

A Metabolic Study of Patients with Lung Cancer and Hyponatremia of Malignancy

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Abstract Purpose: One-third of patients with lung cancer and hyponatremia have no evidence of ectopic arginine vasopressin (AVP) production and the cause of their hyponatremia is not conclusively established. We sought to distinguish patients with hyponatremia caused by elevated AVP versus those with ectopic atrial natriuretic peptide (ANP) via this detailed metabolic study.

Experimental Design: We enrolled 24 patients recently diagnosed with lung cancer in a metabolic study in which patients were placed on sodium and fluid restriction for 4 days. Serum electrolytes, osmolality, urine electrolytes and osmolality, plasma AVP, ANP, aldosterone, urinary cyclic AMP and cyclic guanosine 3',5'-monophosphate were measured daily and tumor tissue was obtained to measure ectopic hormone production. We attempted to characterize the pathophysiology of hyponatremia caused by ectopic ANP production in patients with small cell lung cancer (SCLC) and to determine its effect on the aldosterone axis.

Results: Seven of the nine patients with SCLC presented with hyponatremia and three had elevated ANP levels at presentation without elevation of AVP. All three patients who presented with hyponatremia and elevated ANP showed a decline in serum sodium following fluid restriction, whereas two patients with SCLC and elevated AVP had normalized serum sodium levels. The combination of hyponatremia and elevated ANP was associated with a persistent natriuresis and inappropriately low aldosterone levels despite sodium restriction, suggesting ANP suppression of the aldosterone axis.

Conclusions: Management of patients with hyponatremia and SCLC should be guided by the knowledge that some patients with SCLC have ectopic production of ANP as the cause of their hyponatremia.

Water and sodium homeostasis is dependent upon a complex interplay of hormonal, neural, and physical mechanisms controlling renal sodium and water reabsorption (1, 2). Both atrial natriuretic peptide (ANP) and the renin-angiotensin-aldosterone system play important roles in renal sodium excretion whereas arginine vasopressin (AVP) controls free water clearance in the kidney. Water and sodium homeostasis is abnormal in many patients with lung cancer. Hyponatremia occurs at presentation in ~15% of patients with small cell lung cancer (SCLC) in retrospective studies (3), 30% in our previous

prospective study of patients with SCLC (4), and 1% of patients with non-small cell lung cancer (NSCLC; ref. 3).

Ectopic production of AVP by SCLC cells plays a causal role in the development of hyponatremia in most patients with SCLC (3, 5–11). However, SCLC patients with hyponatremia have been described who have no measurable AVP in their tumors (12), tumor cell lines (13), or in their plasma (10, 14). Ectopic production of ANP mRNA in tumors and tumor cell lines from patients with SCLC and hyponatremia has been documented by Northern blot, S₁-nuclease analysis, RNase protection assay, and PCR (4, 14, 15). ANP has been detected in SCLC tumors and tumor cell lines by RIA (14, 16, 17). Characterization of the peptide from SCLC tumors and tumor cell lines by high-performance liquid chromatography has shown the peptide to be similar to the bioactive 28-amino acid form present in the plasma (17, 18). Plasma ANP levels have been found to be elevated in patients with lung cancer and hyponatremia with elevated AVP levels and in patients with normal AVP levels (16, 17, 19–21). Despite this extensive evidence for ectopic production of ANP, the potential pathologic role of ANP in patients with hyponatremia has not been well defined.

ANP increases the renal excretion of sodium mediated by the ANP receptors in the kidney (1, 2, 22, 23). Our previous prospective study showed that patients with SCLC following a saline load showed increased sodium excretion proportional to their initial ANP levels (4). We found no relationship between ANP levels and plasma renin activity, angiotensin II, and

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aldosterone levels in these patients (4). Ten patients with SCLC and hyponatremia showed no difference in plasma renin activity ($P = 0.67$), angiotensin ($P = 0.47$), and aldosterone ($P = 0.12$) as compared with 21 SCLC patients with normal serum sodium. However, saline loading caused a significant increase in sodium excretion in the hyponatremic patients with elevated baseline levels of ANP ($n = 8$) as compared with patients with normal serum sodium levels ($n = 31$; $P = 0.0045$). These results suggested the contribution of ANP to natriuresis in some patients with lung cancer and hyponatremia.

The results from our previous study prompted us to design this metabolic study to more definitively determine the role of ectopic ANP in sodium homeostasis in patients with lung cancer. In this study, sodium and free water intake was limited by placing patients with lung cancer on a 109 mEq/d sodium diet and a 1,000 mL/d fluid restriction for 4 days prior to their treatment for lung cancer. We chose a 1,000 mL fluid restriction because this is typically administered in the treatment of the syndrome of inappropriate antidiuretic hormone (SIADH; ref. 24) and might sensitively allow discrimination of patients with pathologic AVP elevation versus those with inappropriate ANP elevation. We hypothesized that the controlled low sodium and fluid intake accompanied by an ongoing natriuresis mediated by increased ANP might cause progressive hyponatremia in patients with SCLC and ectopic production of ANP. In order to characterize the metabolic effects of ectopic ANP or AVP production, we measured plasma ANP and AVP levels, urinary cyclic guanosine 3',5'-monophosphate (cGMP) and cyclic AMP (cAMP), which are secondary messengers for ANP and AVP signaling (4), serum aldosterone, and urinary sodium excretion over time. Daily weights and orthostatic hemodynamic measurements were also measured to ascertain whether AVP- or ANP-mediated hyponatremia was associated with clinically relevant hemodynamic effects. The overall aim of this study was to sensitively define the contribution of ectopic ANP and AVP to the hyponatremia observed in patients with lung cancer.

Materials and Methods

Study population. Patients with histologically proven, previously untreated SCLC and NSCLC were entered into the study. Tumor histologies were reviewed prior to study entry by a reference pathologist (J. Williams). The patients' pretreatment evaluation and staging criteria have been previously described (25–27). Eligibility criteria included age >18 years, no prior chemotherapy or radiation therapy, performance status of 0–2 and absence of brain metastases. Patients whose cancer was acutely life-threatening, patients with hypertension, significant myocardial disease, renal insufficiency, and symptomatic hyponatremia were ineligible to participate in the study. Patients taking any medication that was known to alter sodium homeostasis, such as diuretics, antihypertensive agents, medications for congestive heart failure, demeclocycline, and corticosteroids were excluded. Patients with other active cancers, excluding superficial skin carcinomas, were not eligible. The rationale and possible side effects of the study were explained to each patient. The patients were then given the opportunity to participate in the study and sign an informed consent approved by our Institutional Review Board.

Study design. The patients were admitted to the Medical Oncology inpatient unit at the National Naval Medical Center. A history and physical examination including weight, blood pressure, and pulse rate in the supine, sitting and upright position were done daily. Serum electrolytes, blood urea nitrogen, and creatinine were drawn on the first day. The patients were then placed on a 109 mEq sodium diet with a

fluid restriction of 1,000 mL per day. Diet and fluid intake were monitored daily by a staff dietician at the hospital (E. Taylor). If the patients could not eat, salt tablets and fluid were administered orally or i.v. to bring the intake to the prescribed levels. The patients' weight, serum electrolytes, blood urea nitrogen, creatinine, plasma AVP and ANP were measured daily for days 1 to 4 and aldosterone was measured on days 1 to 3. Serum osmolality was measured on days 2 to 4. The 24-hour creatinine clearance, urine electrolytes, urine osmolality, urine cAMP, and cGMP were collected on days 3 to 5.

Definitions and sample processing. Plasma sodium levels ≤ 135 mEq/L were considered low as previously defined by examination of normal controls (28, 29). The reference range of plasma sodium at National Naval Medical Center at the time of this study was 136 to 145 mEq/L. The upper limits of the normal plasma hormone levels were determined from published literature (30, 31). AVP levels were analyzed in relation to serum osmolality as described by Robertson (32). Plasma aldosterone levels ≤ 240 pmol/L were considered normal (33, 34). Reference range cGMP and cAMP levels were estimated based on previously published values (31, 35).

Plasma hormone samples were processed as previously described (4). Twenty-four hour urine collections were completed for each patient on days 3 to 5. Concordantly, daily urine samples were collected for electrolyte and osmolality measurements. All chemistry and osmolality samples were processed by the chemistry laboratory at the National Naval Medical Center.

Tumor cell line studies. Attempts were made to establish tumor cell lines from patients with lung cancer treated on Institutional Review Board–approved protocols at the Medicine Branch (4, 15). Tumor cell lines were studied for AVP and ANP mRNA using reverse-transcriptase PCRs. cDNA strands were generated from the mRNA as previously described (4). The primers for AVP were sense 5'-GGCTAC-TGGCCTTCTCCTCC-3' and antisense 5'-CTCTCGTCGTTGCAGCAAAC which amplified a 293-bp product (4). The primers for ANP were sense 5'-CAACGCAGACCTGATGGATT-3' and antisense 5'-TTAGGAGGGCA-GATCGATCAGA-3' which amplified a 236-bp product (4). The PCRs were done using methods we have previously described (4). Approximately 1.0 mL of packed tumor cells were also harvested during log-phase growth and washed twice in PBS at 4 °C. The lung cancer cell lines were then processed for measurement of immunoreactive AVP and ANP as previously described (4).

RIA. Plasma and urinary ANP and AVP levels were determined after the plasma was extracted as previously described (5). The limit of detection of ANP was 0.2 pmol/L and AVP was 0.01 fmol/L. The interassay and intraassay variabilities have been previously reported (4). Plasma aldosterone immunoreactivity was determined with the RSL [¹²⁵I] aldosterone kit (ICN Biomedicals, Inc., Costa Mesa, CA) with a limit of detection of 2.0 pmol/L (4). RIAs for quantification of urinary cAMP and cGMP were determined as previously described (31, 35).

Data analysis. Comparative analyses of the levels of serum sodium, osmolality, ANP, AVP, aldosterone, and cGMP and cAMP between SCLC and NSCLC patients were done using the two-tailed *t* test and Wilcoxon rank sum test. Associations with hyponatremia within the SCLC patients were assessed using the Wilcoxon rank sum test. Correlations among the various levels were tested by the Spearman rank correlation method. Additional analyses were done for trends in the plasma hormone levels using the samples taken from the patients over the course of the metabolic study. The direction of the change in hormone levels in each patient was analyzed using the two-tailed Wilcoxon signed rank test and repeated measures ANOVA. Where individual tests were done simultaneously in SCLC and NSCLC subgroups, *P* values were corrected for multiple testing by the Bonferroni method.

Results

Study population. Nine previously untreated patients with SCLC and 15 patients with NSCLC seen at the Medicine Branch

from July 1995 to July 1996 participated in the trial. The clinical characteristics of the patients who participated in the trial are shown in Table 1. Seven of the nine SCLC patients (78%) had hyponatremia, whereas only 1 of 15 patients with NSCLC (7%) presented with hyponatremia (Fig. 1A). Three of the nine patients had initial sodium levels ≤ 131 mEq/L and one had an initial sodium value of 127 mEq/L. The mean sodium level at enrollment in the SCLC patients (133.1 ± 3.1 mEq/L) was significantly lower than the mean sodium level in the NSCLC patients (139.4 ± 3.3 mEq/L; $P = 0.0003$). Similarly, the initial mean serum osmolality level in patients with SCLC (281.1 ± 15.5 mOsm/kg) was significantly lower than the initial serum osmolality level in patients with NSCLC (293.3 ± 8.0 mOsm/kg; Fig. 1B; $P = 0.04$). The Spearman rank correlation coefficient of serum sodium and osmolality at enrollment was 0.67 ($P = 0.0006$).

Levels of ANP, AVP, cGMP, cAMP, and aldosterone at enrollment. The parameters of sodium homeostasis were measured in patients at enrollment. Of note, the denominator of patient samples differs slightly across different analyses due to rare errors in sample collection. A level of ANP of >15 pmol/L has been defined as elevated in previous publications (29). The mean ANP level at enrollment in patients with SCLC (15.5 pmol/L ± 12.5 , $n = 8$) was 2-fold higher than that of patients with NSCLC (8.2 pmol/L ± 6.6 , $n = 15$; Fig. 1C) although this difference did not meet significance ($P = 0.07$). Three of the four SCLC patients with ANP levels >15 pmol/L had hyponatremia at presentation. One NSCLC patient had an ANP of 27.6 pmol/L and sodium levels of 138 mEq/L at enrollment. The single NSCLC patient who presented with hyponatremia had an ANP level of 7.5 pmol/L and an AVP level of 319 fmol/L, suggesting ectopic production of AVP. Patients with SCLC had comparable urine sodium excretion levels at initial measurement (mean, 93.1 ± 75.4 mmol) compared with NSCLC patients (mean, 126.3 ± 47.3 mmol; $P = 0.1$; two-tailed t test). Interestingly, the three SCLC patients with elevated ANP levels

and hyponatremia at presentation had significantly higher levels of urinary sodium excretion (121, 220.0, and 125.4 mmol) compared with all other SCLC patients (mean, 56.2 ± 55 mmol; $P = 0.04$).

The mean levels of plasma AVP were 36.3 and 48.6 fmol/L in the SCLC and NSCLC patients, respectively. As described by Robertson et al. (32), we examined AVP levels in the enrolled patients in relation to their initial osmolality measurements. Two SCLC patients with hyponatremia and elevated ANP levels showed hypoosmolality coupled with low AVP levels (Fig. 1D). In these patients, the hypoosmolality seemed to be caused by ANP rather than AVP. Two additional patients with SCLC presented with hypoosmolality and inappropriately elevated AVP levels (Fig. 1D) consistent with SIADH. Only one of the NSCLC patients showed an AVP level which was inappropriately elevated compared with their serum osmolality (Fig. 1D). This patient had hyponatremia and a low ANP (7.5 pmol/L), suggesting SIADH. Spearman rank analysis showed no relationship between AVP levels and hyponatremia in SCLC patients.

The mean levels of aldosterone were 170.9 and 145.2 pmol/L in patients with SCLC and NSCLC, respectively, at enrollment, and this difference was not statistically significant ($P = 0.6$; Fig. 1E). One patient with SCLC and three patients with NSCLC had elevated aldosterone levels (>240 pmol/L) at initiation. We found no correlation between aldosterone levels at enrollment and tumor histology, hyponatremia, ANP, AVP levels, or urine sodium excretion levels. However, among the three patients with SCLC who presented with hyponatremia and elevated ANP, all had aldosterone levels which were inappropriately low (213, 112, and 171 pmol/L), suggesting a blunting of the aldosterone response in these patients. The mean urinary level of cGMP, which is the secondary messenger for ANP, was not significantly different between the patients with SCLC and NSCLC at enrollment (Fig. 1F). Of the three SCLC patients with elevated ANP levels and hyponatremia at presentation, only one had an elevated urinary cGMP level (1.63 pmol/L). Similarly, we found no difference in the levels of urinary cAMP levels between patients with SCLC and patients with NSCLC at presentation (data not shown). The single NSCLC patient who presented with hyponatremia and markedly elevated AVP (318 fmol/L) also did not show elevated urinary cAMP (3.3 pmol/L). We found no correlation between urinary cGMP levels and tumor histology, ANP levels, or hyponatremia in the patients at enrollment.

Tumor cells were also obtained from four enrolled patients to assess tumor mRNA expression for AVP and ANP. We were unable to generate primary tumor cell lines in three of those patients. One patient who presented with SCLC and hyponatremia (Na, 131 mEq/L) had an elevated level of ANP (30.8 pmol/L), the second highest value. The reverse-transcriptase PCR analyses of the patient's tumor cells showed expression of the ANP transcript in the absence of detectable expression of AVP mRNA (Fig. 2A and B).

Metabolic studies. A total of 24 patients (9 SCLC, 15 NSCLC) were enrolled on a 4-day metabolic study in which they were placed on a 109 mEq/d sodium diet and a 1,000 mL/d fluid restriction. One patient was withdrawn from the study after 48 hours due to renal insufficiency, but the remainder participated to completion. During the course of the study, the mean serum sodium levels in the SCLC patients did not change

Table 1. Characteristics of enrolled patients

Characteristic	Patients with SCLC	Patients with NSCLC
No.	9	15
Age median (range)	63 (50-75)	48 (34-71)
Gender		
Male	5	12
Female	4	3
Eastern Cooperative Oncology Group performance status		
0-1	8	14
2	1	1
Stage		
Limited	2	II 0
Extensive	7	IIIa 2 IIIb 3 IV 10
Serum sodium, mean (range)	133 mEq/L (127-140)	139.4 mEq/L (130-146)*

* $P = 0.0003$ for difference in median serum sodium at initiation of trial.

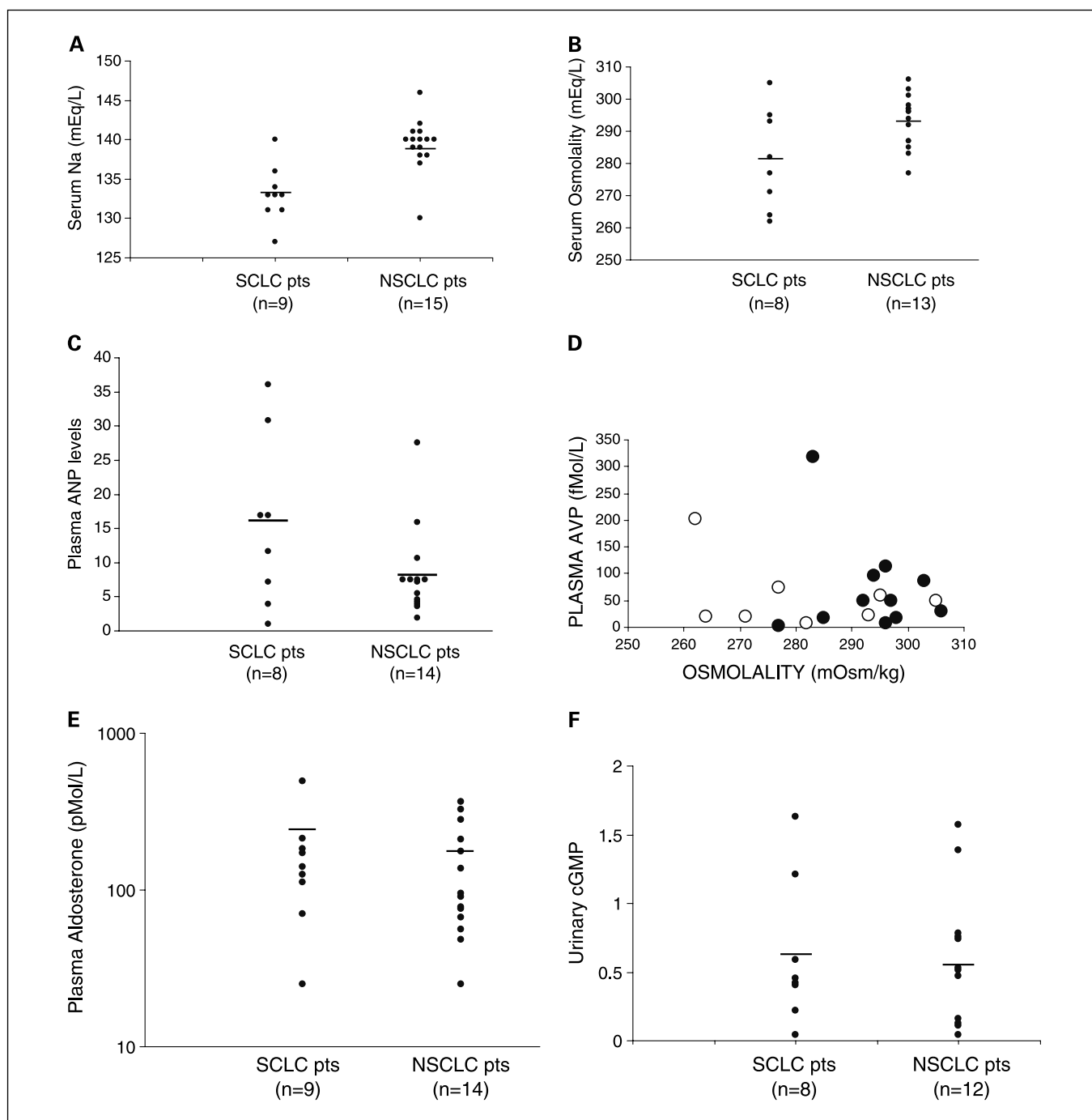


Fig. 1. Metabolic parameters of patients with lung cancer on admission. *A*, serum sodium levels in SCLC patients compared with NSCLC patients on admission. The mean serum sodium levels are represented by horizontal lines. The mean serum sodium level on admission in patients with SCLC was significantly lower than the mean level in patients with NSCLC ($P = 0.0003$). *B*, the initial measured serum osmolality was found to be significantly higher in patients with NSCLC versus patients with SCLC. *C*, plasma ANP levels were measured in patients with SCLC and NSCLC on admission. Scatter plots of ANP levels and the mean ANP levels in each group. *D*, plasma AVP levels in relationship to serum osmolality is shown. ●, patients with NSCLC at day 0; ○, patients with SCLC at day 0. Two SCLC patients show low AVP levels coupled with low serum osmolality measurements; one NSCLC patient shows an inappropriately elevated AVP level compared with a low serum osmolality. *E*, plasma aldosterone levels on admission in patients with SCLC and NSCLC, demonstrating no significant difference between the groups at day 0. *F*, urinary cGMP levels at admission in patients with SCLC and NSCLC, demonstrating no significant difference between the groups.

significantly (133 mEq/L at day 1 and 132.8 mEq/L at day 4; Fig. 3A). Similarly, serum sodium levels in the NSCLC patients remained consistent throughout the study (139.4 mEq/L at day 1 and 138 mEq/L at day 4; Fig. 3B). Among the seven SCLC patients who presented with hyponatremia, four showed a

significant decline in serum sodium between days 1 and 4 (mean, 130.3 mEq at day 1 and 126.3 mEq at day 4; $P = 0.02$; Fig. 3C) despite 1,000 mL fluid restriction. Three of these four patients had elevated plasma ANP levels on admission. For example, the SCLC patient with demonstrable ectopic

transcription of ANP in his tumor showed a decline in sodium levels from 131 to 128 mEq/L during the course of the study. In contrast, two patients with SCLC who presented with sodium levels <135 mEq/L and elevated AVP levels corrected their serum sodium levels to normal during the study (mean, 133.5 mEq/L at day 0 and 140 mEq at day 3; Fig. 3D). The other three patients with SCLC who presented without obvious elevations in ANP or AVP did not show significant changes in their serum sodium levels during the course of the study.

Figure 4 shows the relationship between serum sodium and ANP levels in the patients with SCLC over the course of the study. A trend toward an inverse correlation between increasing ANP levels and decreasing serum sodium measurements was observed at day 1, although the Spearman rank correlation coefficient for this analysis did not reach significance ($P = 0.6$). Comparison of days 2 and 3 sodium and ANP levels showed a similar trend toward an inverse relationship, but again, the

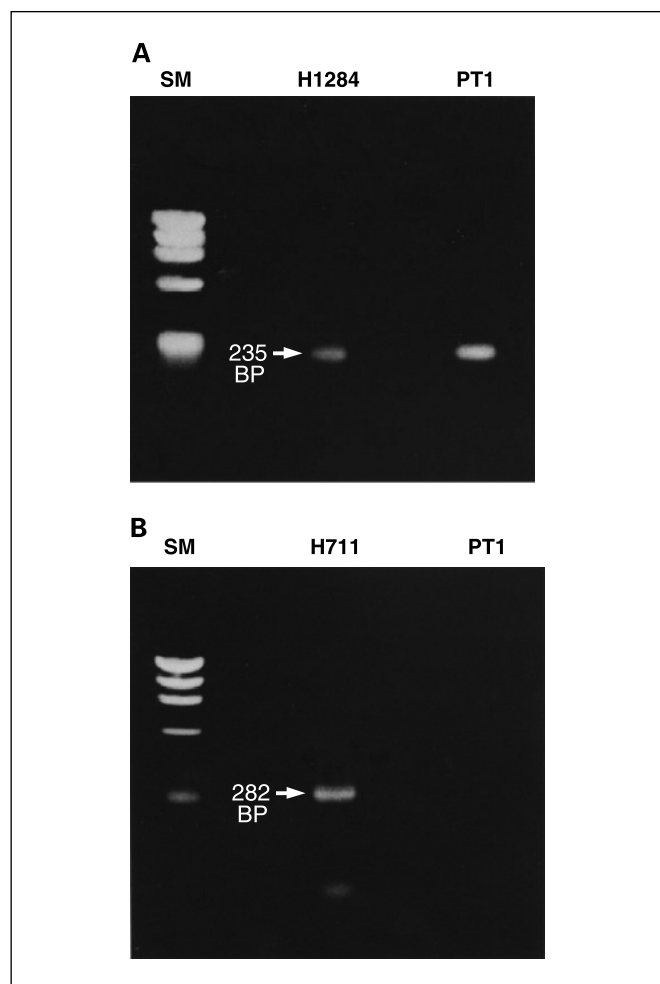


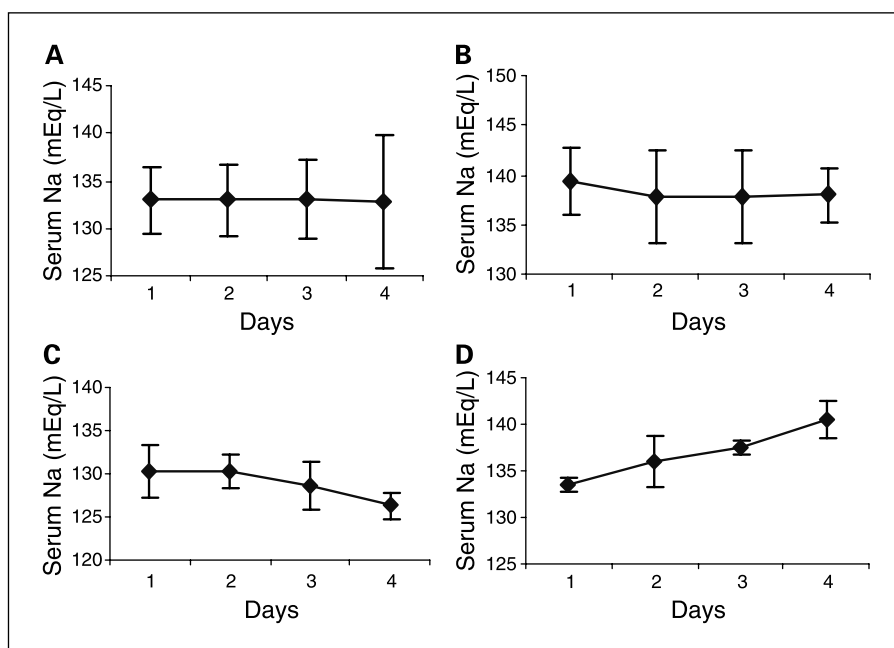
Fig. 2. Evidence of ectopic expression of ANP mRNA in a patient with SCLC and hyponatremia. Reverse-transcriptase PCR analysis of expression of ANP (A) and AVP (B) from an enrolled SCLC patient who presented with hyponatremia and elevated plasma ANP levels. Size markers are shown on the left. Cell line H1284 is an established SCLC cell line which we have previously shown to express ANP (4). The SCLC patient in this study shows expression of the ANP mRNA with a transcription product of 235 bp. B, reverse-transcriptase PCR analysis of AVP mRNA levels in the same SCLC patient as compared with expression in an established cell line, H711. The patient's tumor cells show no evidence of expression of the AVP mRNA, which is expected to be 282 bp.

correlation coefficients were not significant ($P = 0.3$ and $P = 0.8$, respectively; Fig. 4B and C). However, when changes in sodium levels were analyzed compared with the ANP levels at presentation, a significant inverse correlation was shown between ANP levels at presentation and the changes over the course of the study. The change in serum sodium between days 1 to 2 (Fig. 4D), 2 to 3 (Fig. 4E), and 3 to 4 (Fig. 4F) are shown. The Spearman rank correlation coefficients for these data were $P = 0.2$, $P = 0.01$, and $P = 0.2$, respectively. In contrast, in patients with NSCLC, ANP levels at presentation and changes in serum sodium levels in patients were consistent throughout the course of the study (data not shown). A single NSCLC patient had ANP levels of >50 pmol/L throughout the course of the study. This patient had AVP levels of 3.2, 20.5, and 12.9 fmol/L and showed normal serum sodium throughout the study.

Urinary sodium excretion decreased over the course of the study in the patients with SCLC despite sodium and fluid restrictions (mean, 093.1 ± 75.4 mmol at day 3 and 47.5 ± 28.1 mmol at day 5; $P = 0.1$; Fig. 5A). All three patients with SCLC who presented with hyponatremia and elevated ANP levels showed a decrease in urinary sodium excretion from days 3 to 5 (121.0-22.4, 220-54.0, and 125.4-76.5 mmol, respectively). Although decreasing, these urinary sodium excretions remained pathologic in these patients in the face of persistent hyponatremia, perhaps indicating a competing effect of ectopic ANP and a sodium-restricted diet on sodium homeostasis. Patients with NSCLC also showed a decline in urinary sodium excretion over time (126.3 ± 47.3 to 72.5 ± 51.7 mmol; $P = 1.0$; Fig. 5A). Serum osmolality measurements showed no significant change in the SCLC patients over the course of the study ($281-277.4$ mOsm/kg; $P = 0.4$; Fig. 5B). When we analyzed the osmolality in SCLC patients with hyponatremia at presentation, two of these patients showed a decline in osmolality and both had ANP levels >15 pmol/L. The patients with NSCLC showed no change in serum osmolality over time ($293.2-294.4$ mOsm/kg; $P = 0.4$; Fig. 5B). Aldosterone levels declined in SCLC patients over the course of the study, although this did not meet significance (mean, 171-114 pmol/L; $P = 0.2$; Fig. 5C). In the three patients with SCLC who presented with hyponatremia and elevated ANP levels, one patient showed a decline in aldosterone levels (213-90 pmol/L), one showed an increase (112-165 pmol/L), and another showed no change (171-171 pmol/L). However, none of these three patients achieved aldosterone levels >240 pmol/L, which would have been expected in light of the worsening hyponatremia in each patient. In comparison, the NSCLC patients significantly increased their plasma aldosterone levels during the period of sodium and fluid restriction (146-205 pmol/L; $P = 0.02$; Fig. 5C). Trend analysis for the change in plasma aldosterone levels in NSCLC patients was significant as well ($P = 0.01$).

Patients with SCLC showed no change in the cGMP levels over time (0.62-0.65 pmol/L; $P = 0.5$). In the three patients with SCLC who presented with hyponatremia and elevated ANP levels, one had increasing cGMP levels over time (0.42-0.93 nm/mL), whereas two showed a decline in cGMP (1.63-1.2 and 0.45-0.24 nm/mL). cGMP levels in NSCLC patients did not change over time. Cyclic AMP levels did not change significantly over time in either the SCLC patients or NSCLC patients (data not shown).

Fig. 3. Serum sodium levels in patients with lung cancer during the course of the metabolic study. **A**, the mean serum sodium levels in all patients with SCLC during the 4-day course of 1,000 mL/d fluid and 109 mEq/d sodium diet restriction. **B**, the mean serum sodium levels in all patients with NSCLC over time. **C**, the serum sodium in four patients who presented with SCLC and hyponatremia declined over the course of study. Three of these patients had ANP levels >15 pmol/L. **D**, serum sodium levels in two patients with SCLC and hyponatremia who had inappropriately elevated AVP levels in relation to serum osmolality, demonstrating correction to normal serum sodium following fluid restriction.



Patients with SCLC and NSCLC showed a mean 1.7 and 1.4 kg weight loss, respectively, during the metabolic study. Systolic blood pressures at day 1 were not different between SCLC patients and NSCLC patients in the supine or seated position, but the systolic blood pressure was significantly higher (mean, 153) in the patients with SCLC in the standing position compared with the NSCLC patients (mean, 128; $P = 0.01$). There was no difference in diastolic blood pressures between the patient groups in any orthostatic position. Patients with SCLC and NSCLC showed no differences in resting pulse at day 1 or following metabolic study (data not shown). Both SCLC and NSCLC patients were orthostatic by pulse change >10 bpm on admission (mean change of 13.0 and 15.7 bpm, respectively) and these orthostatic states worsened in both groups following fluid and sodium restriction (mean, 21.3 versus 19.6 bpm, respectively; $P = 0.8$). The weight and orthostatic changes observed within the three SCLC patients with hyponatremia and elevated ANP were not significantly different than the other SCLC patients (data not shown). These data indicated that the majority of patients with SCLC and NSCLC have orthostatic changes at presentation, but no differences between these groups were observed following fluid and sodium restriction. Of note, we did observe a positive correlation between ANP at enrollment and the change in orthostatic pulse over time in both patients with SCLC and NSCLC (Spearman correlation coefficient = 0.68, $P = 0.012$; Fig. 6). In both SCLC patients and NSCLC patients, the level of ANP at enrollment predicted for worsening orthostatic changes over the course of the study, indicating that elevated ANP levels could cause clinically important hemodynamic abnormalities in patients with lung cancer.

Discussion

In this prospective study, seven of nine patients with SCLC had serum sodium levels <135 mEq/L at presentation. Among these patients, we identified two patients with elevated plasma AVP levels (>40 fmol/L) who corrected to normal sodium levels

after 4 days of fluid restriction. Conversely, three patients with SCLC, hyponatremia and elevated ANP levels (>15 pmol/L) in the absence of increased AVP, showed persistent decline in serum sodium levels following fluid restriction. We were also able to confirm that tumor cells from one patient with SCLC, plasma ANP, 30.8 pmol/L, and sodium of 131 mEq/L on admission, expressed ANP mRNA in the absence of AVP expression, suggesting a causal relationship between tumor-produced ANP and clinically significant hyponatremia. This patient showed declining serum sodium levels (131 mEq/L at day 0 and 128 mEq/L at day 4) and persistent natriuresis despite fluid and sodium restriction. These data indicate that ANP produced ectopically can cause inappropriate natriuresis and hyponatremia in patients with SCLC.

Despite previous reports which have shown the ectopic tumor production of ANP mRNA (14, 15), ANP immunoreactivity in tumor cells and supernatant (16, 17), and reports of resolution of hyponatremia following surgical resection of ANP expressing tumors (16), no consistent prospective associations between elevated plasma ANP and hyponatremia have been reported in patients with lung cancer (4, 15, 16). Two studies of ANP in plasma and tumor cell lines showed no relationship between high levels of ANP and hyponatremia in patients with lung cancer (15, 16). This has been explained, in part, by the potential effect which sodium intake has on ANP levels, causing wide variations in previously studied patients (22, 23). In our previous study, we found that all 10 SCLC patients (of the 31 total) who presented with hyponatremia also had inappropriately elevated levels of AVP (4). Initial ANP levels were not associated with hyponatremia in those patients, but we did observe that urinary sodium increased during saline infusion proportionally to their initial plasma ANP levels (4). Taken together, these studies suggested that the contribution of ANP and AVP in patients with lung cancer might be more sensitively measured by controlling both the sodium and fluid intake and measuring sodium homeostasis in that setting.

By restricting patients in this study to 1,000 mL/d fluid restriction and a 109 mEq/d sodium diet, we were able to discriminate the metabolic responses of patients with elevated AVP levels versus patients with ANP excess. Interestingly, preclinical studies have shown that sodium-restricted diets significantly decrease the plasma levels of ANP in rats after 1 or 3 weeks (36). Sodium restriction in patients with thyroid disease also decreased plasma ANP levels, but this did not reach statistical significance (37). In this study, three patients with SCLC presented with hyponatremia and elevated ANP levels and, after 4 days of sodium restriction, no significant declines in plasma ANP levels could be detected, suggesting ectopic production of ANP in these patients. Other experimental models of the syndrome of inappropriate antidiuretic hormone have shown that hyponatremia was primarily caused by AVP binding to the V_2 receptor, which increased the free water retention in the kidney, rather than natriuresis (38), in the setting of uncontrolled fluid intake (29, 39, 40). Not surprisingly, the two SCLC patients in this study with elevated AVP levels corrected their hyponatremia in response to fluid restriction. Conversely, ANP has been shown to increase the renal excretion of sodium mediated by the ANP receptors in the kidney. This occurs via increased production of cGMP which increases the glomerular filtration rate, inhibiting renin release in the juxtaglomerulosa cells, and inhibiting sodium and water resorption in the collecting duct (1, 2, 22, 23, 40). ANP infusion over 2 to 4 days has also been shown to decrease the production of renin, angiotensin II, and aldosterone (22, 23, 41). Therefore, it has been postulated that ectopically produced ANP could contribute to hyponatremia by causing natriuresis, negative sodium balance, and nonosmotic release of AVP secondary to decreased intravascular volume (4). In our previous prospective study of patients with lung cancer and hyponatremia, we observed no correlation between the plasma ANP levels and levels of renin, angiotensin II, or aldosterone (4). We concluded, based

on those results, that ANP was unlikely to contribute to hyponatremia via suppression of the renin-angiotensin II-aldosterone axis. In this study, we found that all four patients with SCLC and elevated ANP levels had aldosterone levels <240 pmol/L, and this included three patients who presented with hyponatremia. These three patients showed persistent natriuresis and decreasing serum sodium levels despite sodium-restricted diet and their aldosterone levels remained <240 pmol/L. These results suggest that physiologic aldosterone response to hyponatremia, sodium restriction, and natriuresis failed to occur in patients with elevated ANP. Therefore, ANP may mediate hyponatremia, in part, via suppression of an aldosterone response. Metabolic studies of more prolonged duration might allow further delineation of the effect of ectopic ANP on the aldosterone axis, but such studies are difficult to justify in patients with newly diagnosed lung cancer awaiting treatment.

We also examined the levels of cyclic GMP in patients' urine as a secondary indicator of ANP activity (35). We did not find elevations in cGMP in SCLC patients who had elevated plasma levels of ANP. Similarly, the two patients with SCLC who presented with hyponatremia and elevated AVP showed only low levels of cAMP in their urine (<2 pmol/L). Experimental studies have suggested that cyclic GMP levels in the urine can be blunted in normal volunteers after prolonged infusion of ANP and in the presence of persistent elevations in plasma ANP levels (34). It has been proposed that the blunting of the cGMP response is caused by receptor down-regulation or inhibition of cGMP formation secondary to chronic ANP exposure (34). The low levels of cGMP and cAMP measured in the urine of SCLC patients in this study may therefore have been secondary to the patients' chronic exposure to ectopic ANP and AVP well prior to the diagnosis. Similarly, hemodynamic changes were not significantly worse in the SCLC patients who had hyponatremia and elevated ANP levels at presentation as compared with the other SCLC patients, suggesting that these patients may have

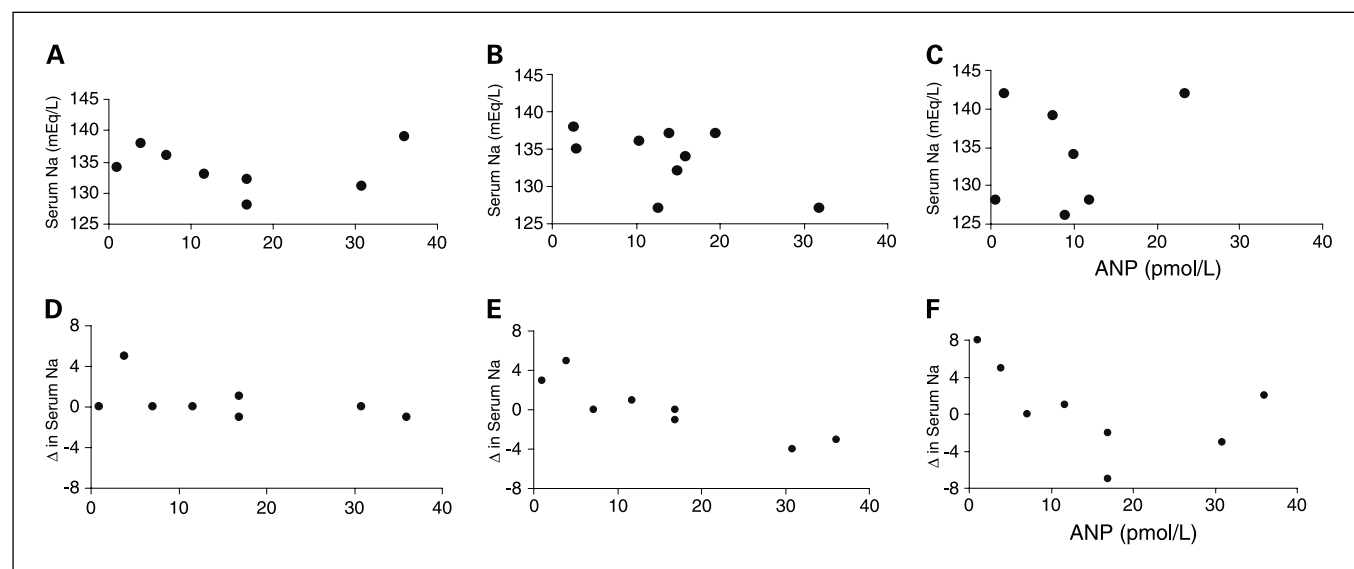


Fig. 4. Serum sodium versus plasma ANP levels over time in patients with lung cancer. *A-C*, plot of serum sodium versus plasma ANP levels on days 1 to 3 in patients with SCLC. The plots suggest a trend toward a linear association between increasing ANP levels and decreasing serum sodium in patients enrolled, but this did not reach significance. *D-F*, scatter plot showing inverse correlation between ANP levels at admission versus serum sodium changes over time. Note that a significant inverse correlation is shown in (*E*), which illustrates the change in serum sodium from days 2 to 3.

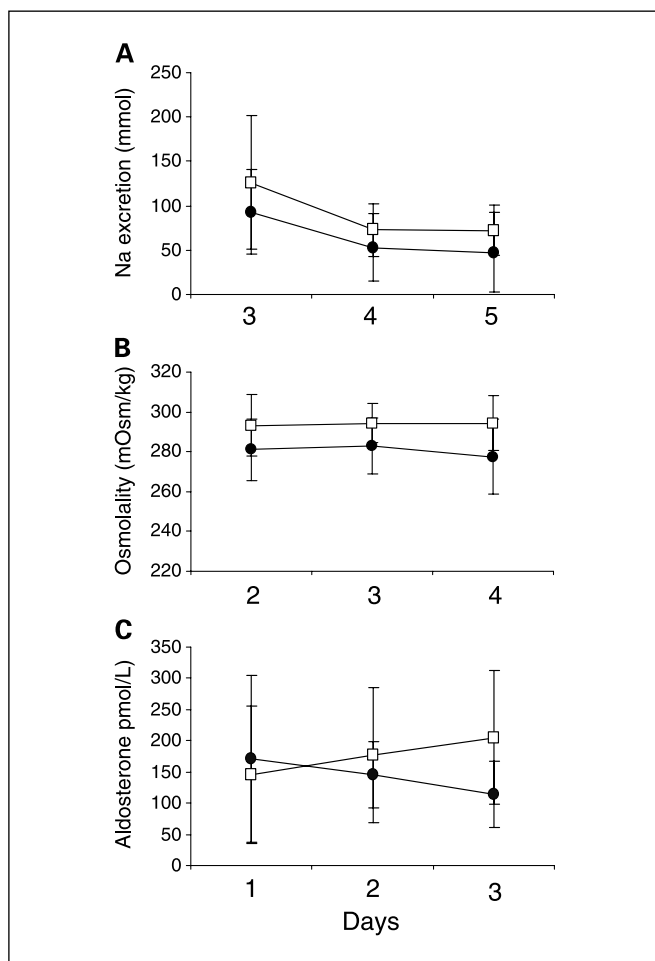


Fig. 5. Metabolic parameters over time in patients with lung cancer. *A*, the mean urinary sodium excretion during days 3 to 5 of sodium and fluid restriction in all SCLC patients (●) and NSCLC patients (□). *B*, serum osmolality over time in all patients with SCLC and NSCLC. *C*, plasma aldosterone levels in patients with SCLC and NSCLC over time, showing an appropriate increase in aldosterone levels in NSCLC patients in response to sodium restriction, whereas SCLC patients showed a pathologic decline in aldosterone levels over time.

accommodated the effects of ectopic ANP over time prior to enrollment. Nonetheless, both SCLC and NSCLC patients showed worsening hemodynamic changes during the course of the metabolic study, which positively correlated with elevated ANP levels at enrollment, indicating that patients with elevated ANP levels are susceptible to clinically important hemodynamic deficits.

The continued definition of the syndrome of "antidiuretic hormone" has been aided recently by the development of antagonists to AVP that block V_2 receptors on the renal collecting ducts (42). Several preclinical studies and phase I and II clinical trials have now been completed and several V_2 receptor antagonists have been shown to be safe and effective in promoting free water excretion and increases in serum sodium in patients with SIADH following a single dose (43–45). The receptor for ANP has also been identified (46, 47). ANP binding of the guanylyl cyclase A receptor leads to an increase in the intracellular second messenger, cGMP (47). However, development and testing of ANP receptor antagonists have not been extensively done. The role of ectopic ANP production in patients

with hyponatremia of malignancy may be established definitively in the future by the administration of ANP receptor antagonists to patients such as those described in this study.

The results of this metabolic study provide compelling evidence that: (a) hyponatremia discovered in SCLC patients is frequently associated with inappropriate elevations in ANP rather than elevations of AVP, (b) SCLC patients with hyponatremia and elevated ANP levels do not respond to fluid restriction; fluid restriction may worsen hyponatremia in such patients if sodium intake is not concomitantly increased, (c) ANP seems to mediate its natriuretic effect, at least in part, by suppression of aldosterone. In the original description of SIADH by Bartter and Schwartz (48), the authors proposed three potential factors which could explain sodium loss: a factor which causes (a) suppression of aldosterone secretion resulting from an increase of extracellular fluid volume, (b) an increase in the filtered load of sodium resulting from an increase in the glomerular filtration rate, and (c) suppression of tubular reabsorption of sodium in response to expansion of the extracellular fluid volume. ANP is known to increase the glomerular filtration rate and inhibits sodium reabsorption into the renal tubules (22, 23), thereby fulfilling two of the original criteria of Bartter and Schwartz. Although further studies will be required to define the suppressive effects of ectopic ANP on aldosterone response, we have shown for the first time, that lung cancer patients with hyponatremia and elevated ANP have pathologic natriuresis as the cause of hyponatremia. Fluid restriction worsens this defect. The appropriate treatment for patients with SIADH and hyponatremia should therefore be different than for patients with SIADH. In patients who present with SCLC and hyponatremia, the syndrome of inappropriate ANP should be considered as well as the possibility that ANP and AVP may both be contributing to the etiology of hyponatremia of lung cancer. In practice, we recommend that the treating physician should apply fluid restriction initially in newly diagnosed patients with SCLC who present with hyponatremia. However, if the hyponatremia fails to improve or worsens after 72 to 96 hours of fluid restriction, plasma levels of AVP and ANP should be measured to determine whether SIADH or SIADH is the causative syndrome.

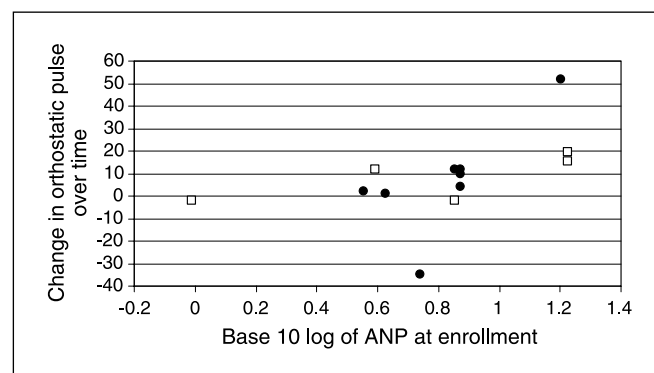


Fig. 6. Positive correlation between ANP levels and orthostatic hemodynamic changes in SCLC and NSCLC patients. Along the Y axis, changes in orthostatic pulse over time are shown for all measurable patients with SCLC and NSCLC as indicated. Along the X axis, the base 10 log of ANP at enrollment is shown for these patients. A positive correlation is identified between the ANP levels at enrollment and the change in orthostatic pulse measurement over time in all patients (Spearman correlation coefficient 0.68, $P = 0.012$).

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