References

Renal Elimination of Troponin T and Troponin I, Reinhard Ziebig,*1 Andreas Lun,1 Berthold Hocher,2 Friedrich Priem,1 Claudia Altermann,1 Gernot Asmus,3 Hartmut Kern,4 Rolfdieter Krause,2,5 Babette Lorenz,2 Rainer Möbes,6 and Pranav Sinha1 (1 lnstitut für Laboratoriumsmedizin und Pathobiocemie, 2 Medizinische Klinik mit Schwerpunkt Nephrologie, 3 Klinik für Anaesthesiologie und operative Intensivmedizin, and 6 Medizinische Klinik mit Schwerpunkt Kardiologie, Pulmologie und Angiologie, Universitätshospital Charité, Campus Charité Mitte, Medizinische Fakultät der Humboldt Universität zu Berlin, Schumannstrasse 20-21, 10117 Berlin, Germany; 4 Kuratorium für Dialyse und Nierentransplantation e. V., Dialysezentrum, Sonnenallee 47, 12045 Berlin, Germany; 5 Kuratorium für Dialyse und Nierentransplantation e. V., Dialysezentrum–Moabit, Turmstasse 20 A, 10559 Berlin, Germany; * author for correspondence: fax 49-030-450-569-912, e-mail reinhard.ziebig@charite.de)

Cardiovascular complications represent the predominant cause of death in patients in the terminal stage of renal failure. Increased concentrations of cardiac troponin T (cTnT) may be a valuable predictor of cardiac risk (1, 2). However, cardiac troponin I (cTnI), clinical symptoms, and electrocardiogram (ECG) indications may be absent in patients with a positive cTnT. This may be attributable to instability of the cTnI molecule (3) or dissimilar glomerular filtration of cTnT and cTnI (4). Positive cTnT values are of cardiac origin because the second generation of cTnT assays will not detect cTnT isoforms expressed in the skeletal muscle of hemodialysis patients (5). We therefore measured the cardiac troponins cTnT and cTnI in the plasma and urine of selected patients differing in their kidney function.

We examined 24 patients with increased plasma cTnT. Patients were grouped according to their basic disease and renal function as follows:

Group A included five patients (patients 1–5) who had suffered an acute myocardial infarction and three patients (patients 6–8) with cardiac damage as a result of heart surgery, all with normal or only slightly restricted glomerular filtration rate of > 80 mL/min. All eight patients were male, with a mean (SD) age of 63 (11) years. Patients had clinically typical chest pain (with the exception of patient 8), and electrocardiography (ECG) showed signs of old myocardial infarction, signs of ST-segment reduction or elevation > 0.1 mV with and without chest pain, or signs any arrhythmia of unknown origin.

Group B included two patients (patients 9 and 10) who had suffered an acute myocardial infarction and six patients (patients 11–16) with cardiac damage as a result of heart surgery; all had a substantially restricted glomerular filtration rate (only patient 9 had values for creatinine and creatinine clearance that were within the appropriate reference intervals). All patients in group B were males, with a mean (SD) age of 63 (11) years. Four patients had clinically typical chest pain, and six had a positive ECG.

Group C included eight patients with terminal renal failure and undergoing chronic hemodialysis; all of these patients had a residual diuresis and a plasma cTnT > 0.03 µg/L. Four patients in group C were female, and four were male. The mean (SD) age of this group was 68 (9) years. None had experienced chest pain, and none had positive signs on ECG.

Albumin was used as a marker of a glomerular disorder and α1-microglobulin (α1M) as a marker of a tubular disorder to classify renal damage using a diagnostic program for quantitative analysis of proteinuria (6). We also calculated the protein ratio in urine and plasma for all of the proteins assayed to demonstrate that the renal elimination of the troponins is comparable to that of other known proteins.

All of the determinations were made immediately on receipt of the samples. Blood and urine were collected exactly 72 h after acute myocardial infarction with clinically typical chest pain or heart surgery. In hemodialysis patients, the blood was collected before dialysis. The analytical methods used were as follows: for cTnT (plasma, urine), the Elecsys 2010 Immunoassay (Roche Diagnostics); for cTnI (plasma, urine), the Dimension RxL Immunoassay (Dade Behring); for creatinine (plasma, urine), a modified Jaffe reaction (Dade Behring) on a Dimension RxL; for albumin, a brom cresol purple (BCP) dye-binding method (Dade Behring) on a Dimension RxL; for α1M, an immunoturbidimetric assay (Roche Diagnostics) on a Hitachi 911; for creatine kinase (CK) and CK isoenzyme MB (CK-MB) in plasma, modifications of the ultraviolet enzymatic and the immunoinhibition methods, respectively, on a Dimension RxL; and for albumin and α1M in urine, an immunoturbidimetric assay (Roche Diagnostics) on a Hitachi 911.

The reference intervals for cTnT and cTnI were derived from the manufacturer and were as follows: for cTnT, 0.03 µg/L (CV = 10%) as the new clinical decision cutoff and < 0.01 µg/L as the 99th percentile of the values for a reference control group (7, 8); and for cTnI, < 1.5 µg/L (for exclusion of acute myocardial infarction) and < 0.05 µg/L (the 97.5th percentile of the values for a reference control group). The upper limits of the reference intervals for the urinary proteins were as follows: albumin, 20...
mg/g of creatinine; α1M, 19 mg/g of creatinine (6). Creatinine clearance was estimated according to the method of Cockcroft and Gault (9) on the basis of serum creatinine concentration and the person’s weight.

The troponin assays used are not designed for the analysis of urine specimens, but in a control group (10 completely healthy individuals), all of the cTnI and cTnT measurements (plasma and urine) were below the detection limit. The within-assay imprecision (as the CV) in urine was, for cTnT, 14% and 3.3% (mean values of 0.016 and 0.263 μg/L, respectively), and for cTnI, 4.8% and 3.2% (mean values of 0.647 and 8.42 μg/L, respectively). The assay was linear at urinary concentrations up to 0.01 μg/L for cTnT and 0.05 μg/L for cTnI. The recovery of troponin from urine (after addition of defined quantities of plasma with cTnT and cTnI to urine) was 96–101% for cTnT and 98–103% for cTnI.

The mean (SD) values for the biochemical markers in the three groups were as follows: for group A, plasma cTnT, 4.18 (2.34) μg/L; plasma cTnI, 70.7 (37.0) μg/L; plasma CK (25 °C), 781 (693) U/L; plasma CK-MB (25 °C), 98 (116) U/L; plasma creatinine, 98.9 (22.4) μmol/L; creatinine clearance, 81 (23) mL/min; urinary albumin, 18.1 (12.4) mg/g of creatinine; urinary α1M, 29.7 (20.2) mg/g of creatinine; for group B, plasma cTnT, 7.80 (4.16) μg/L; plasma cTnI, 121.2 (135.3) μg/L; plasma CK (25 °C), 889 (359) U/L; plasma CK-MB (25 °C), 58 (34) U/L; plasma creatinine, 258.8 (103.4) μmol/L; creatinine clearance, 37 (28) mL/min; urinary albumin, 273 (284) mg/g of creatinine; urinary α1M, 293 (117) mg/g creatinine; for group C, plasma cTnT, 0.077 (0.038) μg/L; plasma cTnI, all <0.05 μg/L except for two patients (0.21 and 0.16 μg/L, respectively); plasma CK (25 °C), 24.4 (9.2) U/L; plasma creatinine, 724.3 (111.6) μmol/L; urinary albumin, 1936 (786) mg/g of creatinine; urinary α1M, 1292 (249) mg/g of creatinine (Fig. 1).

In group A, cTnI was not detectable in urine after acute myocardial damage even in cases with very high plasma concentrations of the cardiac troponins. In only one case was a very low cTnT concentration found in urine. Creatinine, albumin, α1M, and the calculated creatinine clearance indicated normal processes of glomerular filtration and tubular reabsorption. In group B, we observed abnormal values for creatinine, creatinine clearance (with

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Fig. 1. Concentrations of the biochemical markers in patient groups A, B, and C.

(A), plasma concentrations of cTnT (□, in μg/L), cTnI (▲, in μg/L), and creatinine (×, in mg/dL); (B), urinary concentrations of cTnT (□) and cTnI (▲), both in μg/g of creatinine; (C), urinary concentrations of albumin (○, in mg/g of creatinine) and α1M (●, in mg/g of creatinine), and creatinine clearance (+; mL/min); (D), calculated protein ratios in urine and plasma for cTnT (□: 37 kDa), cTnI (▲: 23 kDa), α1M (●: 33 kDa), and albumin (○: 67 kDa), R, reference values.
the exception of one patient), albumin, and α1M. α1M dominated in comparison with albumin, so that the renal function of these patients was rated as restricted tubulo-interstitial reabsorption (one patient with glomerulopathy and restricted tubulo-interstitial reabsorption). The restricted tubular reabsorption may lead to the appearance of cTnT and cTnI in urine. Restricted tubular reabsorption may occur as a result of tubulus ischemic damage or an overload of the tubular reabsorption capacity. A decrease in the glomerular filtration rate to <70 mL/min will cause an overload of the tubular reabsorption capacity for α1M (10), and the α1M concentration in the plasma will increase with increasingly restricted filtration.

Group C patients presented with massive, combined glomerular and tubular renal damage (albumin >638 mg/g of creatinine and α1M >895 mg/g of creatinine). We found cTnT in the plasma and urine of all eight of the patients, but we found cTnI at very low concentrations, in the plasma of only two cases, and no cTnI in the urine of any of the cases.

The calculated protein ratios in urine and plasma (Fig. 1D) considered the initial plasma concentration of the proteins, and a direct comparison between the troponin ratios with α1M and albumin ratios was possible. The α1M and albumin ratios suggested a dependence of the molecular weight and the degree of renal impairment. In group A, troponins were not detectable. In group B, the troponin ratios were in the order of magnitude of α1M and albumin, and the cTnT ratios were higher in group C than in group B. The cTnI ratios for group C could not be calculated because cTnI was not detectable in this group, as is frequently seen in these patients.

Although increased cTnT values are an important prognostic factor for cardiovascular disease in hemodialysis patients, the frequently observed differences in the values between cTnT and cTnI are not clear. The lack of cTnI in these patients could be the result of changes in the molecular structure of antigenic regions caused by degradation, oxidation, phosphorylation, or nonenzymatic glycation.

On the basis of the demonstrated parallels between the troponin and the α1M and albumin results, we cannot exclude an influence of the kidney on troponin kinetics. Usually, a complex of troponin I and troponin C (cTnI-C complex) is the dominant form in blood, but under uremic conditions, the portion of free cTnI could increase and produce alterations in the glomerular filtration compared with cTnT. Other studies could not confirm an influence of kidney function on plasma troponin concentrations (11, 12). Hannemann-Pohl et al. (13), however, found differences in the plasma concentration of myoglobin in patients with renal failure depending on the degree of renal impairment. On the basis of our data, kidney function seems to contribute to the elimination of troponins.

References


Cell-free Fetal DNA in Maternal Circulation after Amniocentesis, Osamu Samura,* Norio Miharu, Maki Hyodo, Hiroshi Honda, Yoko Ohashi, Nao Honda, Tetsuaki Hara, and Koso Ohama (Department of Obstetrics and Gynecology, Hiroshima University Faculty of Medicine, 1-2-3 Kasumi Minami-ku, Hiroshima 734-8551, Japan; * author for correspondence: fax 81-82-257-5264, e-mail osamura@hiroshima-u.ac.jp)

After amniocentesis, 5–20% of patients have evidence of fetal-maternal hemorrhage as indicated by increases in maternal serum α-fetoprotein (1–5) or by the Betke–Kleihauer test (6–8). The Betke–Kleihauer test can differentiate fetal from maternal erythrocytes by the relative resistance of hemoglobin F-containing cells to acid elution, and it is the most popular method of diagnosing and assessing the severity of fetal-maternal hemorrhage (9). The reliability of this test has been questioned, however, because numerous sources of error are associated with it (10). These sources of error possibly contribute to the wide variation in the reported incidence of fetal-maternal hemorrhage; a more accurate method of assessing fetal-maternal hemorrhage is therefore required in the clinical setting of rhesus D-negative pregnant women.

The discovery of cell-free fetal DNA in maternal serum and plasma has opened a new avenue for noninvasive prenatal diagnosis and has provided a useful marker of complicated pregnancies (11–16). The analysis of fetal DNA in maternal serum or plasma has afforded diagnoses of fet al rhesus D status (12) and single-gene disor-