been detected by the MLT, but was more obvious when the patients used this meter.

To evaluate the lot-to-lot variation, we included only the measurements performed by the MLT. For the Glucometer Dex, the analytical quality of one lot was significantly poorer than the quality of the two others, even when we excluded the results for incompletely filled strips. For the GlucoMen Glyco there was no difference in analytical quality among the three lots, but all had a negative bias compared with the reference method, as shown in Supplement 2 in the online Data Supplement. Lot-to-lot variation may be a considerable problem with SMBG devices and could be a major factor in loss of analytical quality (12). From 2004 onward, the quality of all lots on the Norwegian market will be examined.

Educational efforts might influence the performance of SMBG (13–15). For the Glucometer Dex, the precision obtained both at the consultation and at home was better for the patient group trained on meter use by the MLT compared with the group that received only written instructions [CV, 12% and 28% for the trained patients and the untrained patients, respectively, at the consultation (P < 0.05) vs 5.6% for the trained patients and 8.8% for the untrained patients at home (P < 0.05)]. For the GlucoMen Glyco, the precision obtained at home was better for the trained group than for the nontrained group (CV, 5.2% vs 7.6%; P < 0.05). However, 12 patients in the nontrained group compared with 2 patients in the trained group had coded the GlucoMen Glyco incorrectly.

User errors that were assessed in the evaluations were highlighted in the questionnaires (Supplements 3 and 4 in the online Data Supplement). Regarding the Glucometer Dex, ~25% of the patients commented on the problem of poor uptake of blood. In the case of the GlucoMen Glyco, 13% answered that it was difficult to apply blood to the test strip, and 17% found it difficult to code the instrument. Manufacturing of SMBG instruments according to patients’ wishes may lead to improvements in acceptability, compliance, and glucose control (16).

Each evaluation lasted ~5 months. One month was used to prepare the work, 2.5 months were needed to complete the practical work, and 1 month was needed for result evaluation. The costs were estimated to be approximately NOK 150 000 (US $20 000) for each evaluation.

It is essential that important shortcomings of SMBG devices are disclosed before the instruments are made commercially available. A procedure for evaluating new instruments and strips should therefore be standardized, including both a user part and a part that deals with analytical quality in the hands of experienced technologists. The evaluation should not be too costly to perform. We believe that our procedure fulfills these demands, and the Norwegian Health Authorities have decided that all SMBG instruments marketed in Norway should be examined by a procedure similar to the one described in this study. In addition, all lots of strips on the market will be tested in a special survey because they cannot be included in the procedure for practical reasons.

The Glucometer Dex and GlucoMen Glyco instruments and strips used and tested by the MLT and the patients in the study were kindly supplied by Bayer Diagnostics (Tarrytown, NY) and Menarini (Firenzi, Italy), respectively. The National Office for Social Insurance in Norway provided financial support for the study. The study is part of the Global Campaign of Diabetes Mellitus launched by the IFCC.

References

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Elimination of the Cardiac Natriuretic Peptides B-Type Natriuretic Peptide (BNP) and N-Terminal proBNP by Hemodialysis, Hans Günther Wahl,1,2 Stephanie Graf,2 Harald Renz,1 and Winfried Fassbinder2 (1 Klinikum der Philipps-Universität Marburg, Department of Clinical Chemistry and Molecular Diagnostics, 35033 Marburg, Germany; 2 Klinikum Fulda, Department of Internal Medicine III, Fulda, Germany; * author for correspondence: fax 49-6421-286594, e-mail hg.wahl@med.uni-marburg.de)

The measurement of natriuretic peptides for the diagnosis of heart failure has been a major breakthrough in cardi-
B-Type natriuretic peptide (BNP) is synthesized as preproBNP mainly in the ventricular myocardium. On ventricular myocyte stretch, preproBNP is enzymatically cleaved to proBNP and released in the form of the hormonally active BNP and the inactive N-terminal proBNP (NT-proBNP). Both BNP and NT-proBNP have been shown to reflect heart failure severity, but studies on their sensitivity and specificity for different degrees of heart failure produced conflicting results. Both BNP and NT-proBNP can be used for the diagnosis of heart failure, but there are important differences between the two tests, particularly regarding influence of age and renal function. In addition to glomerular filtration, BNP is eliminated from plasma mainly through natriuretic peptide receptors and degraded by neutral endopeptidases. In contrast, NT-proBNP possibly is largely eliminated by glomerular filtration only. This explains the strong influence of renal function on NT-proBNP concentrations. Because of the normal decrease in glomerular filtration rate with increasing age, the diagnostic cutoff for NT-proBNP depends on age. This is also true for BNP, but to a much lesser extent. Importantly, both BNP and NT-proBNP concentrations can be increased in the setting of hemodialysis. The prevalence of chronic heart failure is significantly increased in dialysis patients and is associated with left ventricular hypertrophy, which may be secondary to volume overload and hypertension. Reports on the effect of hemodialysis on plasma concentrations of BNP and NT-proBNP showed significant decreases in BNP and significant increases in NT-proBNP. This different behavior was explained by the different sizes of BNP (3.5 kDa) and NT-proBNP (8.5 kDa) and their different half-lives [-20 min and 60–120 min, respectively]. The decrease in BNP plasma concentrations could be attributable to reduced production/secretion of BNP caused by a reduction in plasma volume, elimination by dialysis, or both of these factors.

In this study we investigated the effect of the dialysis procedure on BNP concentrations by hemodialysis, measuring BNP (ADVIA BNP assay; Bayer) and NT-proBNP (Elecsys proBNP; Roche Diagnostics). To address the unanswered question of elimination, we measured the concentrations in both plasma and, for the first time reported, in the corresponding dialysis fluid. Although these assays have not been validated for dialysis fluid, the results can be used to compare the relative effects of different membranes. Pre- and postdialysis samples (EDTA plasma) were drawn from 17 chronic hemodialysis patients [11 men and 6 women; mean (SD) age, 72.3 (6.2) years; mean (SD) duration of hemodialysis treatment, 5.6 (2.9) years]. Patients were treated with the Genius Therapy System (Fresenius Medical Care) and assigned to either low-flux (Polyflux 14 L; Gambro) or high-flux membranes (F 60 S; Fresenius Medical Care). The term high-flux membrane refers to a membrane with a high ultrafiltration rate. Because high-flux membranes tend to have larger pores, clearance of mid-molecular-weight molecules is usually higher than with low-flux membranes. Aliquots of the dialysis fluid were collected in EDTA-containing tubes for plasma preparation. The mean (SD) duration of dialysis was 4.4 (0.5) h, and the mean volume of the ultrafiltration was 2.6 (0.9) L. All samples were centrifuged immediately and stored at -20 °C, and all were analyzed at the same time. Postdialysis samples were adjusted for volume changes by use of the hematocrit.

All patients (n = 17) showed increased mean (SE) concentrations for BNP [738 (120) ng/L] and NT-proBNP [25 366 (9062) ng/L] in predialysis specimens. Even the postdialysis concentrations of both BNP [555 (159) ng/L] and NT-proBNP [24 933 (9828) ng/L] were increased. The approved cutoffs for the diagnosis of heart failure in non-renal-decreased populations are 100 ng/L for BNP, and 125 ng/L (age <75 years) and 450 ng/L (age ≥75 years) for NT-proBNP. Hemodialysis caused mean (SE) decreases of 21.6 (7.1)% for BNP and 10.1 (4.3)% for NT-proBNP.
NT-proBNP (n = 17). The mean BNP decrease in the group of patients treated with the low-flux membrane (n = 4) was 18.5 (1.9)% compared with 22.5 (9.4)% in the group treated with the high-flux membrane (n = 13). Whereas treatment with the high-flux membrane also caused a decrease in NT-proBNP of 18.4 (2.3)%, treatment with the low-flux membrane led to an increase in NT-proBNP of 16.8 (4.9)%. Moreover, each patient treated with the low-flux membrane showed this increase in NT-proBNP plasma concentration after hemodialysis (Fig. 1), but none of the patients treated with the high-flux membrane showed this postdialysis increase. With the exception of two patients (both treated with the high-flux membrane), all patients had decreased BNP concentrations after hemodialysis (Fig. 1). One of the two patients mentioned above had the lowest predialysis BNP value (92 ng/L). The other patient was the only one with no change in blood volume, as estimated by a hematocrit of 0.35 before and after dialysis. The length of hemodialysis treatment (4 h) for these two patients was within the range for all other patients, as was the ultrafiltrate volume (2.2 L). Without these two cases, the mean decrease of 21.6 (7.1)% for BNP becomes 30.2 (5.8)% for all patients, and for the patients treated with the high-flux membrane (n = 11), it changes from 22.5 (8.1)% to 34.5 (5.5)%.

Natriuretic peptide concentrations in the combined dialysis and ultrafiltrate fluid were 13–183 ng/L (median, 25 g/L) for BNP and 75–846 ng/L (median, 223 ng/L) for NT-proBNP. Mass balances were calculated as the product of these concentrations and the total volume (dialysis fluid and ultrafiltrate). The mean (SE) mass balance for BNP was 3282 (871) ng with higher values for the group treated with the high-flux membrane [3603 (1106) ng] compared with the group treated with the low-flux membrane [2238 (900) ng]. The total amount of NT-proBNP eliminated showed a mean mass balance of 40 382 (14 809) ng with higher values for the group treated with the high-flux membrane [49 910 (18 674) ng] compared with the group treated with the low-flux membrane [9416 (4416) ng].

Recently, Clerico and Emdin (22) published a review on the diagnostic accuracy and prognostic relevance of the measurement of cardiac natriuretic peptides. They pointed out the conflicting results of the few studies published for the clinical relevance of these assays in patients with renal failure. In our study, we therefore investigated the effect of the dialysis procedure on concentrations of BNP and NT-proBNP in hemodialysis patients. Both BNP and NT-proBNP are clearly increased in plasma from hemodialysis patients, with much higher concentrations for NT-proBNP, causing a mean (SE) NT-proBNP:BNP ratio of 28.0 (4.4). After hemodialysis, this ratio increased to a mean value of 36.0 (6.8). The mean NT-proBNP:BNP ratio in ambulatory patients with heart failure was reported to be 8.53 (0.33) (23), but we must emphasize that in the case of dialysis with low-flux membranes, where we observed an increase in plasma NT-proBNP, there is still elimination of NT-proBNP by hemodialysis, as was demonstrated by the results obtained for the dialysis fluid.

Both BNP and NT-proBNP are eliminated during hemodialysis, but they show different behaviors depending on the chosen dialysis membrane. BNP is cleared by both high- and low-flux membranes, with high-flux membranes giving higher clearance (mass balance) and reduction rates. NT-proBNP has clearance and reduction rates similar to BNP when high-flux membranes are used but very low clearance with low-flux membranes, leading to an increase in postdialysis plasma concentrations. This may be explained in part by the different molecular masses of BNP (3.5 kDa) and NT-proBNP (8.5 kDa). Both BNP and NT-proBNP seem to be released into the circulation during the hemodialysis session as shown by increasing postdialysis plasma concentrations in spite of demonstrated clearance. In contrast to NT-proBNP, circulating plasma BNP concentrations seem to be affected by acute intradialytic events. Additional studies are needed to test the influence of dialysis treatment on plasma concentrations of BNP and NT-proBNP and to elucidate the interdependence of the production, release, and elimination of these peptides in dialysis treatment.

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References

Use of PCR-Based Amplification Analysis as a Substitute for the Southern Blot Method for CYP21 Deletion Detection in Congenital Adrenal Hyperplasia, Hsien-Hsiung Lee,1 Yann-junn Lee,2 Peter Chan,1 and Ching-Yu Lin1 (1 King Car Food Industrial Co., Yuan-Shan Research Institute, Taiwan, Republic of China; 2 Department of Pediatrics, Mackay Memorial Hospital, and College of Medicine, Taipei Medical University, Taiwan, Republic of China; address correspondence to this author at: King Car Food Industrial Co., Ltd., Yuan-Shan Research Institute, No. 326 Yuan Shan Rd., Sec. 2, Yuan Shan, Ilan 264, Taiwan, ROC; fax 886-3-9228030, e-mail hhlee@ms2.kingcar.com.tw)

Congenital adrenal hyperplasia (CAH) is a common autosomal recessive disorder caused mainly by defects in the steroid 21-hydroxylase gene (CYP21). The defective CYP21 genes in CAH fall into one of three categories: (a) small-scale conversions from CYP21P; (b) spontaneous mutations; and (c) chimeric RCCX modules that include the chimeric CYP21P/CYP21 gene (1) and the chimeric TNXA/TNXB gene (2–4). The RCCX module in chromosome 6p21.3 of the human MHC class III region is composed of a part of the RP gene (serine/threonine nuclear protein kinase) (5), a full-complement C4 gene, a full CYP21(P) gene, and a portion of the TNX gene (6, 7). The C4 protein is coded by two genes, C4A and C4B. The occurrence of a long (20.4 kb) or short (14.1 kb) C4 gene is attributable to the presence of an endogenous retroviral sequence (6.7 kb), namely HERV-K (C4), in intron 9 (8).

The TNX gene contains 3A (TNXA) and TNXB. TNXB, in the downstream CYP21 gene, is partially duplicated in the downstream CYP21P gene, where a truncated gene is termed TNXA. Both TNXA and TNXB are transcribed on the opposite strand. There are two RP genes, RPI and RP2. The RP2 gene is truncated and corresponds to RPI adjacent to TNXA. These genes are arranged in the RPI-C4A-CYP21P-XA-RP2-C4B-CYP21-TNXB gene sequence and are designated as the bimodule of RCCX (Fig. 1A).

The bimodule is composed of a long module including part of RPI, C4A (long), CYP21P, and TNXA, and a short module containing RP2, C4B (short), CYP21, and part of the TNXB gene (2) (Fig. 1A). In Caucasians, the RCCX module has three possible forms: monomodule, bimodule, and trimodule; the bimodular form is the most frequently occurring form (9). To date, 75 different CYP21 mutations have been reported (10). Approximately 70% of cases of CYP21 mutations, including 15 mutations (10, 11), have been attributed to the intergenic recombination of DNA sequences from the highly homologous CYP21P pseudogene. The other 60 mutations (in ~10% of cases) are spontaneous. On the other hand, gross gene deletions of the ~30-kb genome, which leave behind the C4A and a single CYP21A-like gene, have been reported to occur in 20% of alleles in patients with CAH involving a 21-hydroxylase deficiency (12). Obviously, this kind of deletion (and/or conversion) is caused by loss of the -XA-RP2-C4B- gene array between these two RCCX modules. However, from recent studies, the consequence of such a gene deletion produces at least two different features of gene arrangement. One of them presents a -C4A-CYP21P/CYP21-TNXB gene array, which produces four kinds of chimeric CYP21P/CYP21 genes (1, 13), whereas the other presents a -C4A-CYP21P-TNAXA/TNXB array, which contains two kinds of chimeric TNXA/TNXB genes (2, 4).

Identification of such a 30-kb gross gene deletion (and/or conversion) in the RCCX region has traditionally required the Southern blot method with multiple isotope-labeled probes and separate restriction endonuclease digestion (14); TaqI generates 3.7- (functional) and 3.2-kb (pseudogene) fragments, whereas 2.5- (functional) and 2.0-kb (pseudogene) fragments are produced by double digestion with BglI/EcoRI. Although a nonisotopic Southern method has been used (15), the blocking procedure is still laborious. Furthermore, a heterozygous allele for the deleted CYP21 gene cannot be detected in the presence of a wild-type allele, and the results may be hard to interpret without a family study. It is possible that an allele with the trimodule CYP21P-XA-RP2-C4B-CYP21P-XA-RP2-C4B-CYP21-TNXB may interfere with identification of a bimodule with the -XA-RP2-C4B- deletion. In such cases, densitometric screening of fragments is prone to errors and may produce a discordant diagnosis.

To circumvent these problems, we developed a long PCR product with allele-specific primers covering the 5'