mostly evenly distributed range of eGFR values, whereas observational studies underrepresented patients with renal impairment. Therefore, our study demonstrates that renal function affects NT-proBNP concentrations beyond cardiac structural abnormalities that are frequently present in CKD. Also, it is the first to provide an algorithm for adjusting marker concentrations for this frequent and important comorbidity.

## Effect of adjustment of NT-proBNP for renal dysfunction

Adjustment of NT-proBNP for eGFR according to the proposed formula results in decreased NT-proBNP concentrations in male subjects with reduced eGFR. This also results in a decreased scatter of marker-concentrations with less overlap between the cardiac subgroups and better distinction between healthy patients (CTRL) and those with structural heart disease (History and LVH) and LVD (Figure 2).

The effect of adjustment of NT-proBNP upon the predictive value was tested in patients with LVD and symptomatic LVD, both for the reference value as well as for an optimized cut-point yielding highest sensitivity and specificity as determined from ROC analysis. All findings demonstrate that adjustment of NT-proBNP for eGFR is associated with a marked and statistically significant improvement in the predictive value (Table 4). At the reference cut-point, unadjusted NT-proBNP provides an extremely high negative predictive value (NPV) for both LVD and symptomatic LVD (Table 4, rows 1 and 5). This indicates that NT-proBNP reliably excludes the presence of (symptomatic) LVD at below-threshold concentrations, and underscores its role as an important cardiac marker. Since the proposed algorithm decreases the measured NT-proBNP concentrations in subjects with renal insufficiency, it might cause marker concentrations to be adjusted from elevated to normal. If this reclassification resulted in inappropriate values, it would result in a loss of NPV. However, as indicated in Table 4 (rows 3 and 7), adjustment of NT-proBNP was not associated with any loss of negative predictive value, indicating that the adjustment is appropriate and does not impair the power of NT-proBNP to exclude LVD. Also, adjustment of NT-proBNP was associated with an increase in specificity of $\sim$20% both for LVD and symptomatic LVD, without any loss in sensitivity (Table 4, compare rows 1 with 3 and 5 with 7, respectively). The increase in specificity is equivalent to a decrease in unspecific test-results, which indicates that renal insufficiency may cause increased NT-proBNP concentrations.

The increase in NT-proBNP with decreasing eGFR is depicted in Figure 3, where the adjustment curves for 3 popular cut points are plotted against eGFR. These cut-off thresholds are 125 pg/mL which represents the approved cut-point for the exclusion of LVD, 193.5 pg/mL which is the cut-point for our healthy reference population, and 300 pg/mL which is the rule out cut-point for acute congestive heart failure (CHF) (10). The adjusted curves demonstrate that when eGFR decreases, NT-proBNP concentrations increase exponentially and might indicate pathological concentrations if no adjustment is performed.

## Importance of renal dysfunction

CKD is a predictor of poor prognosis in asymptomatic patients as well as patients with symptomatic cardiac disease or heart failure (14, 18–23). It is likely that cardiac markers will be measured more frequently in patients at risk, but as the current study confirms, will require careful interpretation of individual test results. While a reduction in eGFR may not be as important if significant cardiac disease with LVD is present, it may be crucial for the interpretation of plasma concentrations in individuals without any cardiac abnormalities. In these patients, false positive test results will be generated if the contribution of renal dysfunction on NT-proBNP concentrations is neglected. The relevance of adjustment of NT-proBNP concentrations in patients with CKD is apparent by the fact that 21% of all patients in the group with renal dysfunction were reclassified from ''elevated'' to ''normal'' NT-proBNP following adjustment.

## Mechanism of increased NT-proBNP in renal dysfunction

The strong correlation between renal insufficiency and plasma NT-proBNP concentrations suggests renal elimination as a major possible contributor. Indeed, renal elimination of NT-proBNP, similar to BNP, has been demonstrated by catheter-

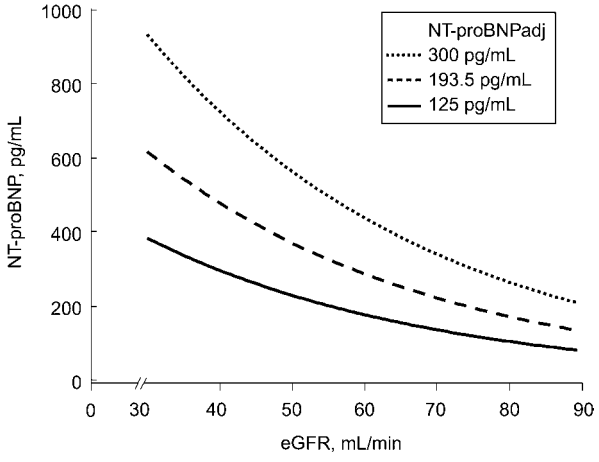


**Figure 3** Association between NT-proBNP and eGFR for three important decision points: 125 pg/mL is the approved decision point for the exclusion of LVD; 193.5 pg/mL is the 97.5th percentile of a healthy reference population; 300 pg/mL is the decision point for ruling out acute CHF in the ER.

ization studies (34–36). In these studies, the renal elimination rate was between 15% and 20% for both BNP and NT-proBNP, and is similar to other bioactive peptides (37). Impairment in glomerular filtration may then lead to impaired renal extraction and higher plasma concentrations. As additional mechanism of increased NT-proBNP and BNP in renal insufficiency, increased plasma concentrations might also be partly attributable to fluid overload in these patients. However, we tried to minimize this problem in the current study by excluding patients with decompensated heart failure during the past 4 weeks, and made sure that patients with decreased eGFR in the control group displayed no clinical signs of heart failure or volume overload.

### Limitations

Despite the stratification of patients and multi-center design, the current study has limitations. NT-proBNP concentrations in the group with normal echocardiographic findings and various degrees of renal impairment may not reflect that of completely ''healthy'' controls. Renal disease as well as high prevalences of diabetes, hypertension, and renal transplantation in this group may impact the molecular biology of the natriuretic peptides. Further, any such condition may cause changes in cardiac structure without being detectable by echocardiography, consequently affecting NT-proBNP concentrations. This dilemma cannot be eliminated in a human study, however. A further limitation is that only male patients were recruited. Due to the effect of gender on NT-proBNP concentrations, the algorithm for adjusting NT-proBNP concentrations in female subjects may differ and need to be evaluated separately. Also, our study does not clarify whether an adjustment for renal insufficiency improves the prognostic properties of NT-proBNP. Since increased NT-proBNP is a predictor of poor prognosis in subjects with LV dysfunction, and renal dysfunction is a predictor of poor prognosis by itself, the prognostic information of NT-proBNP in patients with heart failure may solely not be derived from the association with LV, but also with renal insufficiency.

### Conclusions

NT-proBNP plasma concentrations are affected by renal insufficiency. The current study provides an algorithm for adjusting NT-proBNP concentrations according to eGFR, and demonstrates that adjustment markedly improves the predictive value of this marker in male patients. Most importantly, it helps to increase the specificity of the test without loss of sensitivity or NPV. Individual NT-proBNP test results should always be interpreted in the context of renal function.

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### Conflict of interest statement

**Authors' conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article. The funding source and granting support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.
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### References

1. Hunt PJ, Richards AM, Nicholls MG, Yandle TG, Doughty RN, Espiner EA. Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-PROBNP): a new marker of cardiac impairment. Clin Endocrinol (Oxf) 1997;47:287–96.
2. McDonagh TA, Holmer S, Raymond I, Luchner A, Hildebrant P, Dargie HJ. NT-proBNP and the diagnosis of heart failure: a pooled analysis of three European epidemiological studies. Eur J Heart Fail 2004;6:269–73.
3. Luchner A, Hengstenberg C, Lowel H, Riegger GA, Schunkert H, Holmer S. Effect of compensated renal dysfunction on approved heart failure markers: direct comparison of brain natriuretic peptide (BNP) and N-terminal pro-BNP. Hypertension 2005;46:118–23.
4. Hammerer-Lercher A, Ludwig W, Falkensammer G, Muller S, Neubauer E, Puschendorf B, et al. Natriuretic peptides as markers of mild forms of left ventricular dysfunction: effects of assays on diagnostic performance of markers. Clin Chem 2004; 50:1174–83.
5. Hammerer-Lercher A, Neubauer E, Muller S, Pachinger O, Puschendorf B, Mair J. Head-to-head comparison of N-terminal pro-brain natriuretic peptide, brain natriuretic peptide and N-terminal pro-atrial natriuretic peptide in diagnosing left ventricular dysfunction. Clin Chim Acta 2001;310:193–7.
6. Nielsen LS, Svanegaard J, Klitgaard NA, Egeblad H. N-terminal pro-brain natriuretic peptide for discriminating between cardiac and non-cardiac dyspnoea. Eur J Heart Fail 2004; 6:63–70.
7. Tschope C, Kasner M, Westermann D, Gaub R, Poller WC, Schultheiss HP. The role of NT-proBNP in the diagnostics of isolated diastolic dysfunction: correlation with echocardiographic and invasive measurements. Eur Heart J 2005;26: 2277–84.
8. Falkensammer G, Lechleitner P, Hammerer-Lercher A, Theurl A, Puschendorf B, Mair J. B-type natriuretic peptide and N-terminal pro brain natriuretic peptide are related to systolic and diastolic left ventricular function assessed by radionuclide ventriculography. Int J Cardiol 2005;105:340–1.
9. Januzzi JL Jr, Camargo CA, Anwaruddin S, Baggish AL, Chen AA, Krauser DG, et al. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. Am J Cardiol 2005;95:948–54.
10. Januzzi JL, van Kimmenade R, Lainchbury J, Bayes-Genis A, Ordonez-Llanos J, Santalo-Bel M, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the

International Collaborative of NT-proBNP Study. Eur Heart J 2006;27:330–7.
11. Bayes-Genis A, Santalo-Bel M, Zapico-Muniz E, Lopez L, Cotes C, Bellido J, et al. N-terminal probrain natriuretic peptide (NT-proBNP) in the emergency diagnosis and in-hospital monitoring of patients with dyspnoea and ventricular dysfunction. Eur J Heart Fail 2004;6:301–8.
12. Lainchbury JG, Campbell E, Frampton CM, Yandle TG, Nicholls MG, Richards AM. Brain natriuretic peptide and n-terminal brain natriuretic peptide in the diagnosis of heart failure in patients with acute shortness of breath. J Am Coll Cardiol 2003;42:728–35.
13. Zaphiriou A, Robb S, Murray-Thomas T, Mendez G, Fox K, McDonagh T, et al. The diagnostic accuracy of plasma BNP and NTproBNP in patients referred from primary care with suspected heart failure: results of the UK natriuretic peptide study. Eur J Heart Fail 2005;7:537–41.
14. Luchner A, Hengstenberg C, Lowel H, Trawinski J, Baumann M, Riegger GA, et al. N-terminal pro-brain natriuretic peptide after myocardial infarction: a marker of cardio-renal function. Hypertension 2002;39:99–104.
15. Luchner A, Hengstenberg C, Lowel H, Buchner S, Schunkert H, Riegger GA, et al. NT-ProBNP in outpatients after myocardial infarction: interaction between symptoms and left ventricular function and optimized cut-points. J Card Fail 2005;11: S21–7.
16. Richards M, Nicholls MG, Espiner EA, Lainchbury JG, Troughton RW, Elliott J, et al. Comparison of B-type natriuretic peptides for assessment of cardiac function and prognosis in stable ischemic heart disease. J Am Coll Cardiol 2006;47: 52–60.
17. Vickery S, Price CP, John RI, Abbas NA, Webb MC, Kempson ME, et al. B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with CKD: relationship to renal function and left ventricular hypertrophy. Am J Kidney Dis 2005;46: 610–20.
18. Anavekar NS, McMurray JJ, Velazquez EJ, Solomon SD, Kober L, Rouleau JL, et al. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. N Engl J Med 2004;351:1285–95.
19. McAlister FA, Ezekowitz J, Tonelli M, Armstrong PW. Renal insufficiency and heart failure: prognostic and therapeutic implications from a prospective cohort study. Circulation 2004; 109:1004–9.
20. Akhter MW, Aronson D, Bitar F, Khan S, Singh H, Singh RP, et al. Effect of elevated admission serum creatinine and its worsening on outcome in hospitalized patients with decompensated heart failure. Am J Cardiol 2004;94:957–60.
21. Varghese K, Cherian G, Abraham UT, Hayat NJ, Johny KV. Predictors of coronary disease in patients with end stage renal disease. Ren Fail 2001;23:797–806.
22. Parfrey PS, Foley RN. The clinical epidemiology of cardiac disease in chronic renal failure. J Am Soc Nephrol 1999;10: 1606–15.
23. Baigent C, Burbury K, Wheeler D. Premature cardiovascular disease in chronic renal failure. Lancet 2000;356:147–52.
24. Cockroft D, Gault M. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41.
25. Botev R, Mallie JP, Couchoud C, Schuck O, Fauvel JP, Wetzels JF, et al. Estimating glomerular filtration rate: Cockcroft-Gault and Modification of Diet in Renal Disease formulas compared to renal inulin clearance. Clin J Am Soc Nephrol 2009;4: 899–906.
26. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58:1072–83.
27. Devereux RB, Koren MJ, de Simone G, Okin PM, Kligfield P. Methods for detection of left ventricular hypertrophy: application to hypertensive heart disease. Eur Heart J 1993;14(Suppl D):8–15.
28. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiog 1989;2:358–67.
29. Chenevier-Gobeaux C, Claessens YE, Voyer S, Desmoulins D, Ekindjian OG. Influence of renal function on N-terminal probrain natriuretic peptide (NT-proBNP) in patients admitted for dyspnoea in the emergency department: comparison with brain natriuretic peptide (BNP). Clin Chim Acta 2005;361:167–75.
30. Pfister R, Diedrichs H, Schiedermair A, Rosenkranz S, Hellmich M, Erdmann E, et al. Prognostic impact of NT-proBNP and renal function in comparison to contemporary multi-marker risk scores in heart failure patients. Eur J Heart Fail 2008;10: 315–20.
31. Goei D, Schouten O, Boersma E, Welten GM, Dunkelgrun M, Lindemans J, et al. Influence of renal function on the usefulness of N-terminal pro-B-type natriuretic peptide as a prognostic cardiac risk marker in patients undergoing noncardiac vascular surgery. Am J Cardiol 2008;101:122–6.
32. Das SR, Abdullah SM, Leonard D, Drazner MH, Khera A, McGuire DK, et al. Association between renal function and circulating levels of natriuretic peptides (from the Dallas Heart Study). Am J Cardiol 2008;102:1394–8.
33. Herrmann Z, Uhl W, Steinberg HW, Dworschack R. The influence of renal function on NT-proBNP levels in various disease groups. Clin Lab 2003;49:649–56.
34. Schou M, Dalsgaard MK, Clemmesen O, Dawson EA, Yoshiga CC, Nielsen HB, et al. Kidneys extract BNP and NT-proBNP in healthy young men. J Appl Physiol 2005;99:1676–80.
35. Goetze JP, Jensen G, Moller S, Bendtsen F, Rehfeld JF, Henriksen JH. BNP and N-terminal proBNP are both extracted in the normal kidney. Eur J Clin Invest 2006;36:8–15.
36. Lainchbury JG, Nicholls MG, Espiner EA, Ikram H, Yandle TG, Richards AM. Regional plasma levels of cardiac peptides and their response to acute neutral endopeptidase inhibition in man. Clin Sci (Lond) 1998;95:547–55.
37. Henriksen JH, Schwartz TW, Bulow JB. Endogenous pancreatic polypeptide in different vascular beds: relationship to release and degradation in subjects with normal and decreased kidney function. Metabolism 1986;35:542–6.