Clinical Significance of Urinary Cyclic Guanosine Monophosphate in Diagnosis of Heart Failure

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We measured concentrations of guanosine 3',5'-monophosphate (cGMP) in plasma and urine of healthy subjects and patients with congestive heart failure, renal impairment, neoplastic disease, and hepatic cirrhosis. There was no correlation between cGMP concentrations in urine and in plasma. In all patients except those with renal impairment, urinary cGMP concentrations were significantly higher than in healthy persons. Only patients with heart failure or renal impairment showed significantly increased plasma cGMP concentrations. In contrast, cGMP in urine does not relate to the clinically assessed severity of heart failure (New York Heart Association functional classes). Determination of cGMP in plasma results in higher sensitivity and specificity for diagnosing heart failure than measurement of cGMP in urine.

Indexing Terms: *liver disease/kidney disease/cancer/atrial natriuretic peptide*

Heart failure is not simply a hemodynamic disorder, and its physiologic abnormalities cannot be assessed only by measurement of pressure, volume, and flow. It is also the interplay of neurohormonal and hemodynamic forces (*1*). Any change in atrial filling pressures leads to the release of atrial natriuretic peptides (ANP) from the heart.[5] Once released, atrial peptides exert potent direct vasodilator and natriuretic actions by virtue of their ability to increase their intracellular second messenger, cyclic guanosine monophosphate (cGMP) (*2*). Because cGMP egresses rapidly from target cells after ANP binding to particulate guanylate cyclase-linked receptors, extracellular cGMP may be a useful biological marker for the action of ANP in vivo under pathophysiological conditions (*3*). Exogenously administered ANP was found to increase the plasma cGMP concentration in accordance with its physiologic effects (*4*). Moreover, an increase of urinary excretion of cGMP after an ANP bolus injection in men was reported by Gerzer et al. (*5*). Increased cGMP concentrations in urine of patients with heart failure have been reported as well (*6*). Studies of ANP in heart failure hinted at problems with

storing ANP, because this peptide is highly susceptible to degradation and nonspecific influences in blood (*7*). Instead of ANP, plasma cGMP concentrations can be used to monitor patients with heart failure (*8*). Earlier, we demonstrated that plasma cGMP yields high sensitivity (94%) and specificity (94%) for cardiac diseases in the absence of severe renal impairment (*9*). In patients with heart failure, close correlations have been found between cGMP and ANP plasma concentrations. Both plasma ANP and cGMP correlated closely with the severity of congestive heart failure (*10*).

The recommendation for ethanol extraction of plasma samples prior to measurement of cyclic nucleotides (*11*) hampers the use of plasma cGMP as a substitute for ANP in routine laboratories, because this procedure is time consuming and cannot be automated easily. In urine there is no need for extraction, and urine is easy to collect from patients by noninvasive means. Therefore, we investigated the use of urinary cGMP measurements for the diagnosis of heart failure.

Subjects and Methods

Methods

Specimen collection. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983. Single urine or plasma specimens were collected from healthy individuals and patients at random times. All blood samples were collected into tubes containing EDTA (1.5 g/L blood). After centrifugation for 10 min at room temperature, plasma was stored at −20°C until assayed. Urine was collected and also stored at −20°C until cGMP measurement.

cGMP determination. For plasma extraction we mixed 1000 μL of ethanol and 250 μL of plasma, and after centrifuging the mixture for 15 min collected the supernatant. The precipitate was washed with 500 μL of ethanol and centrifuged again for 15 min. The supernatants were combined and evaporated to dryness at 56°C under a stream of nitrogen. The residues were redissolved in 1000 μL of assay buffer, and 500 μL of this was used in the assay for acetylation.

Urine samples were diluted 100-fold in distilled water before assay. cGMP concentrations in urine were expressed as micromoles of cGMP excreted per gram of creatinine.

Both in urine and in plasma, concentrations of immunoreactive cGMP were measured by RIA (cGMP assay RPA 525; Amersham, Bucks, UK). Creatinine concentrations in plasma and in urine were determined enzymatically (*12*) with reagents from Boehringer Mannheim (Mannheim, Germany).

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[5] Nonstandard abbreviations: ACE, angiotensin-converting enzyme; ANP, atrial natriuretic peptide; cGMP, cyclic guanosine monophosphate; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; and CLL, chronic lymphocytic leukemia.

Radionuclide ventriculography. Supine electrocardiogram-gated equilibrium radionuclide ventriculography was performed after an in vitro/in vivo labeling of erythrocytes with 20–25 mCi of ^{99}Tc. We used an Elscint (Haifa, Israel) Apex SR camera interfaced to an Elscint Apex 1 computer with a semiautomatic operator-interactive ejection fraction program. Resting scintigrams were obtained in the anterior, 40° left anterior oblique, and left lateral positions for a preset time of 4 min. Scintigraphic evaluation of left ventricular ejection fraction (LVEF) in our laboratory correlated well with that obtained angiographically ($r = 0.9$). Normal LVEF values in our laboratory are >0.55 (*13, 14*).

Subjects

Healthy individuals. To establish a reference interval for cGMP in urine, we took urine samples from 76 healthy volunteers (29 female, 47 male), ages 19–60 years (mean ± SD = 33 ± 10 years). For the comparison of plasma cGMP concentrations of controls with those of different patient populations, we used previously published values for healthy individuals (*9*) that were obtained with the same analytical procedure.

Patients with heart failure. Diagnostic sensitivity of cGMP in urine was determined in a group of 50 patients (16 female, 34 male), ages 43–78 years (62 ± 10 years), with congestive heart failure. According to New York Heart Association (NYHA) classification, this group consisted of 14 patients in functional class II, 21 patients in class III, and 15 patients in class IV insufficiency. The underlying causes were ischemic heart disease (n = 25), valvular heart disease (n = 9), cardiomyopathy (n = 12), and congestive heart failure of unknown origin (n = 4). At blood withdrawal, edema was present in 32%, pulmonary congestion in 39%, tachycardia in 16%, orthopnea in 16%, dyspnea in 42%, and cardiac hypertrophy in 13%. In 32 patients, LVEF was measured by radionuclide ventriculography. LVEF of patients ranged from 15% to 66% (33% ± 13%). Plasma creatinine concentrations were 90 ± 27 μmol/L (range 35–150 μmol/L). Patients were treated with nitrovasodilators (32%), angiotensin-converting enzyme (ACE) inhibitors (13%), digitalis (55%), diuretics (77%), calcium antagonists (13%), or various combinations of these substances (91%). Patients who had more than one disease or who received more than one therapeutic agent were listed in each group separately.

Patients with hepatic cirrhosis. This group consisted of nine patients (six female, three male), ages 34–69 years (57 ± 13 years), of whom four already had ascites. Plasma creatinine concentrations in these patients were within the normal reference range (78 ± 10 μmol/L).

Patients with renal impairment. This group consisted of 29 individuals (12 female, 17 male), 34–80 years (55 ± 12 years), all with increased plasma creatinine concentrations ranging from 283 to 919 μmol/L (mean ± SD = 504 ± 150 μmol/L). Of these patients, 11 had chronic glomerulonephritis, 5 had renal cysts, 1 had hepatorenal syndrome, 1 had chronic interstitial nephritis, 1 had

chronic pyelonephritis, 1 had had a nephrectomy, and 1 had diabetic nephropathy.

Patients with malignant tumors. This group comprised 50 patients (15 female, 35 male), ages 24–87 years (58 ± 14 years). The composition of this group was 10 patients with chronic lymphocytic leukemia (CLL), 12 patients with Hodgkin disease (n = 3) or non-Hodgkin lymphoma (n = 9), 8 patients with multiple myeloma, 15 patients with neoplasms of the lung, and 5 patients with malignant tumors of the gastrointestinal tract. In all patients the tumors were progressive despite anticancer treatment. Plasma creatinine concentrations were within the normal reference range in all patients (77 ± 11 μmol/L, range 54–100 μmol/L).

Data Analysis

All results were expressed as mean ± SD. The association between continuous variables was analyzed by the Spearman rank correlation test. Statistical analysis was performed with the Mann–Whitney U-test. P values <0.05 were considered significant.

Sensitivity, specificity, efficiency, positive predictive value, negative predictive value, pre-odds, post-odds, likelihood ratio, and Youden index (*15*) (sensitivity, and specificity − 1) were calculated to describe the performance of cGMP in urine in the diagnosis of heart failure.

Results

cGMP Concentrations in Urine

Healthy volunteers. In 76 healthy volunteers, neither sex nor age influenced cGMP concentrations in urine significantly. cGMP concentrations in urine ranged from 0.002 to 1.28 μmol/g creatinine (0.39 ± 0.24 μmol/g). The distribution of cGMP values in healthy controls is shown in Fig. 1. For determination of the upper cutoff value we calculated the 97.5th percentile for urine cGMP concentration, which was 0.86 μmol/g creatinine. The performance of this cutoff value in diagnosing heart failure is characterized in Table 1.

Patients with heart failure. In patients with heart failure (n = 50), cGMP concentrations in urine were significantly higher (1.49 ± 1.43 μmol/g of creatinine, range 0.14–8.63 μmol/g, $P = 0.0001$) than in healthy

Table 1. Performances of two cutoff values for urinary cGMP concentrations in diagnosing heart failure.

	97.5th percentile of controls, 0.86 μmol/g creatinine	Maximum Youden index, 0.50 μmol/g creatinine
Efficiency	0.78	0.71
Sensitivity	0.64	0.88
Specificity	0.83	0.64
Pos. predictive value	0.56	0.46
Neg. predictive value	0.87	0.94
Youden index	0.47	0.52
Likelihood ratio	3.66	2.47
Pre-odds	0.35	0.35
Post-odds	1.28	0.86

volunteers. The distribution of urinary cGMP values in these patients is shown in Fig. 1. There was no relation between cGMP concentrations in urine and the severity of heart failure according to NYHA functional classes (Fig. 2A). In plasma, by contrast, there was a clear correlation between cGMP concentrations and the severity of heart failure (Fig. 2B). cGMP plasma concentrations in patients with congestive heart failure were
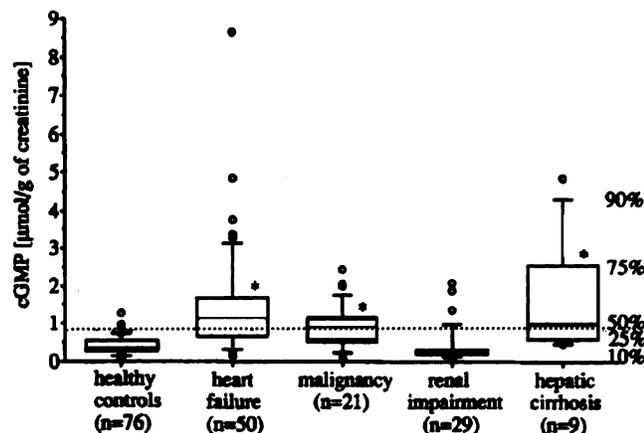

Fig. 1. Distribution of urinary cGMP concentrations in healthy controls and patients.

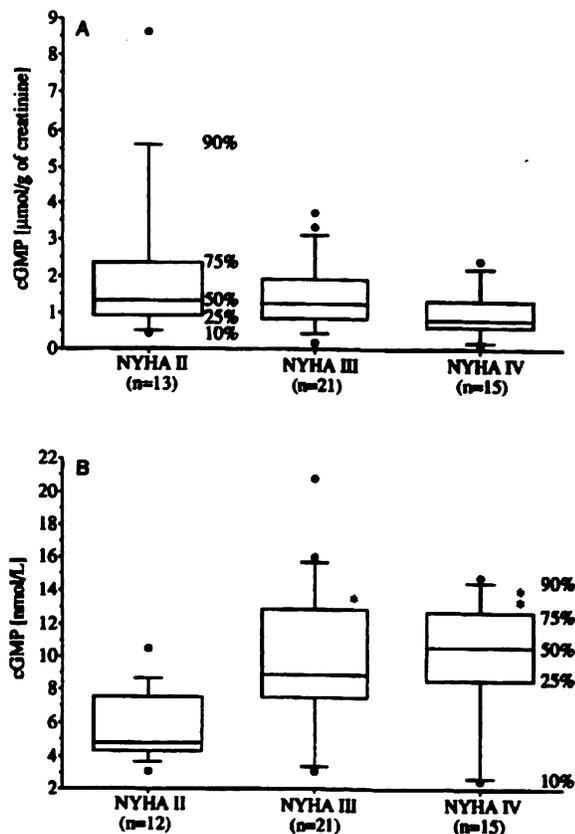*, significantly higher than in control subjects (P = 0.0001). ...., cutoff value (97.5th percentile).

Fig. 2. Distribution of urinary (A) and plasma (B) cGMP concentrations in patients with heart failure, according to NYHA stage.

*, significantly higher than in NYHA II (P = 0.01). ‡, significantly higher than in NYHA II (P = 0.008).

significantly higher than in healthy persons (P = 0.0001). Plasma cGMP concentrations in patients with congestive heart failure in NYHA stages III or IV were significantly higher than in those with heart failure in stage II (P = 0.01 and 0.008, respectively). There was no significant difference between plasma cGMP concentrations in stage III and stage IV patients (P = 0.69). Although cGMP plasma concentrations and urinary cGMP tended to be higher in patients with low LVEF, there were no significant correlations between LVEF and plasma (r = 0.246, P = 0.174) or urinary (r = 0.114, P = 0.53) cGMP concentrations. Grouping of patients according to either etiology of heart failure or clinical symptoms revealed no significant influence of these variables on plasma or urinary cGMP concentrations. Plasma and urinary cGMP concentrations were significantly lower in patients who received ACE inhibitors (P = 0.018 and P = 0.025, respectively; n = 4) and significantly higher in patients who received diuretics (P = 0.047 and P = 0.042, respectively; n = 22).

For determination of diagnostic sensitivity and specificity of urinary cGMP excretion in cardiovascular disease, we quantified cGMP in urine in 50 patients with congestive heart failure and in 143 patients with noncardiac diseases. The upper cutoff value for urinary cGMP that gave the maximal Youden index (15) for diagnosis of heart failure was 0.5 μmol/g creatinine (Fig. 3). This cutoff value provided efficiency of 0.705 with diagnostic specificity of 64% and sensitivity of 88% (Table 1).

Patients with renal impairment. In these patients (n = 29), cGMP concentrations in urine were significantly lower (P = 0.017) than they were in healthy volunteers (0.39 ± 0.5 μmol/g creatinine, range 0.07–2.09 μmol/g). The distribution of cGMP values is shown in Fig. 1. cGMP concentrations in plasma are significantly higher in those patients than in healthy persons (P = 0.0001). There was no significant correlation between plasma creatinine concentrations and cGMP concentrations in plasma (r = 0.048, P = 0.8) or in urine (r = -0.159, P = 0.42).

Patients with malignancies. Except for one patient with CLL who had a cGMP concentration in plasma of
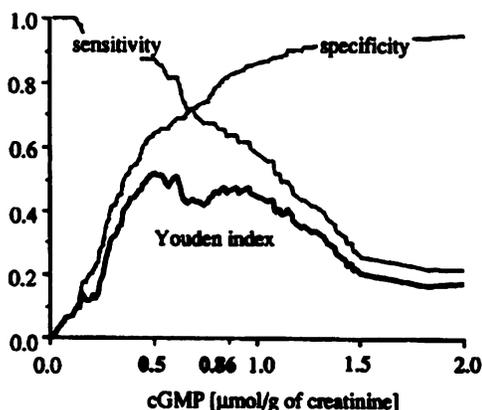

Fig. 3. Sensitivity, specificity, and Youden index of urinary cGMP determination for diagnosis of heart failure, and dependence of these results on the cutoff value used.

7.4 nmol/L, all patients with malignant tumors had plasma cGMP concentrations within the normal reference range [3.83 ± 1.27 nmol/L, range 0.78–6.32 nmol/L, upper cutoff value 6.6 nmol/L (9); data not shown].

In two patients with CLL, three patients with non-Hodgkin lymphoma, three patients with multiple myeloma, nine patients with neoplasm of the lung, and four patients with gastrointestinal tumors, we simultaneously measured cGMP concentrations in urine (0.908 ± 0.561 μmol/g creatinine, range 0.061–2.428 μmol/g). Of the 21 investigated patients (Fig. 1), 11 (1 with CLL, 2 with non-Hodgkin lymphoma, 2 with multiple myeloma, 5 with carcinoma of the lung, and 1 with tumor of the gastrointestinal tract) had increased cGMP concentrations in urine (>0.86 μmol/g creatinine) despite normal cGMP concentrations in plasma.

Patients with hepatic cirrhosis. In these patients (n = 9), cGMP concentrations in urine were significantly higher ($P = 0.0001$) than in healthy volunteers (1.65 ± 1.56 μmol/g creatinine, range 0.46–4.86 μmol/g). The distribution is shown in Fig. 1. cGMP plasma concentrations were within the normal range in all these patients (3.19 ± 1.98 nmol/L, range 0.5–6.24 nmol/L).

Correlation Between cGMP Concentrations in Plasma and in Urine

When we compared cGMP concentrations in plasma and in urine in 114 patients (50 with heart failure, 29 with renal impairment, 29 with malignancies, 6 with hepatic cirrhosis) we found no significant correlation ($r = -0.111, P = 0.24$).

Discussion

cGMP is cleared from the circulation by the kidneys (16). Thus, in patients with renal failure, we found high plasma and low urinary cGMP values. There was no significant correlation between cGMP values of blood and urine samples. Urinary excretion of cGMP cannot be used as a reliable index for either plasma cGMP generated by circulating ANP or for the cGMP generated within the kidneys. It is likely that urinary cGMP is derived partly from circulating cGMP and partly from cGMP formed in the kidney as a consequence of intrarenal urodilatin activity (17).

We found increased urinary cGMP concentrations in some patients with progressive tumors and in some patients with hepatic cirrhosis. Urinary cGMP has been proposed as a tumor marker (18–21). Increased cGMP concentrations in urine have been reported in children with malignancies (18), in adults with acute and chronic leukemia (19) or epithelial ovarian cancer (20, 21), and also in patients with nonmalignant tumors (22). Urinary cGMP may relate to tumor growth rate (23). In patients with raised pretreatment concentrations, urinary cGMP excretion is reported to drop to near normal values during remission of the disease, whereas recurrences are accompanied or preceded by a rise in cGMP excretion (18, 20). However, the mechanism responsible for the increase in urinary cGMP in

tumor patients remains obscure. Increased activity of guanylate cyclase and depressed activity of phosphodiesterase have been demonstrated in tumors. Additionally, it has been shown that the activity of blood phosphodiesterase is considerably suppressed in cancer patients, compared with normal individuals (22).

In human malignancies, the plasma and urine patterns of cyclic nucleotides are variable (24). We found increased urinary cGMP in 52% of patients with progressive tumor, but none had increased cGMP plasma concentrations. There was no significant difference in urinary cGMP values between the various types of neoplasms studied. The low sensitivity for cancer, in addition to the overlap of values from cancer patients with those from controls (25) and from patients with other diseases (poor specificity), renders this marker unsuitable for screening purposes (23). It is also unlikely that urinary cGMP excretion will be used as a tumor marker. However, because urinary cGMP may be increased in some patients with progressive neoplasms, the specificity of this marker for diagnosing heart failure is reduced.

In accordance with previous reports (26) we also found increased urinary cGMP in some patients (56%) with hepatic cirrhosis, whereas cGMP concentrations in plasma were within the normal reference range in all patients. This divergence between urine and plasma cGMP concentrations remains to be investigated; however, it reduces the specificity of cGMP concentration in urine for diagnosis of heart failure.

cGMP measurement in urine provides a sensitivity of 88% and a specificity of 64.3% for diagnosing heart failure, which was lower than the corresponding values (sensitivity 94%, specificity 94%) obtained with determination in plasma samples (9). In heart failure, right atrial pressure is reported to correlate positively with urinary cGMP concentrations and excretion rate (6). In contrast to plasma cGMP, in our patients urinary cGMP was not related to the severity of heart failure assessed clinically according to the NYHA. Plasma cGMP concentrations did not differ significantly between stage III and stage IV patients. In severe heart failure there is an uncoupling of plasma ANP concentrations and cGMP production in the pulmonary and peripheral circulation, probably caused by receptor down-regulation (4, 8). In patients treated with diffent therapeutic agents, plasma and urinary cGMP concentrations differed significantly. Because of the relatively small number studied and the overlap between treatment and severity of congestive heart failure, it is not possible to attribute these observations to the effect of different drugs. However, the use of medications that may affect plasma ANP or cGMP concentrations (ACE inhibitors and diuretics, respectively) may have affected the outcome of this study and thus limited the applicability of these results to pretreated heart failure patients.

In summary, measurement of cGMP in plasma is superior to measurement of this cyclic nucleotide in urine

for diagnosing and monitoring heart failure. However, simultaneous determination of cGMP in a patient's urine and plasma allows one to identify the causes of an increase in plasma cGMP concentrations, i.e., impaired renal clearance or increased systemic production.

We dedicate this paper to Prof. Dr. Franz Dienstl of Innsbruck, Austria, on the occasion of his 65th birthday.

References

1. Packer M. The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. J Am Coll Cardiol 1992;20:248–54.
2. Packer M. Neurohormonal interactions and adaptations in congestive heart failure. Circulation 1988;77:721–30.
3. Margulies KB, Heublein DM, Perrella MA, Burnett JC Jr. ANF-mediated renal cGMP generation in congestive heart failure. Am J Physiol 1991;260:F562–8.
4. Tsutamoto T, Kanamori T, Wada A, Kinoshita M. Uncoupling of atrial natriuretic peptide extraction and cyclic guanosine monophosphate production in the pulmonary circulation in patients with severe heart failure. J Am Coll Cardiol 1992;20:541–6.
5. Gerzer R, Witzgall H, Tremblay J, Gutkowska J, Hamet P. Rapid increase in plasma and urinary cyclic GMP after bolus injection of atrial natriuretic factor in man. J Clin Endocrinol Metab 1985;61:1217–9.
6. Abraham WT, Hensen J, Kim JK, Durr J, Lesnefsky EJ, Groves BM, Schrier RW. Atrial natriuretic peptide and urinary cyclic guanosine monophosphate in patients with chronic heart failure. J Am Soc Nephrol 1992;2:1697–703.
7. Nelesen RA, Dimsdale JE, Ziegler MG. Plasma atrial natriuretic peptide is unstable under most storage conditions. Circulation 1992;86:463–6.
8. Tsutamoto T, Kanamori T, Morigami N, Sugimoto Y, Yamaoka O, Kinoshita M. Possibility of downregulation of atrial natriuretic peptide receptor coupled to guanylate cyclase in peripheral vascular beds of patients with chronic severe heart failure. Circulation 1993;87:70–5.
9. Vorderwinkler KP, Artner-Dworzak E, Jakob G, Mair J, Dienstl F, Pichler M, Puschendorf B. Release of cyclic guanosine monophosphate evaluated as a diagnostic tool in cardiac diseases. Clin Chem 1991;37:186–90.
10. Hirata Y, Ishii M, Matsuoka H, Sugimoto T, Iizuka M, Uchida Y, et al. Plasma concentrations of α-human atrial natriuretic polypeptide and cyclic GMP in patients with heart disease. Am Heart J 1987;113:1463–9.
11. Albano JDM, Barnes GD, Maudsley DV, Brown BL, Etkins RP. Factors affecting the saturation assay of cyclic AMP in biological systems. Anal Biochem 1974;60:130–41.
12. Siedel J, Möllering H, Ziegenhorn J. Sensitive color reagent for the enzymic determination of creatinine. Clin Chem 1984;30:968–9.
13. Maddahi J, Berman D, Matsauka D, Waxman W, Stankus K, Forrester JS, Swan HJC. A new technique for assessing right ventricular ejection fraction using rapid multigated equilibrium cardiac blood pool scintigraphy. Circulation 1979;60:581–6.
14. Pichler M, Mostbeck G, Fridrich L, Eghbalian F, Gassner A. Scintigraphic left ventricular function during exercise in elderly patients with coronary artery disease. Eur Heart J 1984;5:55–7.
15. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32–5.
16. Broadus AE, Kaminsky NI, Hardman JG, Sutherland EW, Liddle GW. Kinetic parameters and renal clearances of plasma adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in man. J Clin Invest 1970;49:2222–36.
17. Goetz K, Drummer C, Zhu JL, Leadley R, Fiedler F, Gerzer R. Evidence that urodilatin, rather than ANP, regulates renal sodium excretion. J Am Soc Nephrol 1990;1:867–74.
18. Puschendorf B, Grunicke H, Jentsch J, Berger H. Urinary excretion of cyclic guanosine 3',5'-monophosphate in children with malignant tumors. J Clin Chem Clin Biochem 1981;19:979–81.
19. Peracchi M, Lombardi L, Maiolo AT, Bamonti-Catena F, Toschi V, Chiorboli O, et al. Plasma and urine cyclic nucleotide levels in patients with acute and chronic leukemia. Blood 1983;61:429–34.
20. Luesley DM, Chan KK, Newton JR, Blackledge GR. Serial urinary cyclic guanosine monophosphate measurements in the assessment of response to treatment in epithelial ovarian cancer. Br J Obstet Gynaecol 1987;94:461–6.
21. Turner GA, Greggi S, Guthrie D, Benedetti-Panici P, Ellis RD, Scambia G, Mancuso S. Monitoring ovarian cancer using urine cyclic GMP. A two-centre study. Eur J Gynaecol Oncol 1990;11:421–7.
22. Turner GA, Ellis RD, Guthrie D, Latner AL, Monaghan JM, Ross WM, et al. Urine cyclic nucleotide concentrations in cancer and other conditions; cyclic GMP: a potential marker for cancer treatment. J Clin Pathol 1982;35:800–6.
23. Luesley DM, Blackledge GR, Chan KK, Newton JR. Random urinary cyclic 3',5'-guanosine monophosphate in epithelial ovarian cancer: relation to other prognostic variables and to survival. Br J Obstet Gynaecol 1986;93:380–5.
24. Peracchi M, Toschi V, Bamonti-Catena F, Lombardi L, Bareggi B, Cortelezzi A, et al. Plasma cyclic nucleotide levels in acute leukemia patients. Blood 1987;69:1613–6.
25. Hunt NH, Smith B, Pembrey R. Cyclic nucleotide excretion in human malignancies. Clin Sci 1980;58:463–7.
26. Wood PJ, Ross G, Smith CL. Plasma and urine cyclic nucleotide levels in malignant disease and cirrhosis of the liver. J Clin Pathol 1979;32:998–1002.