

*Supplementary materials for*

## **Sodium and potassium concentrations and somatic cell count of human milk produced in the first six weeks postpartum and their suitability as biomarkers of clinical and subclinical mastitis**

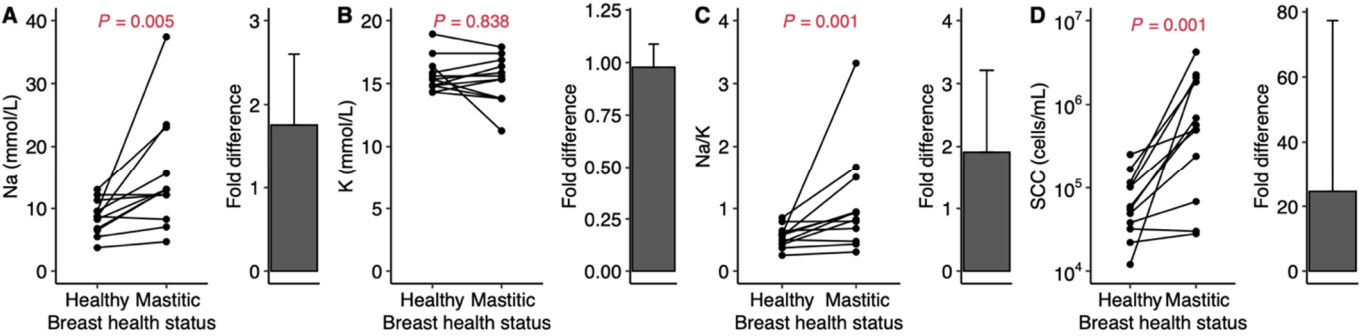
**Figure S1.** Comparisons of Na concentrations, K concentrations, Na/K, SCC between breasts within subjects during clinical mastitis.

**Figure S2.** Analysis of biomarkers in milk produced by women with and without clinical mastitis.

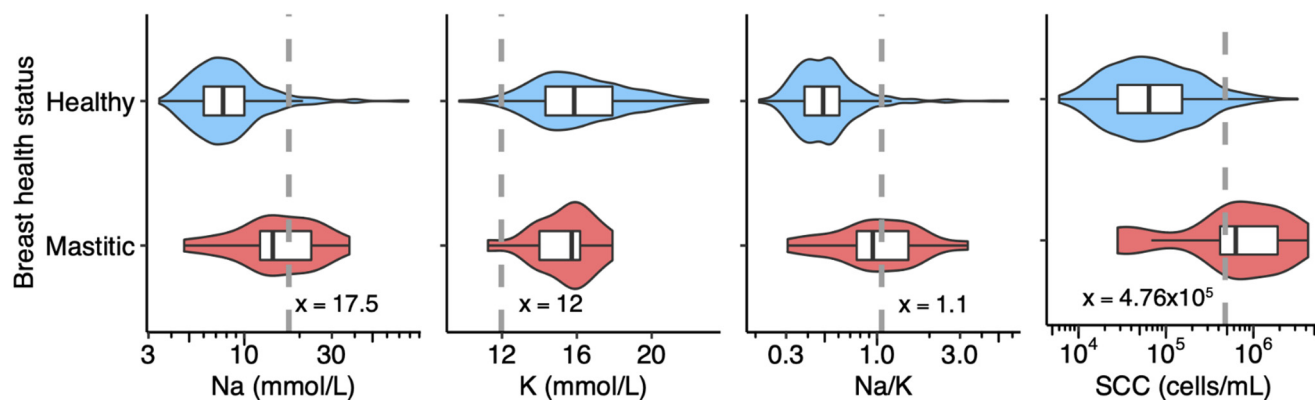
**Table S1.** Self-reported perinatal- and lactation-related characteristics of participants who were healthy for the entire study and those who developed clinical mastitis.

**Table S2.** Na concentrations, K concentrations, Na/K, and SCC summary measurements during the first 6 weeks postpartum in lactating women without clinical mastitis ( $n=31$ ).

**Table S3.** Na concentrations, K concentrations, Na/K, and SCC summary measurements of milk samples produced during clinical mastitis in the first 6 weeks of lactation.



**Figure S1.** Comparisons of Na concentrations, K concentrations, Na/K, SCC between breasts within subjects during clinical mastitis. (A) Na, (B) K, (C) Na/K, and (D);  $n=12$  paired healthy and mastitic breast observations.  $P$ -values were calculated from Wilcoxon signed rank tests.



**Figure S2.** Analysis of biomarkers in milk produced by women with and without clinical mastitis. Distribution of biomarker concentrations in healthy (Na, K, and Na/K,  $n=468$ ; SCC,  $n=473$ ) and clinical mastitis (Na, K, Na/K, and SCC,  $n=16$ ) milk samples. The inner box-and-whiskers plots show the median (middle line), first and third quartiles (lower/upper line), and 1.5 times the first and third quartiles (whiskers). The vertical dotted lines along the x-axes correspond to the logistic regression-derived biomarker thresholds for clinical mastitis.

**Table S1.** Self-reported perinatal- and lactation-related characteristics of participants who were healthy for the entire study and those who developed clinical mastitis.

Characteristic	Total	Healthy	Mastitis	P
Participants	41 (100)	31 (76)	10 (24)	-
Alcohol use during pregnancy	12 (29)	8 (26)	4 (40)	0.441
Tobacco use during pregnancy	2 (5)	2 (8)	0	>0.999
Marijuana use during pregnancy	1 (2)	1 (3)	0	>0.999
Complications during pregnancy/delivery	24 (59)	16 (52)	8 (80)	0.152
Infections during pregnancy/delivery	13 (32)	10 (32)	3 (30)	>0.999
Bacterial vaginosis	1 (2)	0	1 (10)	-
Chorioamnionitis	1 (2)	0	1 (10)	-
Group B <i>Streptococcus</i>	4 (10)	3 (10)	1 (10)	-
Herpes	2 (5)	2 (6)	0	-
Lower urinary tract infection	3 (7)	3 (10)	0	-
Other	2 (5)	2 (6)	0	-
Perceived stress during birth, median (IQR), 1-10 scale <sup>1</sup>	6 (3-8)	6 (3-8)	6 (5-7)	0.748
Skin-to-skin within 1 hr of birth	34 (83)	25 (81)	9 (90)	0.660
Infant feeding plans at delivery				0.376
Exclusively at the breast	30 (73)	24 (77)	6 (60)	
Mix of at the breast and expressed milk	10 (24)	6 (19)	4 (40)	
Mix of at the breast, expressed milk, and formula	1 (2)	1 (3)	0 (0)	
Infant first breastfed after delivery				0.712
<1 hr	31 (76)	23 (74)	8 (80)	
1-3 hr	8 (20)	6 (19)	2 (20)	
>3 hr	2 (5)	2 (6)	0	
Reported barriers to breastfeeding <sup>2</sup>	6 (15)	5 (16)	1 (10)	>0.999
Pumping milk, first 120 hours postpartum	23 (56)	16 (52)	7 (70)	0.467
Time between delivery and first pumping, median (IQR), h	72 (48-96)	84 (66-96)	72 (48-72)	0.150
Within 3 days of delivery				
Had access to a lactation consultant	19 (46)	15 (48)	4 (40)	0.727
Experienced nipple damage	20 (49)	15 (48)	5 (50)	>0.999
Infant feeding status, first week <sup>3</sup>				0.304
Exclusively at the breast	25 (63)	21 (68)	4 (44)	
Mix of at the breast and expressed milk	10 (25)	6 (19)	4 (44)	
Mix of at the breast and/or expressed milk with formula	5 (12)	4 (13)	1 (11)	
Exclusively fed infant human milk during entire study	35 (85)	26 (84)	9 (90)	>0.999
Exclusively fed infant at the breast during entire study	9 (22)	6 (19)	3 (30)	0.662
Nipple shield use	10 (24)	7 (23)	3 (30)	0.683
Nipple cream use	33 (80)	24 (77)	9 (90)	0.653
Infant diagnosed with tongue tie	2 (5)	1 (3)	1 (10)	0.433

IQR, interquartile range. Values are given as numeric counts and percentages or IQR in parentheses. Percentages may not sum to 100 due to rounding. *P*-values were calculated from Wilcoxon rank sum tests for continuous data and Fisher's exact tests for categorical data. <sup>1</sup>Stress during birth was ranked from 1 (no stress) to 10 (severe stress); <sup>2</sup>Reported barriers to breastfeeding included cesarean section, difficulty latching, neonatal intensive care unit stay, and infant respiratory problems; <sup>3</sup>Missing data from 1 participant with clinical mastitis.

**Table S2.** Na concentrations, K concentrations, Na/K, and SCC summary measurements during the first 6 weeks postpartum in lactating women without clinical mastitis ( $n=31$ ).

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
<b>Na (mmol/L)</b>						
Left breast	11.8 (10.3-13.5) <sup>a</sup>	9.5 (8.3-10.9) <sup>b</sup>	8.5 (7.4-9.8) <sup>bc</sup>	7.4 (6.5-8.5) <sup>cd</sup>	7.3 (6.3-8.3) <sup>cd</sup>	6.2 (5.4-7.1) <sup>d</sup>
Right breast	12.0 (10.5-13.8) <sup>a</sup>	9.5 (8.3-10.8) <sup>b</sup>	8.4 (7.3-9.6) <sup>bc</sup>	7.7 (6.8-8.9) <sup>bcd</sup>	6.9 (6.0-7.9) <sup>cd</sup>	6.5 (5.7-7.4) <sup>d</sup>
Left/right mean	11.9 (10.5-13.4) <sup>a</sup>	9.5 (8.4-10.7) <sup>b</sup>	8.5 (7.5-9.5) <sup>bc</sup>	7.6 (6.7-8.6) <sup>cd</sup>	7.1 (6.3-8.0) <sup>de</sup>	6.3 (5.6-7.2) <sup>e</sup>
<b>K (mmol/L)</b>						
Left breast	18.8 (18.0-19.6) <sup>a</sup>	16.4 (15.7-17.1) <sup>b</sup>	15.5 (14.9-16.2) <sup>bc</sup>	15.4 (14.8-16.0) <sup>bc</sup>	15.2 (14.6-15.8) <sup>c</sup>	14.8 (14.2-15.5) <sup>c</sup>
Right breast	18.6 (17.8-19.4) <sup>a</sup>	16.2 (15.5-16.8) <sup>b</sup>	15.3 (14.7-16.0) <sup>bc</sup>	15.6 (14.9-16.2) <sup>bc</sup>	15.3 (14.6-15.9) <sup>bc</sup>	14.6 (14.0-15.3) <sup>c</sup>
Left/right mean	18.7 (18.0-19.4) <sup>a</sup>	16.3 (15.7-16.9) <sup>b</sup>	15.4 (14.9-16.0) <sup>c</sup>	15.5 (14.9-16.0) <sup>c</sup>	15.2 (14.7-15.8) <sup>cd</sup>	14.7 (14.2-15.3) <sup>d</sup>
<b>Na/K</b>						
Left breast	0.63 (0.55-0.72) <sup>a</sup>	0.58 (0.51-0.67) <sup>ab</sup>	0.55 (0.48-0.63) <sup>ab</sup>	0.48 (0.42-0.55) <sup>bc</sup>	0.48 (0.42-0.55) <sup>bc</sup>	0.42 (0.36-0.48) <sup>c</sup>
Right breast	0.65 (0.56-0.74) <sup>a</sup>	0.59 (0.51-0.67) <sup>ab</sup>	0.55 (0.48-0.63) <sup>abc</sup>	0.50 (0.43-0.57) <sup>bc</sup>	0.45 (0.39-0.52) <sup>c</sup>	0.44 (0.38-0.51) <sup>c</sup>
Left/right mean	0.64 (0.57-0.72) <sup>a</sup>	0.58 (0.52-0.66) <sup>ab</sup>	0.55 (0.49-0.62) <sup>bc</sup>	0.49 (0.44-0.55) <sup>cd</sup>	0.46 (0.41-0.52) <sup>d</sup>	0.43 (0.38-0.48) <sup>d</sup>
<b>SCC (cells/mL, <math>\times 10^5</math>)</b>						
Left breast	0.95 (0.65-1.38) <sup>a</sup>	0.95 (0.65-1.39) <sup>a</sup>	0.85 (0.58-1.23) <sup>a</sup>	0.75 (0.52-1.10) <sup>ab</sup>	0.70 (0.48-1.03) <sup>ab</sup>	0.42 (0.29-0.62) <sup>b</sup>
Right breast	0.80 (0.55-1.17) <sup>a</sup>	0.79 (0.54-1.15) <sup>a</sup>	0.81 (0.55-1.17) <sup>a</sup>	0.88 (0.60-1.28) <sup>a</sup>	0.61 (0.42-0.89) <sup>a</sup>	0.56 (0.38-0.82) <sup>a</sup>
Left/right mean	0.87 (0.63-1.19) <sup>a</sup>	0.87 (0.63-1.19) <sup>a</sup>	0.83 (0.60-1.13) <sup>a</sup>	0.81 (0.59-1.12) <sup>a</sup>	0.66 (0.48-0.90) <sup>ab</sup>	0.49 (0.35-0.67) <sup>b</sup>

Values represent model-based estimates, means (95% confidence intervals). There were no differences in any measure between breasts ( $P \geq 0.05$ ). Letters denote significance groups within breast over time, Tukey's method  $P < 0.05$ .

**Table S3.** Na concentrations, K concentrations, Na/K, and SCC summary measurements of milk samples produced during clinical mastitis in the first 6 weeks of lactation.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
<b>Na (mmol/L)</b>						
Left breast	17.4 ± 12.9	23.3 ± 0.3	15.4 ± 3.4	7.1	-	-
Right breast	24.8	24.8 ± 17.8	16.8 ± 5.2	13.9 ± 2.5	-	8.9 ± 5.9
Left/right mean	21.1 ± 5.2	24.0 ± 1.1	16.1 ± 0.9	10.5 ± 4.9	-	-
<b>K (mmol/L)</b>						
Left breast	16.8 ± 0.9	14.7 ± 1.3	15.9 ± 0	16.4	-	-
Right breast	16.1	13.3 ± 2.9	13.9 ± 0.2	17.4 ± 0.7	-	14.6 ± 1.1
Left/right mean	16.4 ± 0.5	14.0 ± 1.0	14.9 ± 1.4	16.9 ± 0.7	-	-
<b>Na/K</b>						
Left breast	1.06 ± 0.83	1.59 ± 0.12	0.97 ± 0.21	0.43	-	-
Right breast	1.54	2.06 ± 1.79	1.19 ± 0.36	0.80 ± 0.18	-	0.63 ± 0.45
Left/right mean	1.30 ± 0.34	1.82 ± 0.33	1.09 ± 0.16	0.62 ± 0.26	-	-
<b>SCC (cells/mL, x10<sup>5</sup>)</b>						
Left breast	10.20 ± 14.03	13.83 ± 12.47	14.54 ± 9.12	0.68	-	-
Right breast	4.99	3.62 ± 4.70	9.25 ± 5.01	22.37 ± 28.21	-	11.80 ± 9.63
Left/right mean	7.60 ± 3.68	8.73 ± 7.22	11.90 ± 3.74	1.23 ± 0.78	-	-

Values represent means ± standard deviations (when multiple observations occur). Milk samples were collected from 10 participants. In total, there were 16 milk samples: 3 in week 1, 4 in week 2, 4 in week 3, 3 in week 4, 0 in week 5, 2 in week 6.