Serum Chloride Is an Independent Predictor of Mortality in Hypertensive Patients

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Abstract—Chloride (Cl^-) is the major extracellular anion in the body, accompanying sodium (Na^+), and is primarily derived from dietary sources. Data suggest that increased dietary Cl^- intake increases blood pressure, yet paradoxically, higher serum Cl^- appears associated with lower mortality and cardiovascular risk. This implies that serum Cl^- also reflects risk pathways independent of blood pressure, serum Na^+, and bicarbonate (HCO_3^-). We analyzed 12 968 hypertensive individuals followed up for 35 years, using Cox proportional hazards model to test whether baseline serum Cl^- was an independent predictor of mortality. To distinguish the effect of Cl^- from Na^+ and HCO_3^-, we adjusted for these electrolytes and also performed the analysis stratified by Na^+/HCO_3^- and Cl^- levels. Generalized estimating equation was used to determine the effect of baseline Cl^- on follow-up blood pressure. The total time at risk was 197 101 person-years. The lowest quintile of serum Cl^- (<100 mEq/L) was associated with a 20% higher mortality (all-cause, cardiovascular and noncardiovascular) compared with the remainder of the subjects. A 1 mEq/L increase in serum Cl^- was associated with a 1.5% (hazard ratio, 0.985; 95% confidence interval, 0.98–0.99) reduction in all-cause mortality, after adjustment for baseline confounding variables and Na^+, K^+, and HCO3$^-$ levels. The group with Na^+>135 and Cl^->100 had the best survival, and compared with this group, the Na^+>135 and Cl^-<100 group had significantly higher mortality (hazard ratio, 1.21; 95% confidence interval, 1.11–1.31). Low, not high Serum Cl^- (<100 mEq/L), is associated with greater mortality risk independent of obvious confounders. Further studies are needed to elucidate the relation between Cl^- and risk. **(*Hypertension*. 2013;62:836-843.)** ● **Online Data Supplement**

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The pressure natriuresis hypothesis,[1] monogenic forms of hypertension,[2] and the evidence from dietary salt reduction[3–5] all point to Na^+ as a major determinant of blood pressure (BP) and by extension mortality. Cl^- is the major extracellular anion accompanying Na^+ and is primarily derived from dietary sources. This has resulted in the commonly held view that any clinical relevance of the independent effect of Cl^- on BP and prognosis is largely academic.[6] However, there is a large body of evidence in animals and humans suggesting that the rise of BP in response to salt intake may be more specific to the anionic component namely Cl^- instead of Na^+.[7–13] Also, there is growing evidence that rather than being an inert bystander in electrochemical equilibrium across cell membranes, chloride movement across the cell plasma membrane is involved in regulating cell volume, transepithelial fluid transport, smooth muscle cell contraction, and synaptic transmission.[14] There is also evidence that Cl^- plays a role in inflammation with thick ascending limb of Henle cells showing an upregulation of cyclo-oxygenase-2 in low Cl^- solution and tumor necrosis factor-α–induced inflammatory response partly associated with low intracellular Cl^-.[15,16] In the kidneys, extracellular Cl^- plays a pre-eminent role in the macula densa regulation of renin secretion.[17] Recently, blockade of vascular endothelial growth factor receptor 3 was shown to be associated with selective Cl^- accumulation in the skin of mice along with increased BP, and this was independent of Na^+ and water content.[18] Changes in serum Cl^- occur with concurrent changes in serum Na^+, potassium (K^+), and HCO_3^-, and it has been difficult to disentangle the independent effects of Cl^- on outcomes. In a Belgian general population cohort (n=9106) followed up

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for 10 years, serum Cl^- <100 mEq/L was associated with a 48% (hazard ratio [HR], 1.48; 95% confidence interval [CI], 1.01–2.19) higher risk of all-cause mortality after adjustment for age, body mass index (BMI), and serum Na^+ levels.[19] Interestingly, a post hoc analysis of the CHARM (Candesartan in Heart Failure-Assessment of Reduction in Mortality and Morbidity) study to identify novel prognostic markers in heart failure found that Cl^- was the best predictor of all-cause mortality in heart failure patients with a adjusted HR of 0.78 (95% CI, 0.71–0.85) per SD increase in serum Cl^-.[20] This implies that serum Cl^- predicts risk independently of BP and serum Na^+. Despite the involvement of Cl^- in a variety of physiological functions in the human body and its potential association with BP, the epidemiological significance of Cl^- in relation to long-term mortality outcomes has not been studied in detail. In this study, association of serum Cl^- with cause-specific mortality independent of other electrolytes and BP was tested in a large hypertensive cohort followed up for 35 years.

Methods

Study Setting and Study Population

The study setting and population are explained in detail elsewhere.[21] Briefly, the Glasgow blood pressure clinic provides secondary and tertiary level service to individuals with hypertension from the West of Scotland. Data from all patients attending the clinic at every visit are stored in a computerized database that contains information on individuals attending the clinic from the early 1970s until 2011. A detailed study flowchart is presented in Figure S1 in the online-only Data Supplement. All patients are treated at Glasgow blood pressure clinic until they achieve target BP, after which continued care was offered either in the clinic or in primary care. Use of the anonymized database for analyses is approved by the West of Scotland research ethics service of the National Health Service.

Clinical Measurements

BP measurements were taken manually 3 times, using calibrated mercury sphygmomanometers at each visit by specialist hypertension nurses; the mean of the last 2 measurements was recorded at each visit. Patients attending the clinic were advised to take their regular medications as usual before clinic visit. Height and weight of all patients were measured using standardized equipment during each visit. Blood samples were collected at baseline and at regular intervals for estimation of routine hematologic and biochemical indices, including renal function tests. All biochemical investigations were performed, at the Western Infirmary clinical laboratory service, on blood samples obtained at the first visit as part of routine screening. Serum Chloride was measured using Technicon SMAC continuous-flow analyzer and ion selective electrodes in the 1970s. From mid-1980s, ion selective electrodes were routinely used for chloride measurements. While the chloride assays were performed over a long-time scale, the assays were performed in the certified hospital laboratories on automated analyzers with robust attention to external quality control schemes (NEQAS [National External Quality Assessment Service]), and the normal range of chloride reported by the laboratories has not changed. A structured format was used to assess tobacco (any versus none) and alcohol use (quantity and frequency of consumption). All data were electronically captured and maintained as a large single database.

Outcome Assessment

Records kept by the General Register Office for Scotland ensured notification of a subject's death (provided that it occurred in the United Kingdom) together with the primary cause of death according to the *International Classification of Diseases*, *10th Revision*, Version for 2007 (ICD-10), codes. We considered cardiovascular disease (CVD) deaths (ICD-10 codes I00–I99), ischemic heart disease deaths (ICD-10 codes 120–I25), and stroke deaths (ICD-10 codes I60–I69) in the analysis. Deaths other than attributable to CV causes were classified as non-CVD deaths. Mortality data were collected up to April 2011, allowing a maximum of 35 years for participants who had been under follow-up for the longest time.

Statistical Methods

All analyses were restricted to hypertensive individuals in the database with serum Cl^- assessed at the registration visit (n=12 968). Analyses were initially performed on the measured serum electrolytes and then on adjusted serum Cl^- and HCO_3^- values (after correcting for free water changes). In the presence of free water disturbances, which are common in treated hypertensive patients, serum Na^+, Cl^-, and HCO_3^- concentrations are expected to move concordantly, except when a competing acid–base disorder is present. Adjusted Cl^- and HCO_3^- were calculated using the method of Feldman et al.[22] The ratio of serum Cl^- to Na^+ in normal controls was 0.76±0.02,[22] and from this it can be estimated that the fall (rise) in serum Cl^- with a water excess (deficit) should be close to three-fourths the fall (rise) in serum Na^+. Thus, adjusted serum Cl^- ($Cl^-_{adjusted}$)=measured Cl^-+0.76×(140−measured Na^+). Similarly, the fall (rise) in serum HCO_3^- with a water excess (deficit) should be close to one-fifth the fall (rise) in Na^+, using the ratio of HCO_3^- to Na in normal controls of 0.19±0.01.[22] Accordingly, adjusted serum HCO_3^- ($HCO_{3\ adjusted}^-$)= measured HCO_3^-+ΔAnion Gap+0.19×(140−measured Na^+), where ΔAnion Gap is the difference between the patient's anion gap and the normal anion gap (8 mEq).

The study population was divided into 5 groups based on quintiles of serum Cl^-. The characteristics of the study population across these 5 groups were compared using 1-way ANOVA for continuous variables and χ^2 test for categorical variables. Spearman correlation coefficients were generated to study the overall association between serum electrolytes in the whole cohort and among individuals who were on treatment with diuretics.

The risks of cause-specific mortality (all-cause, CVD, ischemic heart disease, stroke, and non-CVD mortality) were assessed separately. We selected the predictors in our Cox regression models if they crossed a threshold P value <0.1. Our initial model included age, sex, BMI, smoking status, systolic BP (SBP), diastolic BP (DBP), alcohol use, estimated glomerular filtration rate, and CVD (which included diabetes mellitus and prevalent CVD). From these, age, sex, BMI, smoking status, SBP, diastolic BP (DBP), alcohol use, and estimated glomerular filtration rate were the significant predictors that were included in the analyses. All models were adjusted for a variable called epochs, and it represented the year of first or the baseline visit. This variable was used to adjust the secular trend in mortality and was divided into 5 categories (first visit 1977 or before, between years 1978 and 1985, 1986 and 1993, 1994 and 2001, 2002 and thereafter). Initially, serum Cl^- was assessed as a quantitative trait (model 1). Serum Cl^-, SBP, and DBP were considered as time-dependent variables. A second model was generated after adding anion gap as a predictor variable to model 1 (model 2). The anion gap variable in model 3 was replaced with individual serum electrolytes values (model 3). Model 4 contained the same covariates as 3, with the exception of Cl^- that was coded as quintiles. Finally, model 4 was modified by inclusion of baseline serum albumin (model 5). The Kaplan–Meier (KM) and Cox proportional hazards (Cox-PH) models were repeated by replacing the measured serum Cl^- in quintiles with serum $Cl^-_{adjusted}$ and serum $HCO_{3\ adjusted}^-$ in quintiles. Given the potential for a nonlinear relationship of serum Cl^- with time to mortality, regression spline Cox-PH models were also set up to smoothen the hazard functions of each of the predictor variables.

Participants were also categorized into 4 groups based on serum Cl^- and HCO_3^- measured at baseline to examine the effects of both variables on mortality. The 4 groups were classified using Cl^-=100 mEq/L and HCO_3^-=25 mEq/L (median of HCO_3 distribution) as cutoffs. Similarly, 4 groups based on serum Cl^- and Na^+ measured at baseline were defined based on Cl^-=100 mEq/L and Na^+=135 mEq/L (lower normal limit of Na^+).

The survival probabilities of serum Cl^- groups in quintiles and in groups stratified based on serum Na^+ and HCO_3^-/Cl^- levels were studied using both KM and Cox-PH models.

A generalized estimating equation model was used to study the association of baseline serum Cl^- and repeated annual serum Cl^- (and other electrolytes) with change in BP during the follow-up period after adjusting for age, sex, year of first visit, BMI, electrolytes, and estimated glomerular filtration rate . To evaluate the overall adequacy of risk prediction for all-cause and CV mortality, the C-statistics,[23] net reclassification improvement,[24] and integrated discrimination improvement[24] were used. Stata, Version 12.0, Statacorp, TX, was used for all statistical analyses.

Results

Demographics

Baseline characteristics of the 12 968 subjects are presented in Table 1 and the study flowchart in Figure S1. The population was hypertensive (166±29/98±17 mm Hg), middle aged (50.5±14.2 years), and overweight (BMI=27.48±5.66) with approximately equal sex distribution (female, 52%). Forty-five percent were smokers, and 61.3% of the population reported consuming >6 U of alcohol per week. Less than one fifth of the population (18.2%) reported coexisting CVD morbidity at the time of registration. Around one fifth (22.0%) of the population were on diuretics. The achieved SBP and DBP were significantly lower than the baseline BP.

Subjects in the lowest quintile of serum Cl^- (≤100 mEq/L) were older and had higher BP, cholesterol, and CVD prevalence, regardless of HCO_3^- and Na^+ levels (Tables S1–S3). The proportions of individuals with estimated glomerular filtration rate <60 mL/min per 1.73 m^2, and alcohol users, were lowest in the highest quintile of serum Cl^- (Table S1). As expected, serum Na^+ and K^+ showed a positive, and serum HCO_3^- an inverse linear association across serum Cl^- quintiles (P for trend <0.001). Consequently, the mean anion gap was higher at lower levels of serum Cl^- (P for trend <0.001).

The highest Spearman correlation coefficients was observed between serum Cl^- and Na^+ (0.39, P<0.001; Table S4) and HCO_3^- (-0.37, P<0.001). However, adjusting serum Cl^- and HCO_3^- for free water changes resulted in a near perfect correlation (–0.99, P<0.001) between them. The results were similar when stratified by diuretic use.

Association of Serum Cl^- With Longitudinal BP Changes

Though lower serum Cl^- was associated with higher baseline BP, the proportional reduction of achieved BP from baseline BP were similar in all quintiles (Table S1). A more comprehensive analysis of longitudinal BP changes over time using follow-up BP and electrolyte measurements was performed using the generalized estimating equation model in 3591 subjects who had at least 3 annual BP recordings in the first 5 years. After adjustment for conventional covariates including Na^+, K^+, Cl^-, and HCO_3^-, the annual rate of change in BP in the first 5 years of follow-up was –3.95/–2.0 mm Hg (95% CI, –4.1 to –3.8 mm Hg). Serum Cl^- (both baseline and repeated annual) showed no independent effect on SBP change over time. Among the electrolytes, only

Table 1. Characteristics of the Study Population

Variables	Total (N=12 968)	Men (n=6193)	Women (n=6775)
Age at first visit, y	50.55 (14.16)	49.68 (12.94)	51.34 (13.90)
BMI, kg/m^2	27.48 (5.66)	27.49 (5.06)	27.48 (6.16)
SBP, mm Hg	166.22 (29.25)	164.07 (27.41)	168.18 (30.71)
DBP, mm Hg	98.38 (16.97)	99.35 (15.19)	97.50 (18.41)
Total cholesterol, mmol/L	5.95 (1.46)	5.84 (1.58)	6.05 (1.35)
eGFR <60 mL/min per 1.73 m^2, n (%)	2922 (23.70)	1099 (18.70)	1823 (28.26)
Alcohol use, n (%)	7549 (61.31)	4450 (75.73)	3099 (48.15)
Tobacco use, n (%)	5691 (44.80)	2972 (49.08)	2719 (40.91)
CVD, n (%)	2354 (18.15)	1266 (20.45)	1088 (16.06)
Year of first visit			
First visit before and during year 1987, n (%)	6204 (47.94)	3028 (48.97)	3176 (47.94)
First visit between years 1988 and 1997, n (%)	3127 (24.16)	1447 (23.40)	1680 (24.86)
First visit during and after year 1998, n (%)	3610 (27.90)	1709 (27.64)	1901 (28.13)
Serum Na^+, mEq/L	140.04 (3.47)	140.25 (3.16)	139.84 (3.72)
Serum Cl^-, mEq/L	102.67 (3.52)	102.45 (3.47)	102.87 (3.55)
Serum K^+, mEq/L	4.11 (0.73)	4.12 (0.62)	4.10 (0.82)
Serum HCO_3^-, mEq/L	25.92 (3.28)	26.31 (3.21)	25.56 (3.30)
Anion gap	15.81 (3.28)	15.91 (3.20)	15.72 (3.35)
Diuretic use, n (%)	2857 (22.03)	1306 (21.09)	1551 (22.89)
Achieved SBP, mm Hg	151.20 (24.41)	149.68 (22.25)	152.59 (25.34)
Achieved DBP, mm Hg	86.87 (13.03)	90.60 (12.98)	89.20 (13.04)

Data are presented as mean (SD), unless specified otherwise. BMI indicates body mass index; CVD, prevalent cardiovascular disease at baseline; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate based on MDRD (Modification of Diet in Renal Disease) equation; and SBP, systolic blood pressure.

Table 2. Longitudinal Association Between Serum Electrolytes and Systolic Blood Pressure

Serum Electrolytes	GEE β (SBP)*	95% CI β	*P* Value
Serum Cl^-	0.01	−0.19 to 0.21	0.893
Serum Na^+	0.16	−0.03 to 0.35	0.105
Serum K^+	−0.65	−1.82 to 0.53	0.281
Serum HCO_3^-	−0.34	−0.53 to −0.15	0.001

Model includes serum Cl^-, Na^+, HCO_3^- K^+, age, sex, body mass index, epochs, smoking status, alcohol use, and estimated glomerular filtration rate. Number of groups in the analyses were 3599, and median (interquartile range) of number of blood pressure observations in each groups was 5 (4–6). GEE, generalized estimating equation; and SBP, systolic blood pressure.

*Per 1 unit (1 mEq/L) increase in serum electrolytes.

serum HCO_3^- showed a significant independent effect on longitudinal BP, with each unit rise in serum HCO_3^- associated with a 0.34 mm Hg reduction in SBP over 5 years (Table 2). For DBP, serum Cl^- showed no effect whereas serum Na^+, K^+, and HCO_3^- showed small effects on DBP over time (Table 3). The results were similar for both Cl^- and $Cl^-_{adjusted}$.

Association of Serum Cl^- and Mortality Outcomes

The total time at risk was 197 102 person-years with median survival time of 30.3 years and 3434 all-cause deaths. The highest unadjusted event rate was seen in the first quintile (29.4 all-cause deaths/1000 person-years; 18.19 CVD deaths; 11.16 non-CVD deaths; Table S5).

KM survival analysis showed the first quintile of serum Cl^- to be associated with the shortest survival time (log-rank $P<0.001$; Figure S2A). Similar relationship was seen in KM analysis with serum $Cl^-_{adjusted}$ quintiles (Figure S2B). To distinguish whether the Cl^- related risks were specific to Cl^- and not because of the effect of concomitant changes in serum Na^+ and HCO_3^-, univariate survival analyses were performed in groups partitioned by high and low Cl^-, Na^+, and HCO_3^- levels. The KM plots are presented in Figure 1, which shows that individuals with $Cl^- \leq 100$ mEq/L have the lowest survival regardless of Na^+ or HCO_3^- levels (log-rank $P<0.001$).

Multivariate adjusted Cox-PH models showed an inverse association between serum Cl^- and all-cause mortality. A 1 mEq/L increase in serum Cl^- was associated with a 1.5% (HR, 0.985; 95% CI, 0.980–0.990; Table 4) reduction in all-cause mortality, after adjustment for baseline confounding variables and Na^+, K^+, and HCO_3^- levels (model 3). Similar associations were observed for CVD, ischemic heart disease, and non-CVD mortality outcomes. Adjustment for anion gap did not affect the relationship of Cl^- with mortality. The HRs for cause-specific mortality were significantly lower in all other quintiles in comparison to quintile 1 after adjustment for all other factors (model 4). Adjustment for serum albumin in model 5 did not substantially change the relationship. Similar results were observed for CVD mortality as well as non-CVD mortality (Table 4). Regression spline plots in Figure 2 contrast the risk of all-cause mortality attributable to different serum electrolytes.

Table 3. Longitudinal Association Between Serum Electrolytes and Diastolic Blood Pressure

Serum Electrolytes	GEE β (DBP)*	95% CI β	*P* Value
Serum Cl^-	−0.01	−0.11 to 0.10	0.876
Serum Na^+	0.15	0.05 to 0.25	0.003
Serum K^+	−1.23	−1.85 to −0.61	<0.001
Serum HCO_3^-	−0.19	−0.29 to −0.09	<0.001

Model includes serum Cl^-, Na^+, HCO_3^- K^+, age, sex, body mass index, epochs, smoking status, alcohol use, and estimated glomerular filtration rate. Number of groups in the analyses were 3599, and median (interquartile range) of number of blood pressure observations in each groups was 5 (4–6). DBP indicates diastolic blood pressure; and GEE, generalized estimating equation.

*Per 1 unit (1 mEq/L) increase in serum electrolytes.

To understand influence of diuretic use on the association between serum Cl^- and all-cause mortality, the analyses were repeated by including diuretic use as a covariate in all the above models and separately in diuretic users and nonusers at baseline. Including diuretic use as a covariate in all the models had no effect on the results. The adjusted HR for all-cause mortality (model 4) for quintiles 2 to 5 compared with quintile 1 were 0.802 (0.670–0.959), 0.733 (0.601–0.893), 0.824 (0.652–1.041), 0.749 (0.553–1.014), respectively, in diuretic users and 0.743 (0.663–0.883), 0.715 (0.634–0.806), 0.663 (0.576–0.763), 0.762 (0.637–0.911), respectively (similar to the overall analysis). Finally, to address possible confounding attributable to the poor CV risk profile of the lowest Cl^- group (Table S1), the Cox-PH analysis was repeated after excluding deaths occurring in the first 5 years of follow-up. There were 2787 all-cause deaths in the 8484 individuals eligible for this analysis. The adjusted HR for all-cause mortality (model 4) for quintile 2 to 5 compared with quintile 1 were 0.742 (0.671–0.843), 0.744 (0.632–0.778), 0.761 (0.658–0.872), 0.774 (0.665–0.892), respectively (similar to the overall analysis).

Reclassification analyses showed that inclusion of serum Cl^- improved CV mortality risk discrimination by 0.9% over and above conventional CV risk factors when assessed using C-statistics (0.792, 95% CI, 0.781–0.802 versus 0.783, 95% CI, 0.771–0.792; $P<0.001$) or integrated discrimination improvement (0.0096; $P=0.035$). The net reclassification improvement was 4.6% ($P=0.021$).The corresponding statistics for all-cause mortality were (C-statistics=0.777, 95% CI, 0.769–0.785 versus 0.772, 95% CI, 0.0.763–0.781; $P<0.001$; integrated discrimination improvement=0.016, $P<0.001$ and net reclassification improvement=0.023, $P<0.001$; Figure S3).

Discussion

In a large cohort of 12 968 hypertensive patients followed up for 35 years, serum Cl^- <100 mEq/L was an independent predictor of all-cause mortality as well as both CVD and non-CVD mortality and this association was independent of concomitant serum Na^+ and HCO_3^- levels and diuretic use.

Although serum Cl^- is part of the standard screening biochemistry panel in outpatient clinics and is routinely measured in hypertensive patients, it does not feature in routine risk stratification. Our data would suggest that serum Cl^- is a risk marker, and this is supported by other studies. Similar findings to ours were reported in a general population cohort of the Belgian Interuniversity Research on Nutrition and Health (BIRNH) study, who were followed up for 10 years, in which serum Cl^- <100 mEq/L was associated with higher risk of all-cause, CVD, and non-CVD mortality.[19] Consistent findings were also reported in heart failure subjects.[20] Risk discrimination

Table 4. Cox Regression Analysis for the Association Between Serum Cl^- and Mortality

	All-Cause Mortality		CVD Mortality		IHD Mortality		Stroke Mortality		Non-CVD Mortality	
	n=3552/11 051		n=2087/11 051		n=1163/11 051		n=500/11 057		n=1465/11 051	
Serum Cl^-	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Baseline Serum Cl^- (model 1)	0.987*	0.983–999	0.988*	0.983–0.993	0.988*	0.981–0.995	0.996	0.985–1.007	0.985*	0.979–0.991
	n=3392/9037		n=2007/9037		n=1126/9037		n=481/9037		n=1385/9037	
Baseline Serum Cl^- (model 2)	0.988*	0.984–0.992	0.989*	0.984–0.995	0.989*	0.982–0.997	0.996	0.985–1.007	0.986*	0.979–0.992
	n=3392/9037		n=3392/9037		n=3392/9037		n=3392/9037		n=3392/9037	
Baseline Serum Cl^- (model 3)	0.985*	0.980–0.990	0.985*	0.978–0.991	0.985*	0.976–0.995	0.996	0.981–1.010	0.985*	0.977–0.990
Baseline Serum Cl^- (model 4)	n=3392/9037		n=3392/9037		n=3392/9037		n=3392/9037		n=3392/9037	
Serum $Cl^-\leq 100$	1		1		1		1		1	
Serum $Cl^-=101–102$	0.762*	0.692–0.839	0.766*	0.677–0.867	0.759*	0.642–0.896	0.767*	0.591–0.995	0.757*	0.650–0.881
Serum $Cl^-=103–104$	0.723*	0.629–0.800	0.729*	0.638–0.832	0.824*	0.694–0.980	0.724*	0.549–0.955	0.717*	0.610–0.842
Serum $Cl^-=105–106$	0.709*	0.628–0.801	0.708*	0.605–0.827	0.700*	0.566–0.866	0.904	0.666–1.225	0.714*	0.592–0.861
Serum $Cl^-\geq 107$	0.771*	0.661–0.898	0.762*	0.622–0.930	0.758*	0.576–0.998	0.987	0.671–1.453	0.789	0.622–1.000
Baseline Serum Cl^- (model 5)	n=2092/6499		n=1156/6499		n=603/6499		n=304/6499		n=936/6499	
Serum $Cl^-\leq 100$	1		1		1		1		1	
Serum $Cl^-=101–102$	0.729*	0.645–0.825	0.787*	0.670–0.925	0.865	0.693–1.080	0.681*	0.485–0.955	0.659*	0.545–0.799
Serum $Cl^-=103–104$	0.672*	0.587–0.768	0.646*	0.538–0.775	0.713*	0.554–0.917	0.762	0.538–1.081	0.701*	0.575–0.855
Serum $Cl^-=105–106$	0.649*	0.553–0.755	0.667*	0.541–0.822	0.696*	0.519–0.933	0.828	0.559–1.228	0.624*	0.494–0.788
Serum $Cl^-\geq 107$	0.682*	0.561–0.827	0.681*	0.523–0.887	0.698	0.479–1.017	0.956	0.592–1.544	0.684*	0.514–0.911

Model 1 is adjusted for age at first visit, sex, body mass index, baseline cardiovascular disease, chronic kidney disease, tobacco smoking, alcohol use, year of first visit (epochs), systolic blood pressure (SBP), diastolic blood pressure (DBP) (SBP, DBP, and serum chloride as time-dependent variables). Model 2 is adjusted for all variables as in model 1 and anion gap. Model 3 is adjusted for all variables in model 1 and serum Na^+, serum K^+, and serum HCO_3^- as time-dependent variable. Model 4 is same as model 3 but the serum Cl^- was considered as a categorical variable in quintiles. Model 5 is adjusted for all variables in model 4 and baseline serum albumin. CI indicates confidence interval; CVD, cardiovascular disease; HR, hazard ratio; and IHD, ischemic heart disease.

*$P<0.05$.

analyses show that inclusion of serum chloride improved risk discrimination over and above traditional CV risk factors when assessed using C-statistics, net reclassification improvement, and integrated discrimination improvement. Indeed, the net reclassification improvement was 4.6% (P=0.021), and this needs to be validated in independent studies.

The mechanism by which low serum Cl^- increases mortality is unclear. We find that the risk posed by low Cl^- is independent of concomitant Na^+ or HCO_3^- levels, suggesting that this is a Cl^--specific finding and does not reflect risks associated with hyponatremia or acid–base disturbances. As our study population were treated hypertensive patients, confounding attributable to diuretic use is a likely possibility. However, only 22% of our patients were on diuretics at the first visit when serum Cl^- was measured, and the relationship between low Cl^- and mortality persisted even when the analyses were stratified for diuretic use. Moreover, we adjusted Cl^- levels for free water changes that are commonly induced by diuretic use

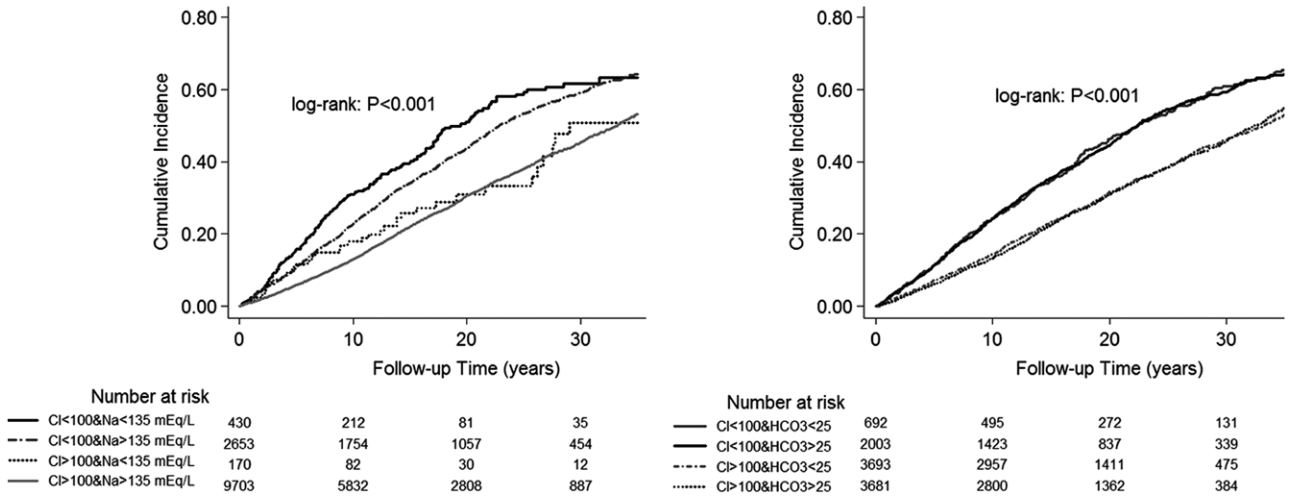

Figure 1. Kaplan–Meier plots for all-cause mortality stratified by Serum Cl^- and Na^+ (**left**) and Serum Cl^- and HCO_3^- (**right**).

and found that the relationship persisted. Only 730 individuals (5% of the entire cohort) showed biochemical evidence of metabolic alkalosis (defined by serum HCO_3^- >30 mEq/L), implying that reverse causation, attributable to severe volume contraction or hyperaldosteronism, is unlikely. Our findings are also supported by the recent report that higher anion gap (which can be associated with low Cl^-) in early chronic kidney disease is a marker of early mortality.[25] High anion gap and the corresponding low serum Cl^- levels are associated with higher BP in a normotensive subset of the NHANES (National Health and Nutrition Examination Survey) study.[26] Our data in treated hypertensive patients also show the same relation between anion gap and serum chloride and between these and baseline BP and achieved BP. To determine that our results are specific to Cl^-, we analyzed groups stratified by the measured anions (Cl^- and HCO_3^-), and showed that Cl^- levels alone are

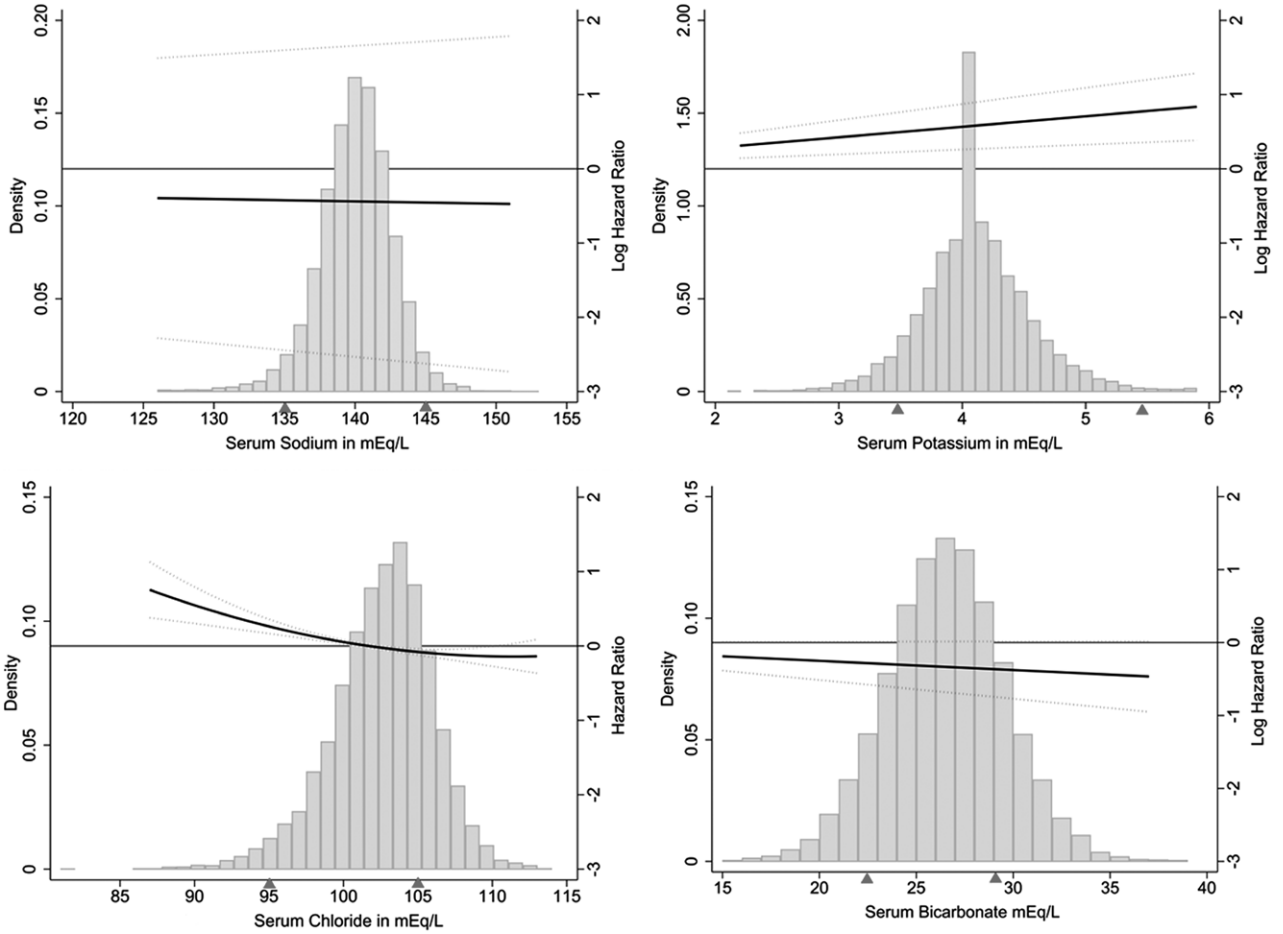

Figure 2. Association between serum Na^+, K^+, Cl^-, HCO_3^- and all-cause mortality—regression spline analyses.

sufficient to classify anybody as high risk without resorting to the calculation of anion gap. This is not contrary to previous studies but reflects the broad range of conditions that can elevate anion gap (metabolic acidosis, hyperalbuminemia, hyperphosphatemia, anionic paraproteinemia, metabolic alkalosis). Additionally, using generalized estimating equation to analyze longitudinal BP changes over 5 years we show that while the annual change in BP was −4 mm Hg, serum Cl^- (both baseline and longitudinal repeat measurements over 5 years) showed no independent effect on BP change over time.

Our findings on serum Cl^- are in contrast to observations on the effect of dietary Cl^-. There are contrasting findings in the literature on the association between dietary serum Cl^- and BP in animal studies with experimental manipulation of diet. While some studies report positive associations,[27–29] others report no significant relationship between dietary Cl^- intake and BP.[30,31] It is also relevant to note that normal BP is a characteristic feature of Bartter syndrome despite its association with selective Cl^- deprivation and salt wasting. It is, however, not clear whether dietary Cl^- increases BP by some mechanisms other than by influencing the renal tubular reabsorption of Cl^-. Emerging evidence that the immune system plays an extrarenal regulatory role in Na^+ homeostasis[32,33] and the intriguing finding that when this immune mechanism was blocked there was selective Cl^- accumulation in the skin salt-sensitive hypertension,[18] would indicate that our findings may have a more complex underpinning mechanism than just renal salt balance.

The strengths of this study include a large cohort of nearly 13 000 hypertensive adults, 35 years of follow-up with median survival time of 32 years, the longitudinal measures of BP and electrolytes. Our study has some limitations: this was an observational study of a treated hypertensive cohort, and hence the results may not be generalizable even though the prevalence of hypertension in the adult population is ≈27%. The exclusion of individuals (≈2000) without serum Cl^- assessed at baseline from our analysis may have resulted in bias. We also do not have any measurements of urinary electrolytes, arterial blood gases, or renin–aldosterone status. We have only analyzed mortality in this study. Finally, residual confounding attributable to unmeasured factors may still exist.

Perspectives

Serum Cl^- is a marker of risk that appears to be dissociated from serum Na^+ and HCO_3^- levels. The underlying mechanism for this risk is unclear. A simple explanation would be that serum Cl^- reflects abnormal physiology better than serum Na^+, levels of which are perhaps more homeostatically regulated than Cl^-. If other studies validate and extend our findings, further studies are now necessary to elucidate the underlying mechanisms involved in the association of low serum Cl^- levels with mortality outcomes. However, as Cl^- measurement is part of routine clinical screening, our results are potentially translatable into clinical practice to identify high-risk hypertensive patients. In view of the inverse linear association between serum chloride level <100 mEq/L and mortality, the normal lower limit of the reference range for serum chloride may be redefined from 95 mEq/L to 100 mEq/L.

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Disclosures

None.

References

1. Guyton AC. Blood pressure control–special role of the kidneys and body fluids. *Science*. 1991;252:1813–1816.
2. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001;104:545–556.
3. Cook NR, Cutler JA, Obarzanek E, Buring JE, Rexrode KM, Kumanyika SK, Appel LJ, Whelton PK. Long term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the trials of hypertension prevention (TOHP). *BMJ*. 2007;334:885–888.
4. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. *J Hum Hypertens*. 2002;16:761–770.
5. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER 3rd, Simons-Morton DG, Karanja N, Lin PH; DASH-Sodium Collaborative Research Group. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med*. 2001;344:3–10.
6. Kaplan NM, Victor RG. *Kaplan's Clinical Hypertension*. 10th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2010.
7. Kurtz TW, Morris RC Jr. Dietary chloride as a determinant of "sodium-dependent" hypertension. *Science*. 1983;222:1139–1141.
8. Kurtz TW, Al-Bander HA, Morris RC Jr. "Salt-sensitive" essential hypertension in men. Is the sodium ion alone important? *N Engl J Med*. 1987;317:1043–1048.
9. Luft FC, Steinberg H, Ganten U, Meyer D, Gless KH, Lang RE, Fineberg NS, Rascher W, Unger T, Ganten D. Effect of sodium chloride and sodium bicarbonate on blood pressure in stroke-prone spontaneously hypertensive rats. *Clin Sci (Lond)*. 1988;74:577–585.
10. Luft FC, Zemel MB, Sowers JA, Fineberg NS, Weinberger MH. Sodium bicarbonate and sodium chloride: effects on blood pressure and electrolyte homeostasis in normal and hypertensive man. *J Hypertens*. 1990;8:663–670.
11. Shore AC, Markandu ND, MacGregor GA. A randomized crossover study to compare the blood pressure response to sodium loading with and without chloride in patients with essential hypertension. *J Hypertens*. 1988;6:613–617.
12. Whitescarver SA, Ott CE, Jackson BA, Guthrie GP Jr, Kotchen TA. Salt-sensitive hypertension: contribution of chloride. *Science*. 1984;223:1430–1432.
13. Wyss JM, Liumsiricharoen M, Sripairojthikoon W, Brown D, Gist R, Oparil S. Exacerbation of hypertension by high chloride, moderate sodium diet in the salt-sensitive spontaneously hypertensive rat. *Hypertension*. 1987;9(6 Pt 2):III171–III175.
14. Duran C, Thompson CH, Xiao Q, Hartzell HC. Chloride channels: often enigmatic, rarely predictable. *Annu Rev Physiol*. 2010;72:95–121.
15. Cheng HF, Wang JL, Zhang MZ, McKanna JA, Harris RC. Role of p38 in the regulation of renal cortical cyclooxygenase-2 expression by extracellular chloride. *J Clin Invest*. 2000;106:681–688.
16. Yang H, Huang LY, Zeng DY, Huang EW, Liang SJ, Tang YB, Su YX, Tao J, Shang F, Wu QQ, Xiong LX, Lv XF, Liu J, Guan YY, Zhou JG. Decrease of intracellular chloride concentration promotes endothelial cell inflammation by activating nuclear factor-κB pathway. *Hypertension*. 2012;60:1287–1293.
17. Lorenz JN, Weihprecht H, Schnermann J, Skøtt O, Briggs JP. Renin release from isolated juxtaglomerular apparatus depends on macula densa chloride transport. *Am J Physiol*. 1991;260(4 pt 2):F486–F493.
18. Wiig H, Schröder A, Neuhofer W, et al. Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. *J Clin Invest*. 2013;123:2803–2815.

19. De Bacquer D, De Backer G, De Buyzere M, Kornitzer M. Is low serum chloride level a risk factor for cardiovascular mortality? *J Cardiovasc Risk*. 1998;5:177–184.
20. Felker GM, Allen LA, Pocock SJ, Shaw LK, McMurray JJ, Pfeffer MA, Swedberg K, Wang D, Yusuf S, Michelson EL, Granger CB; CHARM Investigators. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. *J Am Coll Cardiol*. 2007;50:40–47.
21. Paul L, Jeemon P, Hewitt J, McCallum L, Higgins P, Walters M, McClure J, Dawson J, Meredith P, Jones GC, Muir S, Dominiczak AF, Lowe G, McInnes GT, Padmanabhan S. Hematocrit predicts long-term mortality in a nonlinear and sex-specific manner in hypertensive adults. *Hypertension*. 2012;60:631–638.
22. Feldman M, Soni NJ, Dickson B. Use of sodium concentration and anion gap to improve correlation between serum chloride and bicarbonate concentrations. *J Clin Lab Anal*. 2006;20:154–159.
23. Harrell FE Jr, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. *JAMA*. 1982;247:2543–2546.
24. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med*. 2008;27:157–172; discussion 207.
25. Abramowitz MK, Hostetter TH, Melamed ML. The serum anion gap is altered in early kidney disease and associates with mortality. *Kidney Int*. 2012;82:701–709.
26. Taylor EN, Forman JP, Farwell WR. Serum anion gap and blood pressure in the national health and nutrition examination survey. *Hypertension*. 2007;50:320–324.
27. Schmidlin O, Tanaka M, Bollen AW, Yi SL, Morris RC Jr. Chloride-dominant salt sensitivity in the stroke-prone spontaneously hypertensive rat. *Hypertension*. 2005;45:867–873.
28. Tanaka M, Schmidlin O, Yi SL, Bollen AW, Morris RC Jr. Genetically determined chloride-sensitive hypertension and stroke. *Proc Natl Acad Sci U S A*. 1997;94:14748–14752.
29. Schmidlin O, Tanaka M, Sebastian A, Morris RC Jr. Selective chloride loading is pressor in the stroke-prone spontaneously hypertensive rat despite hydrochlorothiazide-induced natriuresis. *J Hypertens*. 2010;28:87–94.
30. Boegehold MA, Kotchen TA. Importance of dietary chloride for salt sensitivity of blood pressure. *Hypertension*. 1991;17(1 suppl):I158–I161.
31. Wyss JM, Liumsiricharoen M, Sripairojthikoon W, Brown D, Gist R, Oparil S. Exacerbation of hypertension by high chloride, moderate sodium diet in the salt-sensitive spontaneously hypertensive rat. *Hypertension*. 1987;9(6 pt 2):III171–III175.
32. Machnik A, Dahlmann A, Kopp C, Goss J, Wagner H, van Rooijen N, Eckardt KU, Müller DN, Park JK, Luft FC, Kerjaschki D, Titze J. Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhancer binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. *Hypertension*. 2010;55:755–761.
33. Machnik A, Neuhofer W, Jantsch J, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med*. 2009;15:545–552.

Novelty and Significance

What Is New?

- Serum Cl^- is an independent predictor of mortality in hypertensive patients irrespective of serum Na^+ or HCO_3^- levels.

What Is Relevant?

- Serum Cl^- levels (<100 mEq/L) are associated with higher blood pressure and higher mortality risk independent of other electrolytes and cardiovascular risk factors.

Summary

Serum Cl^- levels (<100 mEq/L) identify high-risk patients. Further studies are required to elucidate the mechanism underpinning this increased risk.