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Strong ion and weak acid analysis in severe preeclampsia: potential clinical significance†

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Abstract

Background: The influence of common disturbances seen in preeclampsia, such as changes in strong ions and weak acids (particularly albumin) on acid-base status, has not been fully elucidated. The aims of this study were to provide a comprehensive acid-base analysis in severe preeclampsia and to identify potential new biological predictors of disease severity.
Methods: Fifty women with severe preeclampsia, 25 healthy non-pregnant- and 46 healthy pregnant controls (26–40 weeks′ gestation), were enrolled in this prospective case-control study. Acid-base analysis was performed by applying the physicochemical approach of Stewart and Gilfix.
Results: Mean [SD] base excess was similar in preeclamptic- and healthy pregnant women (−3.3 [2.3], and −2.8 [1.5] mEq/L respectively). In preeclampsia, there were greater offsetting contributions to the base excess, in the form of hyperchloraemia ($BE_{(Cl)}$ −2 [2.3] *vs* −0.4 [2.3] mEq/L, $P<0.001$) and hypoalbuminaemia ($BE_{(Alb)}$ 3.6 [1] *vs* 2.1 [0.8] mEq/L, $P<0.001$). In preeclampsia, hypoalbuminaemic metabolic alkalosis was associated with a non-reassuring/abnormal fetal heart tracing ($P<0.001$). Quantitative analysis in healthy pregnancy revealed respiratory and hypoalbuminaemic alkalosis that was metabolically offset by acidosis, secondary to unmeasured anions and dilution.
Conclusions: While the overall base excess in severe preeclampsia is similar to that in healthy pregnancy, preeclampsia is associated with a greater imbalance offsetting hypoalbuminaemic alkalosis and hyperchloraemic acidosis. Rather than the absolute value of base excess, the magnitude of these opposing contributors may be a better indicator of the severity of this disease. Hypoalbuminaemic alkalosis may also be a predictor of fetal compromise.
Clinical trial registration: clinicaltrials.gov: NCT 02164370.

Key words: acid-base balance; pre-eclampsia; pregnancy

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Editor's Key points

- Preeclampsia remains a challenging condition to manage to achieve best fetal and maternal outcomes.
- Defined biochemical changes that could be used to guide management would have clinical utility.
- This study explores using changes in acid-base status as a predictive biomarker of disease severity.
- Preeclamptic, and healthy women had similar base excess but greater hypoalbuminaemic alkalosis and hyperchloraemic acidosis.
- This preliminary work needs to be confirmed in a larger study.

Introduction

Preeclampsia is a major cause of maternal mortality worldwide[1] and is characterized by multi-organ involvement leading to acute and long-term morbidity of mother and newborn.[2 3] In early onset preeclampsia (<34 weeks gestation), expectant management is usually attempted, and fetal lung maturation with a course of 48 h steroids is standard of care.[4] To date, there are few specific biochemical predictors of disease severity, or criteria to guide clinicians in their decision to offer expectant management *vs* prompt delivery. Management is mostly guided by expert opinion-based guidelines.[5]

In other clinical arenas, acid/base measures have been powerful tools in predicting outcomes.[6–8] However, studies evaluating acid-base imbalances in preeclamptic women are scarce, and to our knowledge no comprehensive analysis of independent factors determining acid/base status has been performed. In a previous study, Dyer and colleagues[9] found a mean arterial base excess (BE) of −6.6 [2.8] mEq/L in preeclamptic women undergoing urgent Caesarean delivery for a non-reassuring fetal heart trace, which is greater than the base excess described in healthy pregnancy. This might indicate maternal abnormalities of acid-base status in preeclampsia. As in previous investigations on acid-base status in preeclampsia, these authors focused mainly on describing plasma pH, HCO_3, base excess (BE) or the anion gap (AG).[10–12] Although sufficient to simply describe an alteration in acid/base status in uncomplicated patients, in critically ill patients simultaneous acid/base derangements may offset each other and ultimately lead to a minimally deranged pH, HCO_3, BE and AG.[13–16] Thus conventional analysis may overlook important and informative pathologic processes. A quantitative physicochemical approach analyses the difference in strong plasma cations and anions, the concentration of weak acids (mainly albumin and phosphate), and the Pco_2.[17–19] By applying this approach, multiple severe acid-base disorders have been demonstrated in various disease processes, despite normal pH and BE.[13–15 20 21] As changes in albumin, volume status, and sodium or chloride homeostasis are common in preeclampsia,[1] we hypothesized that they significantly alter acid-base status, but may go unrecognized because of offsetting effects. Therefore, the primary aim of this study was to analyse acid/base balance in women with severe preeclampsia, by applying the physicochemical methods of Stewart[17] and Gilfix.[19] The secondary aim was to identify potential biological predictors for adverse maternal and neonatal outcomes.

Methods

After approval by two institutional Human Research Ethics Committees, and written informed consent, women diagnosed with severe preeclampsia were enrolled in this prospective case control study. The investigation was conducted at the University of Cape Town (UCT), Cape Town, South Africa, [SA, (#IRB 698/2013)], in collaboration with the University of Washington, Seattle, USA, (#IRB 43603, clinicaltrials.gov: NCT 02164370) in accordance with the Declaration of Helsinki and Good Clinical Practice. This observational study was reported using the STROBE guidelines.[22]

Subjects

Preeclampsia group

Women diagnosed with severe preeclampsia, admitted to the Maternity Centres of UCT, were screened for possible enrolment by one of two study investigators (C.O., B.C.) not providing clinical care. Preeclampsia was defined according to the recommendations of the American College of Obstetricians and Gynaecologists[4] and regarded as severe if the systolic blood pressure exceeded 160 mm Hg and/or the diastolic blood pressure exceeded 110 mm Hg on at least two separate occasions, if symptoms of imminent eclampsia were present, or if proteinuria on urine dipstick was 3+ or more. Early onset disease was defined as diagnosis before 34−, and late onset disease after 34 weeks of gestation.

After informed consent, a blood specimen was drawn at the time of diagnosis, as soon as possible after admission. Blood draws were repeated before delivery in women diagnosed with early onset disease. A second blood sample was obtained at the time of the decision to start induction of labour or to undergo Caesarean delivery, if this was more than 24 h after the initial blood sample was obtained.

Recent evidence showing good correlation between arterial and venous metabolic acid-base status,[23 24] prompted the local institutional review board to request a pilot study of 25 paired arterial and venous blood samples to be analysed. This revealed a mean [SD] venous-arterial difference in pH, Pco_2, PO_2, HCO_3^-, BE and SBE of, respectively, −0.00 [0.01], 0.2 kPa [0.19], −6.0 kPa [3.1], 0.6 mmol/l [0.7], 0.4 mEq/L [0.4], and 0.5 mEq/L [0.7], and yielded a statistically significant difference only in PO_2. Therefore, and in order to reduce parturients' discomfort, it was decided to use venous blood gas data for the analysis.

Antenatal management was according to the established protocol of the local institutions at UCT. At the time of diagnosis of severe preeclampsia, seizure prophylaxis was administered, consisting of magnesium sulphate administered as a loading dose of 4 g i.v., followed by 1 g/h i.v. Magnesium sulphate dissolved in 0.9% normal saline and administered at a rate of 50 ml/h, after the loading dose in a 200 ml bolus. Preeclamptic women were otherwise fluid restricted. Blood pressure was managed according to a standardized protocol, using alpha-methyldopa, nifedipine or dihydralazine, and fetal cardiotocography (CTG) was interpreted according to the guidelines of the Royal College of Obstetricians and Gynaecologists.[25] One non-reassuring (early or variable decelerations, fetal basal heart rate 100–119 or 160–179 beats min^{-1} , or variability less than 5 beats min^{-1} for up to 40 min) and 2 normal/reassuring features on CTG defined category II fetal heart tracing. Two or more non-reassuring features or 1 or more abnormal features on CTG (late or prolonged [>3 min] deceleration, fetal basal heart rate <100 or >180 beats min^{-1}, or variability less than 5 beats min^{-1} for greater than 90 min) defined a category III fetal heart tracing, and was considered to be an indication for Caesarean delivery. The decision to proceed with Caesarean delivery was made by the obstetrics team, independent of the investigators.

Women in labour or unable to understand the study procedure were not included in the study. Women with chronic pulmonary disease, collagen disorders, a history of lithium intoxication, a history of methanol, ethanol or salicylate ingestion, regular ingestion of antacids, chronic renal or hepatic disease, urinary tract infection, chorioamnionitis, intrauterine fetal death, a BMI >50 kg/m^2, or acute asthma, were not considered eligible.

Control groups

Twenty-five healthy non-pregnant controls of childbearing age, and 46 healthy pregnant controls were recruited. Healthy pregnant controls were matched in gestational age by recruiting three women for each week of gestation, ranging from 26 to 40 gestational weeks.

Acid-base analysis

Acid-base analysis was based on Stewart's principle that plasma pH and HCO_3^- are dependent variables of the following four factors:

1. the difference in concentration of fully dissociated cations and anions (=strong ion difference [SID]),
2. the Pco_2,
3. the concentration of non-volatile weak acids (albumin and phosphate), and
4. the presence of unmeasured anions (UMA).

Combining Stewart's principle and the traditional approach in acid-base interpretation, which uses BE to summarize a patient's net metabolic acid-base state, Gilfix[19] sequentially quantified the contribution of plasma dilution/concentration, and changes in chloride, albumin, lactate and unmeasured anions on net BE:

$$BE = BE_{(Na)} + BE_{(Cl)} + BE_{(Alb)} + BE_{(Lac)} + BE_{(UMA)}.$$

Accordingly, BE is defined by five subsets: (1) changes in free water (=$BE_{(Na)}$), (2) changes in Cl^- relative to sodium concentrations (=$BE_{(Cl)}$), (3) changes in albumin concentration (=$BE_{(Alb)}$), (4) changes in lactate concentration (=$BE_{(Lac)}$), and (5) an accumulation of unmeasured anions (=$BE_{(UMA)}$). (Any change in BE not caused by changes in free water, chloride, albumin or lactate is attributed to unmeasured anions. Unmeasured anions, for example, accumulate in renal insufficiency or if there is ketone body excess).

The calculation of the BE subsets and a detailed description of the physico–chemical acid–base analysis is given in the Appendix.

Statistical analysis

Within-group means and standard deviations were used to summarize variables. Student's *t*-tests were used to test for difference between non-pregnant- and healthy pregnant volunteers, and, as a separate test, to evaluate a difference between women with severe preeclampsia and healthy pregnant women. Additional Student's *t*-tests were performed to compare data from patients with early *vs* late onset preeclampsia, and to evaluate a potential association between acid-base parameters and clinical disease features, CTG findings, and delivery outcome.

In order to explore whether results could be related to i.v. magnesium sulphate administered, acid-base variables were compared between women receiving magnesium sulphate before (n=37) and after (n=13) the blood draw, and an analysis of covariance was used to compare groups while controlling for measures of disease severity (blood pressure, symptoms of imminent eclampsia or >3+ proteinuria on urine dipstick).

χ^2 tests were used to test for between-group differences in ethnicity distribution. In order to adjust for multiple testing, an α-value of 0.05 was divided by the number of parameters compared (n=44) and the P-value for statistical significance was adjusted to P<0.001.

Sample size calculation

Sample size was based on the observation in a pilot sample of 25 venous blood specimens in women with severe preeclampsia of mean venous $BE_{(Alb)}$=+4.4 [0.75] mEq/L. Considering a power of 80% and an alpha value of 0.001, a sample size of 25 women in each group was needed to show a difference of 20% in $BE_{(Alb)}$ between the case- and control groups.

Results

Fifty women diagnosed with features of severe preeclampsia were enrolled between December 2013 and April 2014 at UCT, consisting of 25 women with early onset- and 25 with late onset disease (Fig. 1). In addition, 25 healthy non-pregnant- and 46 healthy pregnant controls (26–40 weeks' gestation) were enrolled in this prospective case-control study.

None of the healthy pregnant controls were subsequently diagnosed with preeclampsia. Preeclamptic women and healthy pregnant controls were of similar mean gestational age and showed comparable patient characteristics.

Conventional acid base analysis showed a mean [SD] pH in normal range in healthy pregnancy (7.39 [0.03] and severe preeclampsia (7.41 [0.03], P=0.01) and similar mean base excess (−2.8 [1.5], and −3.3 [2.3] mEq/L respectively, P=0.18). Quantitative analysis using the method of Gilfix showed that, compared with healthy pregnancy, preeclampsia was associated with greater offsetting contributors to the base excess. Hypoalbuminaemic alkalosis, evidenced by $BE_{(Alb)}$, was greater (3.6 [1] mEq/L *vs* 2.1 [0.8] mEq/L, P<0.001). Hyperchloraemic acidosis, evidenced by $BE_{(CL)}$, was also greater (−2 [2.3] mEq/L *vs* −0.4 [2.3] mEq/L, P<0.001) (See Table 1, Fig. 2 for detailed analysis). There was no difference in $BE_{(Na)}$, $BE_{(Lac)}$, and $BE_{(uma)}$. Calculations of SIDa, SIDe and SIG suggest that preeclampsia is a state of a hypoalbuminaemic alkalosis compared with normal pregnancy (P<0.001), and an acidosis secondary to an increase in SIG (P<0.001). The apparent strong ion difference (SIDa) remained unchanged (Table 1, Fig. 3).

No differences were found in acid-base parameters when comparing early *vs* late onset disease. All laboratory values were within normal range in preeclamptic women, except for a decrease in mean albumin level and an increase in uric acid and magnesium (Table 2).

$BE_{(Alb)}$ measured in women with severe preeclampsia that developed a category II or III fetal heart tracing within 24 h of diagnosis, was higher than in women with a category I tracing ($BE_{(Alb)}$ 4.2 [0.86] mEq/L *vs* $BE_{(Alb)}$ 2.9 [0.84] mEq/L, respectively, P<0.0001). Women undergoing an urgent Caesarean delivery had a mean $BE_{(Alb)}$ of 4 [1] mEq/L *vs* a $BE_{(Alb)}$ of 3.2 [1.2] mEq/L in women delivering non-urgently (P=0.02). Plasma albumin level was lower in women developing a category II or III tracing (28.6 [3.0] g/L *vs* 33 [3.0] g/L respectively, P<0.001). $BE_{(Cl-)}$ was −1.17 [2.2] mEq/L in women with a category I CTG compared with a $BE_{(Cl)}$ of −2.4 [2.2] mEq/L in women developing a category II or III CTG (P=0.06). Mean $BE_{(Cl-)}$ was −2.2 [2] mEq/L in women requiring emergency Caesarean delivery within 24 h, compared with −0.8 [2.8] mEq/L in women delivering non-urgently (P=0.05). There

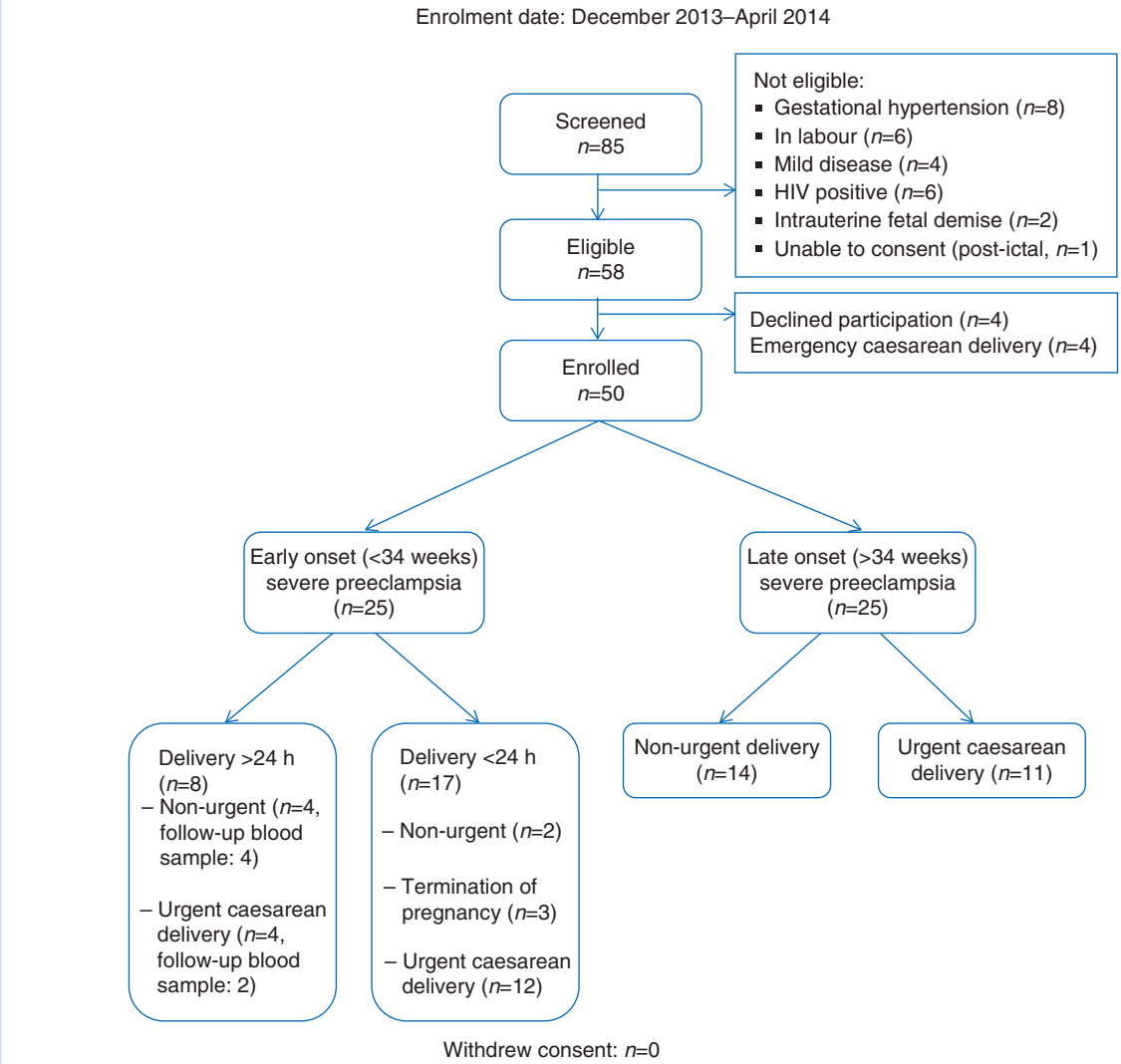

Fig 1 Flow chart showing patient disposition of fifty women diagnosed with severe preeclampsia and enrolled between December 2013 and April 2014 at the University of Cape Town. Among women with early onset preeclampsia (<34 weeks), 17 delivered within 24 h of diagnosis, out of which 12 required an urgent Caesarean delivery, 2 had a non-urgent Caesarean delivery, and 3 had an induction of labour for termination of pregnancy. Expectant management was successful in 8 women, with an average delivery time of 5 days after admission, before which a second blood sample was drawn in 6 women (2 blood samples were missed in women urgently delivering at night). In women with late onset preeclampsia, 11 underwent an urgent Caesarean delivery within 24 h of diagnosis, for either Category III fetal heart rate tracing or worsening maternal symptoms. The remaining 14 women underwent induction of labour and delivered either vaginally or by non-urgent Caesarean delivery.

was no association between $BE_{(alb)}$ or $BE_{(Cl)}$ with any of the features of preeclampsia, such as: arterial blood pressure, proteinuria (dipstick), visual disturbances or eclamptic seizure. Plasma albumin level or proteinuria (dipstick) did not correlate with any clinical disease symptom.

In 13 women, blood samples were drawn before-, and in 37 women after the administration of magnesium sulphate. Women receiving magnesium sulphate before blood draw were younger, of earlier gestational age, more likely nulliparous, had a higher mean arterial blood pressure, and a higher likelihood of presenting with features of severe disease. A comparison of these 2 subgroups showed differences in $PvCO_2$, HCO_3^-, SIDe, SIG and $BE_{(Alb)}$. After controlling for disease severity, there were no significant differences between these parameters.

Healthy pregnancy was associated with a decrease in $PvCO_2$ and net BE compared with non-pregnant controls ($P<0.001$, Table 1, Fig. 2). Quantitative analysis revealed a respiratory and hypoalbuminaemic alkalosis that was metabolically offset by an acidosis secondary to unmeasured anions ($BE_{(UMA)}$ −2.8 [2.7] mEq/L) and dilutional acidosis ($BE_{(Na+)}$ −1.2 [0.5] mEq/L). $BE_{(Lac)}$ was less than in non-pregnant women, but within the normal range ($BE_{(Lac)}$=−0.4 [0.4] mEq/L). Analysis based on Stewart's equations similarly showed pregnancy to be a state of alkalosis secondary to a decrease in $PvCO_2$ and plasma albumin, compensated by the acidotic effect of a decreased apparent strong ion difference (SIDa), $P<0.001$) and a significant increase in the strong ion gap (SIG), $P<0.001$). No correlation between gestational- or patient age or any metabolic acid-base parameter was observed.

Table 1 Measured and calculated acid base parameters. Data presented as mean, standard deviation (SD) and 95% confidence intervals [95% C.I.]. Student t-tests were performed comparing data from non-pregnant volunteers with healthy pregnant controls ([!]=P<0.001) and comparing data from healthy pregnant controls with women with severe preeclampsia (*=P<0.001). BE, base excess; cAG, anion gap corrected for albumin level; SBE, standardized base excess; SIDa, apparent strong ion difference; Expected SBE=0.4×($pvCO_2$ – $pvCO_{2\ normal}$); SIDe, effective strong ion difference; AG, anion gap (including K^+); SIG, strong ion group

	Non-pregnant volunteers (*n*=25) [95% C.I.]	Healthy pregnant controls (*n*=46) [95% C.I.]	Patients with severe preeclampsia (*n*=50) [95% C.I.]
pH	7.35 (0.02)	7.39 (0.03)[!]	7.41 (0.03)
	[7.34, 7.36]	[7.38, 7.40]	[7.40, 7.42]
pvCO2 (kPa)	6.4 (0.7)	4.8(0.7)[!]	4.4 (0.7)
	[6.18, 6.70]	[4.63, 5.03]	[4.19, 4.56]
HCO_3^- (mmol/l)	25.6 (1.6)	21.1 (2.1)[!]	20.1 (2.6)
	[24.8, 26.5]	[20.5, 21.9]	[19.5, 20.7]
BE (mEq/L)	−0.2 (1.2)	−2.8 (1.5)[!]	−3.3 (2.3)
	[−0.9, 0.5]	[−3.3, −2.2]	[−3.8, −2.8]
SBE (mEq/L)	0.4 (1.4)	−3.2 (1.8)[!]	−3.8 (2.5)
	[−0.4, 1.3]	[−3.8, −2.6]	[−4.4, −3.3]
AG	14.1 (2)	16.4 (3)[!]	16.1 (3)
	[13.0, 15.2]	[15.5, 17.1]	[15.3, 16.8]
cAG	12.5 (2)	17.5 (3)[!]	18.4 (3)
	[11.4, 13.6]	[16.7, 18.3]	[17.6, 19.2]
expected SBE	1.3 (2)	−3.6 (2)	−4.9 (2)
	[0.5, 2.1]	[−4.2, −3.0]	[−5.4, −4.3]
Sequential Stewart-Gilfix analysis:			
BE(Na^+)	−0.5 (0.6)	−1.3 (0.5)[!]	−1.3 (0.71)
	[−0.7, −0.2]	[−1.4, −1.1]	[−1.5, −1.1]
BE(Cl^-)	0.9 (1.9)	−0.4 (2.3)	−2 (2.3)*
	[0.1, 1.8]	[−1.1, 0.2]	[−2.6, −1.3]
BE(Alb)	−0.7 (0.6)	2.1 (0.8)[!]	3.6 (1)*
	[−1.1, −0.4]	[1.9–2.4]	[3.4, 3.8]
BE(Lac)	−0.06 (0.4)	−0.4 (0.4)[!]	−0.3 (0.5)
	[−0.2, 0.1]	[−0.6, −0.3]	[−0.5, −0.2]
BE(uma)	0.16 (1.70)	−2.8 (2.7)[!]	−3.3 (2.3)
	[−0.8, 1.1]	[−3.5, −2.1]	[−4, −2.6]
Stewart analysis:			
SIDa (mEq/l)	42.8 (2)	39.9 (2.5)[!]	39.9 (2.4)
	[41.8, 43.7]	[39.1, 40.5]	[39.2, 40.5]
PO_4^- effect	2.1 (0.3)	2.2 (0.6)	2.2 (0.5)
	[1.9, 2.3]	[2.0, 2.3]	[2.1, 2.3]
$Albumin^-$ effect	12.6 (0.6)	10 (0.8)[!]	8.6 (1)*
	[12.3, 13.0]	[9.7, 10.2]	[8.3, 8.8]
HCO_3^-	26.5 (1.6)	21.8 (2.1)[!]	20.8 (2.7)
	[25.6, 27.4]	[21.2, 22.6]	[20.1, 21.4]
SIG (mEq/l)	1.6 (1.7)	5.9 (2.7)[!]	8.3 (3.0)*
	[0.5, 2.6]	[5.0, 6.6]	[7.6, 9.0]

In both control groups, healthy non-pregnant and healthy pregnant volunteers, all mean standard laboratory parameters were within normal reference range as defined by the UCT laboratory. Laboratory parameters in healthy pregnant women reflected physiologic plasma volume expansion associated with pregnancy and were (except for uric acid, K+, AST and PO_4^-), less than in non-pregnant controls (Table 2).

Discussion

The approach developed by Stewart and Gilfix for the analysis of acid/base status has provided new insights into acid/base physiology in a variety of clinical settings.[15 21 26 27] We provide here a comprehensive analysis of acid/base status in severe preeclampsia, compared with healthy pregnancy and the non-pregnant state. While acid/base status, and in particular the absolute value of base excess, in severe preeclampsia seems to be similar to that in healthy pregnant women, the opposing contributors to the base excess appear larger than in healthy pregnancy. In addition, our findings indicate that the degree of alkalosis secondary to BE (alb) or decreased serum albumin may be a biological predictor to identify severity of preeclampsia, and potentially which women have fetal compromise requiring urgent Caesarean delivery.

Our findings are consistent with previous studies reporting similar values of pH, pCO_2 and BE in women with severe preeclampsia.[28 29] More recently, a decrease in anion gap secondary to changes in sodium and chloride concentrations has been demonstrated in women with severe preeclampsia.[10 11] We found a similar change in electrolyte levels, but as we also observed a

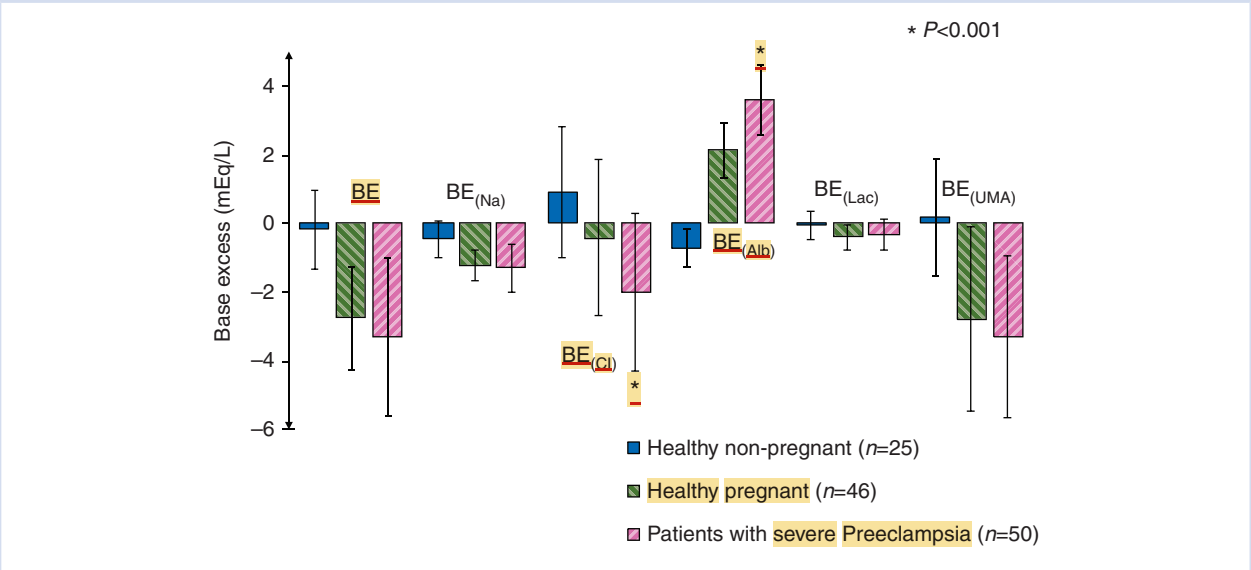

Fig 2 A Comparison of the contributors to base excess in healthy pregnancy and serve preeclampsia. Metabolic acid-base (AB) status in non-pregnant volunteers (n=25), healthy pregnant volunteers (n=46) and women diagnosed with severe preeclampsia (n=50). AB analysis was performed applying Gilfix methodology, based on the concept that net base excess (BE) is determined by effect of free water excess ($BE_{(Na^+)}$), changes in concentrations of chloride ($BE_{(Cl)^-}$), albumin ($BE_{(Alb)}$), lactate ($BE_{(Lac)}$) and the accumulation of unmeasured anions $BE_{(UMA)}$: $BE = BE^+_{(Na)} + BE^-_{(Cl)} + BE_{(Alb)} + BE_{(Lac)} + BE_{(UMA)}$ Comparing AB-status between healthy pregnant women and women with severe preeclampsia, an increased hypoalbuminaemic alkalosis (P<0.001) could be identified in the latter, that was offset by hyperchloraemic acidosis (P<0.001). No difference was found in ($BE_{(Na^+)}$), $BE_{(Lac)}$ or $BE_{(UMA)}$. Data are presented as mean values with error bars representing standard deviations.

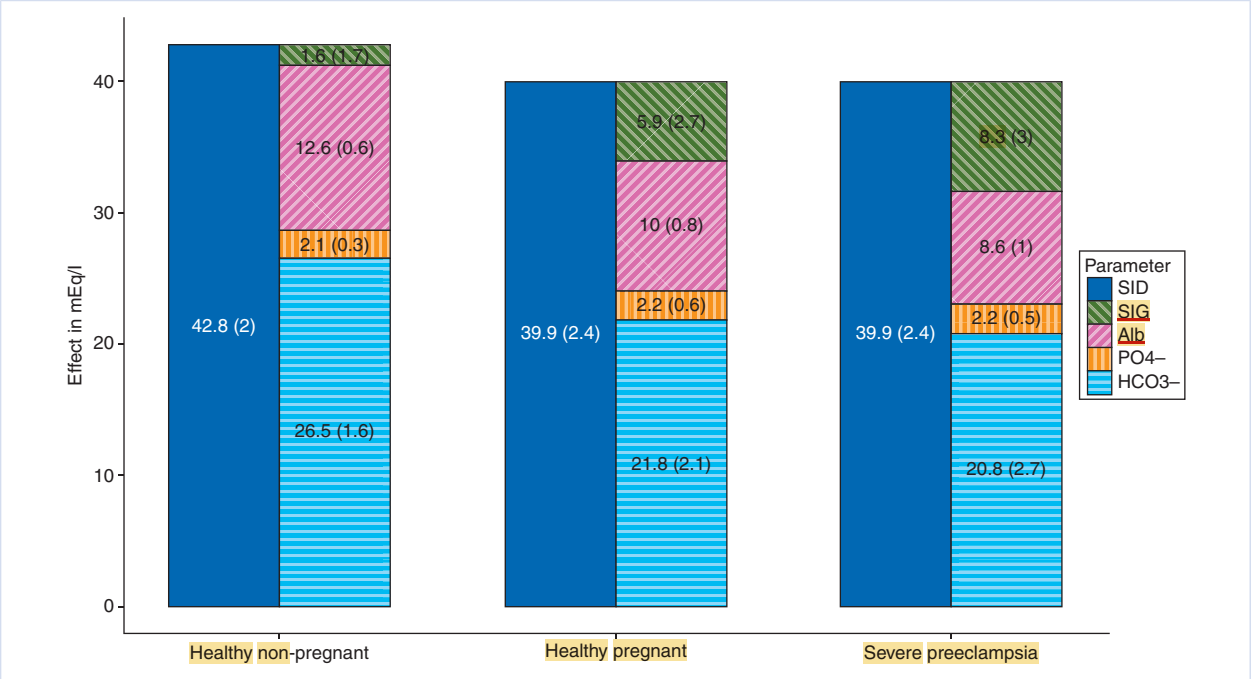

Fig 3 Strong ion difference, weak acids and bicarbonate in healthy pregnancy and serve preeclampsia. Anionic and cationic charges in the three study groups. The mean apparent strong ion difference (SID) and the anionic effect of plasma albumin (Alb), inorganic phosphate PO_4^- and bicarbonate ($HCO_3^- = CO_2$ effect) in healthy non-pregnant volunteers (n=25), healthy pregnant volunteers (n=46), and women diagnosed with severe preeclampsia (n=50). In healthy pregnancy SID, Albumin, HCO_3^-, was less and strong ion gap (SIG) higher than in non-pregnant women. In preeclampsia, albumin concentration was lower and SIG was higher as compared with healthy pregnant women.

Table 2 Patient characteristics & laboratory results. Data presented as mean and standard deviation (SD). n.a.: non applicable. Student *t*-tests were performed comparing data from non-pregnant volunteers with healthy pregnant controls ([!]P<0.001) and comparing data from healthy pregnant controls with women with severe preeclampsia (*P<0.001)

	Non-pregnant volunteers (*n*=25)	Healthy pregnant controls (*n*=46)	Patients with severe preeclampsia (*n*=50)
Patient characteristics			
Ethnicity:			
Black	5 (20%)	31 (67%)	34 (68%)
Coloured	3 (12%)	12 (26%)	14 (28%)
Caucasian	13 (52%)	1 (2%)	2 (4%)
Indian	2 (8%)	2 (4%)	0
Asian	2 (8%)	0	0
Age (yr)	31 (18–41)	27 (18–39)[!]	26 (18–39)
Height (cm)	165 (5)	165 (8)	161 (7)
Weight (kg)	64 (10)	77 (16)[!]	77 (18)
Gestational age (weeks)	n.a.	33 (4.4)	33 (4.5)
G/P	n.a.	G2/P1	G2/P1
Standard laboratory results			
Hb (g/L)	14.6 (1)	12.4 (1.4)[!]	12.6 (1.9)
Na^+ (mmol//)	139 (2)	137 (1.6)[!]	136 (2.5)
K^+ (mmol/l)	4.2 (0.3)	4.0 (0.4)	4.2 (0.5)
Ca^{++} (mmol/l)	1.29 (0.07)	1.20 (0.03)[!]	1.18 (0.06)
Cl^- (mmol/l)	104 (2.4)	103 (2.1)	104 (2.4)
Creatinine (μmol/l)	66 (10)	47 (9)[!]	64 (17)*
Urea (mmol/l)	4.5 (1.1)	2.4 (0.6)[!]	4.2 (2.4)*
Uric acid	0.23 (0.05)	0.23 (0.05)	0.36 (0.11)*
ALT	18 (7)	14 (6)	29 (36)
AST	20 (5)	19 (4)	37 (38)
LDH	342 (62)	397 (52)[!]	641 (273)*
Mg^{++} (mmol/l)	0.81 (0.06)	0.73 (0.09)[!]	1.4 (0.48)*
PO_4^- (mmol/l)	1.17 (0.15)	1.2 (0.35)	1.2 (0.25)
Total Protein (g/l)	72 (3.5)	64 (3.2)[!]	60 (6)*
Albumin (g/l)	46 (2.1)	36 (2.9)[!]	31 (3.7)*
Lactate (mmol/l)	1.16 (0.4)	1.5 (0.4)[!]	1.43 (0.47)

decrease in HCO_3^- of 1 mEq/L, the AG remained unchanged in our cohort. The mild decrease in HCO_3^- mirrors the decrease in Pco_2 observed in preeclamptic women, which may be interpreted as a respiratory compensation for metabolic acidosis associated with the disease.

Calculating the components contributing to BE, patients with severe preeclampsia were found to have significant hypoalbuminaemic alkalosis in combination with hyperchloraemic acidosis. Therefore, we were able to demonstrate that despite a relatively unchanged overall BE in preeclampsia, there are significantly greater opposing contributing factors to this value. Whether the compensation of hypoalbuminaemic alkalosis by hyperchloraemic acidosis should be interpreted as a complex compensation of disorders implying a pathophysiological connection, remains to be further investigated. Similarly to the healthy pregnant cohort, a significant acidosis secondary to unmeasured anions ($BE_{(UMA)}$=−3.3 mEq/L) was found in preeclampsia, and both approaches, Stewart's and Gilfix', indicate greater acidosis in preeclampsia. As a secondary outcome, we found that decreased serum albumin and increased $BE_{(Alb)}$ was associated with abnormal cardiotocography and might possibly indicate a need for emergency Caesarean delivery 24 h after diagnosis. As these findings were not primary outcomes, these associations must be interpreted with caution. Nevertheless, our observations are in keeping with the notion that low serum albumin level is associated with adverse outcome in preeclampsia.[30] Future research will show to what extent BE(alb) may add discrimination when compared with serum albumin levels.

Our findings confirm that healthy pregnancy is a state of chronic respiratory alkalosis with partial metabolic compensation, and we also identify for the first time a significant accumulation of unmeasured anions. Acid-base status in healthy pregnancy results from physiological changes occurring at an early gestational age and reaching a steady state at the end of the 1st trimester.[31] We assessed acid/base status in women during 2nd and 3rd trimester of pregnancy and found decreases in electrolyte-, albumin-, Pco_2-, SBE- and HCO_3^- levels, as described in previous studies on acid-base balance in pregnancy.[32] In addition, we analysed the components determining BE and quantified the effect of changes in albumin, sodium and chloride levels. We confirmed significant hypoalbuminaemic alkalosis and found, in comparison with non-pregnant women, dilutional acidosis. However, the negative $BE_{(Na)}$ and $BE_{(Cl)}$ did not sufficiently compensate for the increase in $BE_{(Alb)}$ to explain the overall decrease in BE. Therefore, there seems to be a significant component of unmeasured anions. This is also shown by the increase in SIG, which partly offsets the decrease in Pco_2 and weak acids. As with other patient groups, such as ICU patients, the exact source of these unmeasured anions remains unknown. One can hypothesize that these may be as a result of accumulation of Kreb's Cycle components or fixed acids from the uteroplacental metabolism, which have both been described in previous

studies to accumulate in pregnancy.[32–34] As the accumulation of unmeasured anions has been associated with negative outcome in other clinical settings,[6 8 33] further studies are needed to elucidate the true origin and physiologic range of this component.

We recognize the limitations of our study. Non-pregnant controls differed in patient weight, age and ethnicity from healthy pregnant controls and patients with severe preeclampsia. However, these patient characteristic parameters have, to our knowledge, no impact on acid/base physiology, and the obtained laboratory acid/base values were within normal limits and the expected range. In addition, the administration of magnesium sulphate might have contributed to unmeasured anions. However, as in previous studies,[35] comparing $BE_{(UMA)}$ between preeclamptic women and healthy pregnant volunteers, no difference in $BE_{(UMA)}$ could be found. Nevertheless, the role of magnesium sulphate should be clarified in future studies, by measuring sulphate concentrations that can be measured in clinical chemistry laboratories, but are not measured routinely.

There is an ongoing debate as to whether early- and late onset preeclampsia should be seen as two different entities.[36] However we decided to evaluate all preeclamptic women as a single group, as acid/base status was similar regardless of gestational age on presentation. Another limitation is that while we have used the most widely applied approach to estimating the acid-base effect of albumin, this is an area of continuing advances and the accepted approach to estimating the acid-base variables may change over time.

In conclusion, this study provides a comprehensive analysis of acid/base status in severe preeclampsia, compared with healthy pregnancy and the non-pregnant state. Healthy pregnancy is associated with a significant accumulation of unmeasured anions. While acid/base status, in particular the base excess, in severe preeclampsia appears to be similar to that in healthy pregnant women, there is in fact a greater offsetting balance of hypoalbuminaemic alkalosis and hyperchloraemic acidosis. These contributors to the base excess may be more important markers of disease severity than its absolute value. In addition, our findings indicate that either increased $BE_{(Alb)}$ or a decreased serum albumin level may be a biological predictor that could be used in the future to identify preeclamptic women at increased risk for fetal compromise and urgent Caesarean delivery.

Authors' contributions

Study design/planning: C.M.O., S.A., R.L., K.C.C., R.A.D.
Study conduct: C.M.O., B.C., S.A., R.A.D.
Data analysis: C.M.O., D.S., R.L., K.C.C., R.A.D.
Writing paper: C.M.O., B.C., S.A., D.S., R.L., K.C.C., R.A.D.
Revising paper: All authors.

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Declaration of interest

None declared.

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Appendix: Blood sampling and acid-base analysis

Blood samples were obtained from the basilic vein on the opposite side to the cannulated peripheral vein. The pH, partial pressure of carbon dioxide (pCO_2), ionized calcium (Ca^{++}), and lactate were measured from one blood specimen immediately after blood draw, with a designated blood gas analyser (ABL 725, Radiometer®, Copenhagen, Denmark). Samples of separated plasma were sent to the central hospital laboratory services and analysed for concentrations of sodium (Na^+), potassium (K^+), chloride (Cl^-), magnesium (Mg^{++}), inorganic phosphate (Pi), albumin (Alb), serum alanine aminotransferase activity (ALT), serum aspartate aminotransferase activity (AST), uric acid, blood urea nitrogen (BUN), creatinine and uric acid by a fully automated analyser (Hitachi 917, Roche Diagnostics® GmbH, Mannheim, Germany). Sodium (Na^+) and (Cl^-) were measured using ion selective electrodes. Lactate was measured with an amperometric electrode.

Calculation of HCO_3^-, base excess (BE), and standard base excess (SBE)

HCO_3^- was calculated using the Henderson-Hasselbalch equation[37] from the measured pH and Pco_2. In order to assess the net metabolic acid-base state, BE and SBE was calculated using the Van Slyke[37] and Sigaard-Andersen equations[38] and used to exclude an apparent metabolic acid-base disorder. Because pregnancy is known to involve chronic respiratory alkalosis, we used the work of Schlichtig, Grogono and Severinghaus[39] to estimate the expected base excess. This was regarded as conventional analysis:

- $[HCO_3^-] = 0.03 \times pCO_{2\times} 10^{(pH-6.1)})$.
- $BE = (HCO_3^- - 24.4 + [2.3 \times Hb + 7.7] \times [pH - 7.4]) \times (1 - 0.023 \times Hb)$
- $SBE = 0.9287 \times (HCO_3^- - 24.4 + 14.83 \times [pH - 7.4])$
- Schlichtig formula to calculate expected BE in respiratory alkalosis:

$$\text{Expected base excess (mmol/L)} = 0.4 \times (pCO_2 - 40).$$

Calculation of anion gap (AG) and correction of the anion gap for albumin (cAG)

- $AG = ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$
- $cAG = AG + [2.5 \times (4 - \text{Albumin(g/dl)}]$

Acid-base analysis based on Stewart-Gilfix equations

1. $BE_{(Na)}$: Excess of free water causes acidosis. Na^+, as the regulated variable that controls the extracellular fluid volume, is used to assess the free water effect (dilution or plasma concentration):[19]

$$BE_{(Na)} = (Na^+_{Measured} - Na^+_{Normal}) \times (Na^+_{Normal} + K^+_{Normal} - Cl^-_{Normal})/Na^+_{Normal}$$

2. $BE_{(Cl)}$: An increase or decrease of chloride relative to sodium concentration is associated with hyperchloraemic acidosis or hypochloraemic alkalosis respectively. The effect of changes in Cl^- is obtained by correcting Cl^- concentration

for changes in free water:

$$Cl^-_{Na+corrected} = Cl^- \times \left(\frac{Na^+_{Normal}}{Na^+_{Measured}} \right).$$

Changes in base excess caused by changes in Cl^- concentration (=$BE_{(Cl)}$) are then calculated as follows:

$$BE_{(Cl)} = Cl^-_{Normal} - Cl^-_{Na+corrected}$$

3. $BE_{(Alb)}$: Albumin is a weak non-volatile acid. A decrease in albumin level results in hypoalbuminaemic alkalosis. In the biologically significant range of pH (i.e. 6.80–7.80), the relationship of ionised albumin [A^-] to total albumin as a function of pH is linear,[18] such that [A^-]=(0.148×pH–0.818). $BE_{(Alb)}$ can therefore be calculated (Albumin in g/l):

$$BE_{(Alb)} = (0.148 \times pH - 0.818) \times (Alb_{Normal} - Alb_{Measured}).$$

4. $BE_{(Lac)}$: An increase of lactate concentration causes an equimolar decrease in BE. For example, an increase of lactate concentration by 5 mmol/l causes BE to decline by 5 mmol/l. Therefore BE attributable to lactate can be calculated:

$$BE_{(Lac)} = lactate_{Normal} - lactate_{Measured}$$

5. $BE_{(UMA)}$: Any change in BE not caused by changes in free water, chloride or albumin, is attributed to unmeasured anions. Unmeasured anions are, for example, accumulating with renal insufficiency or ketone bodies. This can be quantified as:

$$BE_{(UMA)} = BE - (BE_{Na} + BE_{Cl} + BE_{Alb} + BE_{Lac}).$$

In summary, BE comprises $BE_{(Na)}$, $BE_{(Cl)}$, $BE_{(Alb)}$, $BE_{(Lac)}$, $BE_{(UMA)}$:

$$BE = BE_{(Na)} + BE_{(Cl)} + BE_{(Alb)} + BE_{(Lac)} + BE_{(UMA)}$$

Normal laboratory values were derived from age- and gender based mean reference values from the UCT laboratories: $Na^+_{Normal} = 141\ mmol/l$, $Cl^-_{Normal} = 106\ mmol/l$, $Albumin_{Normal} = 43.5\ g/l$, $Lactate_{Normal} = 1.1\ mmol/l$, and $K^+_{Normal} = 4.3\ mmol/l$. A BE parameter $\geq \pm 2\ mEq/L$was defined as abnormal.

Acid-base analysis based on Stewart-Fencl equations

An acid-base analysis based on Stewart's equations[17 27] was performed as follows:
Apparent strong ion difference (SIDa):

$$SIDa = Na^+ + K^+ + 2 \times Mg^{2+} + 2 \times Ca^{2+} - Cl^- - lactate(\text{all concentrations in mmol/l})) \quad (1)$$

The amount of unmeasured ions was determined by summing the ionic effects of all the measured clinical chemistry variables to calculate the strong ion gap (SIG) first described by Kellum and colleagues.[40] A positive value of the SIG represents unmeasured anions (e.g. uric acid, lactate, sulphate, ketone bodies, citrate).

$$SIG(meq/L) = SIDa - Albumin\ anionic\ effect - Phosphate\ anionic\ effect - Bicarbonate \quad (2)$$

- The albumin anionic effect, meq/L=Albumin (g/L)×(0.123×pH–0.631)
- Phosphate anionic effect, meq/L=Phosphate (mmol/L)×(0.309×pH–0.469)

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