RESEARCH

Biochemical markers of renal function and maternal hypothyroidism in early pregnancy

Lise Husted[1], Sidsel Rødgaard-Hansen[1], Maja Hjelm Lundgaard[2], Nanna Maria Uldall Torp[2,3] and Stine Linding Andersen[2,3]

[1]Department of Clinical Biochemistry, Viborg Regional Hospital, Viborg, Denmark
[2]Department of Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark
[3]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

Correspondence should be addressed to S L Andersen: stine.a@rn.dk

Abstract

Objective: The physiological adaptations during a normal pregnancy affect renal and thyroid function and levels of associated biochemical markers. An association between cystatin C (CysC), creatinine, and thyroid function has been considered in nonpregnant individuals but not in pregnant women specifically.
Methods: Cohort study within the North Denmark Region Pregnancy Cohort (2011–2015) with assessment of thyroid function and autoantibodies (ADVIA Centaur XPT, Siemens Healthineers) in serum residues from the early pregnancy. Consecutive samples ($n = 1112$) were selected for measurement of CysC and creatinine (Atellica CH 930, Siemens Healthineers), and results were linked to information in Danish nationwide registers for (i) establishment of pregnancy-specific reference intervals for CysC and creatinine and (ii) evaluation of the prevalence of maternal hypothyroidism in early pregnancy according to levels of CysC and creatinine.
Results: The established reference intervals (2.5–97.5 percentiles) differed by week of pregnancy (week 4–8, 9–11, 12–15) and were CysC: 0.58–0.92 mg/L; 0.54–0.91 mg/L; 0.52–0.86 mg/L; creatinine: 46.9–73.0 µmol/L; 42.0–68.4 µmol/L; 38.8–66.4 µmol/L. The prevalence of maternal autoimmune hypothyroidism in early pregnancy differed by the level of CysC and creatinine (<25th percentile; 25th–75th percentile; >75th percentile) and was for CysC 1.7%, 3.8%, 7.4% and for creatinine 2.5%, 4.1%, 7.1%.
Conclusions: Reference intervals for CysC and creatinine were dynamic in early pregnancy and decreased with increasing gestational age. Furthermore, higher levels of CysC and creatinine associated with a higher prevalence of maternal autoimmune hypothyroidism. Results encourage considerations on the underlying mechanisms for the association between markers of renal and thyroid function.

Keywords:

- gestation
- kidney
- cystatin C
- creatinine
- TSH

Introduction

A normal pregnancy entails physiological alterations to ensure maternal and fetal health. The adaptations are profound and affect nearly every organ system including maternal thyroid and renal function (1). The high levels of human chorionic gonadotropin (hCG) in early pregnancy mimic the role of thyroid-stimulating hormone (TSH). Thereby, the production of thyroid hormones increases with a concomitant decrease in

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TSH (2). This physiological effect is pronounced, and TSH shows considerable dynamics within the first trimester of a pregnancy which necessitates the use of pregnancy-specific reference intervals in the assessment of maternal thyroid function (3). In the kidney, an increase in renal plasma flow and the glomerular filtration rate (GFR) occurs in pregnancy (1). This glomerular hyperfiltration causes a decrease in maternal plasma levels of creatinine in pregnancy (4). Another marker of renal function is cystatin C (CysC) which is drawn into clinical consideration when plasma creatinine is deemed invalid for the estimation of GFR (5). Like plasma creatinine, a decrease in the levels of CysC during pregnancy has been described (6); however, for both renal function markers, any dynamics within the first trimester of pregnancy remain uncertain.

In nonpregnant individuals, a link between thyroid disease and markers of renal function has been proposed and is considered as part of clinical guidelines on the biochemical assessment of renal function (5). Thus, it is stated as part of the recommendations that disorders of thyroid function may affect levels of CysC (5) and in particular that hypothyroidism is associated with lower and hyperthyroidism with higher levels of CysC (7). However, the underlying mechanisms are not clear, and uncertainties prevail regarding the role of thyroid function and thyroid autoimmunity. More recent studies have pointed toward a specific association between autoimmune hypothyroidism and increased levels of CysC (8, 9), which contrasts the general hypothesis that lack of thyroid hormones reduces levels of CysC (7, 10). With a focus on immunological mechanisms, another thought is on the interaction with the physiological immune suppression seen in a normal pregnancy (11).

The North Denmark Region Pregnancy Cohort (NDRPC) is a large, consecutive cohort of Danish pregnant women with stored biobank samples from the early pregnancy (12, 13). The blood samples were drawn as part of routine care in early pregnancy which allowed for the establishment of reference intervals within shorter time periods of the first trimester of pregnancy. Reference intervals for thyroid function tests were previously established (12), and we now established reference intervals for CysC and creatinine within the cohort and evaluated the association between levels of CysC, creatinine, and maternal hypothyroidism, while considering measurement of thyroid autoantibodies as marker of autoimmune thyroid disease.

Materials and methods

We performed a cohort study of Danish pregnant women enrolled in the NDRPC (12, 13). The NDRPC includes a biobank of serum samples drawn as part of prenatal screening for chromosomal anomalies in early pregnant women. In Denmark, all pregnant women are offered prenatal screening in the first trimester of pregnancy which includes a blood sample and a fetal ultrasound examination. After ethical permission, serum residues collected as part of the prenatal screening program among 17,666 pregnancies in the North Denmark Region from 2011 to 2015 (Fig. 1) were used years after the pregnancy for measurement of maternal thyroid function and thyroid autoantibodies. For the present study, we included serum samples from singleton pregnant women drawn before pregnancy week 15 and in the women's first pregnancy within the study cohort period. Furthermore, women with low TSH in the early pregnancy sample were not included in the present study because these biobank samples were previously used for investigations on maternal hyperthyroidism. Thus, a total of 13,575 pregnant women were eligible for the present study and in this cohort, we enrolled a consecutive sample of 1112 women for measurement of CysC and creatinine (Fig. 1). The study was approved by the North Denmark Region Committee on Health Research Ethics (N-20150015) and registered according to the General Data Protection Regulation in the North Denmark Region (2015-34).

The biochemical measurements were performed in medical laboratories with ISO 15189 accreditation. All samples were stored at -80 degrees Celsius until analyses. As previously described in detail (12, 13), TSH, free thyroxine (fT4), thyroid peroxidase antibodies (TPO-Ab), and thyroglobulin antibodies (Tg-Ab) were analyzed from September 2015 to May 2016 on an ADVIA Centaur XPT (Siemens Healthineers), in the Department of Clinical Biochemistry, North Denmark Regional Hospital. Pregnancy-specific reference intervals previously established within the cohort for this specific biochemical method were used for identification of maternal hypothyroidism and classification of thyroid autoantibody status. Hypothyroidism was defined as TSH above the pregnancy week-specific upper reference limit, and the women were considered thyroid autoantibody positive if TPO-Ab (> 60 U/mL) and/or Tg-Ab (> 33 mU/L) were above the pregnancy-specific cutoff established within the cohort (14). Following this, autoimmune hypothyroidism was defined as the

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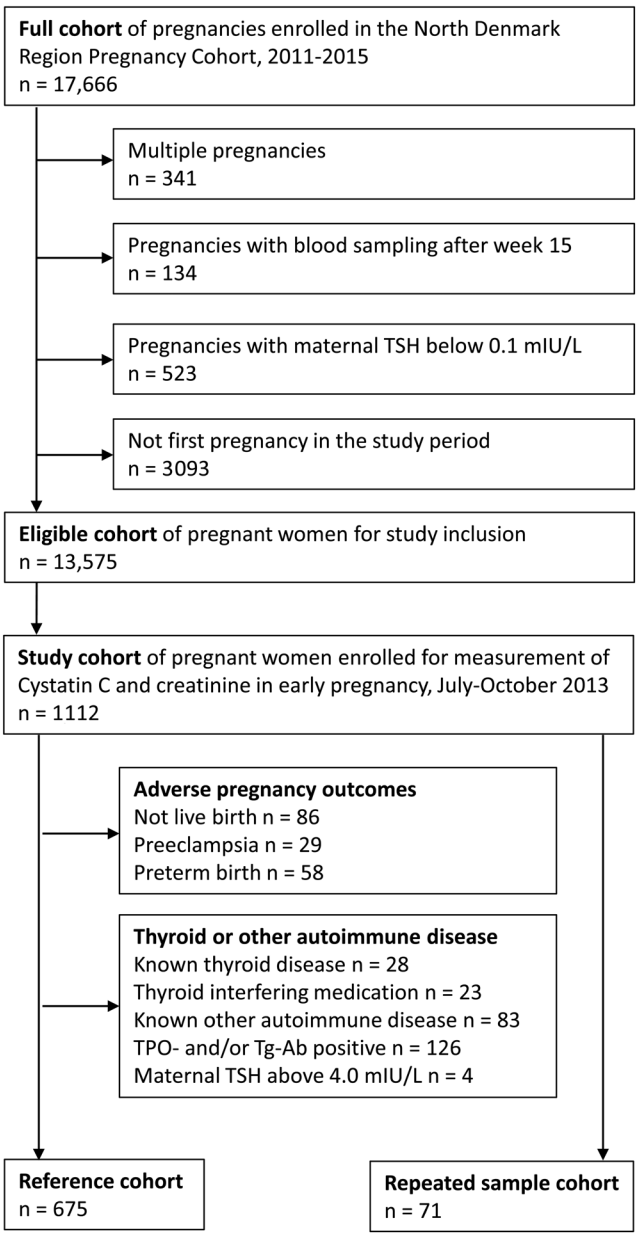

Figure 1
Flowchart of the study population including the selection of the study cohort, the reference cohort, and the repeated sample cohort.

presence of hypothyroidism in thyroid autoantibody positive women.

CysC (Atellica CH Cystatin C_2) and creatinine (Atellica CH Enzymatic Creatinine_2) were analyzed from January to October 2022, on an Atellica CH 930 (Siemens Healthineers, Erlangen, Germany). The measurement range for CysC was 0.25-26.79 mg/L ranging from the limit of quantification defined by a coefficient of variation (CV) less than 10%. The reference interval for nonpregnant adults recommended by the manufacturer was 0.64–1.23 mg/L. The measurement range for creatinine was 9.0–13,260 µmol/L, and the reference interval recommended by the manufacturer for nonpregnant adult women was 44–71 µmol/L. CysC and creatinine were measured in duplicate, and the individual mean of the repeat measurements was used for data analyses. Results of duplicate measurements were used to calculate normalized CV according to the Dahlberg procedure (15). Normalized CV was 4.0% for CysC and 0.8% for creatinine.

The biochemical results were linked to information in Danish nationwide registers via Statistics Denmark, and all data were analyzed in encrypted form meaning that no individuals could be identified by the researcher. The Danish nationwide registers provided information on outcome of pregnancy and maternal and child characteristics (the Medical Birth Registry), maternal hospital diagnoses of disease (the Danish National Hospital Register (16)) and redeemed prescriptions of drugs (the Danish National Prescription Register (17)). As previously described in detail (12, 13), information from the registers was used to identify maternal thyroid disease, use of thyroid interfering medication (antiepileptic drugs, dopamine agonists, lithium, and prednisolone), and other autoimmune diseases (diabetes mellitus, rheumatoid arthritis, and inflammatory bowel disease). For the present study, we specifically assessed the Danish National Hospital Register for any diagnosis of acute or chronic renal failure (according to the tenth edition of the International Classification of Disease: N17-N19), and none of the women studied had such diagnoses registered.

For establishment of reference intervals for CysC and creatinine, we identified a reference cohort of pregnant women among study participants (Fig. 1) who had no chronic kidney disease, no adverse outcome of pregnancy, and no known thyroid disease or thyroid autoantibody positivity. These criteria for the reference cohort were based on the existing literature indicating a link between CysC levels and adverse outcomes of pregnancy (18) as well as thyroid disease (10). Within the full study cohort and the reference cohort, we established percentile levels for CysC and creatinine with 90% confidence interval (CI) among all individuals and stratified by the pregnancy week of blood sampling (4–8, 9–11, and 12–15). Evaluation of outliers using Tukey's inner and outer fences revealed no outliers in the distribution of CysC or creatinine. Multivariate linear regression was used to evaluate the association between categories of maternal CysC and creatinine levels in

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pregnancy (defined by the first and third quartile in pregnancy weeks 4–8, 9–11, and 12–15, respectively), and continuous outcomes of the log-transformed TSH and fT4. Results of the regression analyses were back-transformed and reported as the geometric mean and the adjusted exponentiated beta coefficient (aβ), which is the ratio of the outcome variable between the exposure and the reference group. The adjusted model included pregnancy week of blood sampling, maternal age, origin, diabetes, and thyroid autoantibody status for evaluation in all pregnancies, as well as body mass index, parity, and smoking status, when evaluated among live births. In a subanalysis, CysC and creatinine were included in the model as independent continuous variables. Furthermore, any nonlinear association was assessed using restricted cubic splines with five knots. Finally, the frequency of thyroid autoantibody positivity as well as having autoimmune or nonautoimmune hypothyroidism in pregnancy was evaluated by categories of CysC and creatinine and compared using the chi-squared test.

Of the 1112 participants, a total of 71 had a repeated blood sample drawn in early pregnancy (Fig. 1). The blood sample was repeated as part of the prenatal screening program for chromosomal anomalies to ensure correct timing of the sample in relation to prenatal ultrasound assessment (19). Thus, samples were not drawn for thyroidal or renal reasons. The repeated sample cohort was used to evaluate the longitudinal changes in CysC and creatinine as well as thyroid function parameters in early pregnancy among women with no known thyroid disease ($n = 68$), and results of sample 1 and 2 were compared using Wilcoxon signed rank test.

Analyses were performed using STATA version 17 (StataCorp, College Station, TX, USA).

Results

Altogether 1112 pregnant women were consecutively included in the study cohort corresponding to an 8.2% sample of the eligible cohort (Fig. 1). The study cohort was comparable to the eligible cohort on maternal characteristics (Table 1). From the study cohort, a reference cohort of 675 pregnant women was identified (Fig. 1). Women selected for the reference cohort tended to be younger and to have a lower body mass index, whereas no striking difference was observed regarding other maternal characteristics (Table 1).

Table 1 Maternal characteristics among pregnant women in the North Denmark Region Pregnancy Cohort who were part of the eligible cohort, the study cohort, and the reference cohort. Data are presented as *n* (%).

	Eligible cohort	Study cohort	Reference cohort
Pregnant women, *n*	13,575	1,112	675
Age, years			
<30	6930 (51.0)	594 (53.4)	378 (56.0)
≥30	6645 (49.0)	518 (46.6)	297 (44.0)
Born in Denmark[a]			
Yes	12,008 (88.7)	982 (88.6)	602 (89.2)
No	1533 (11.3)	127 (11.4)	73 (10.8)
Live births, *n*	12,641	1,026	675
Parity[a]			
Nulliparous	5949 (47.2)	499 (48.6)	326 (48.3)
Multiparous	6650 (52.8)	527 (51.4)	349 (51.7)
Prepregnancy BMI[a]			
<30 kg/m^2	10,552 (83.8)	844 (82.3)	577 (85.5)
≥30 kg/m^2	2039 (16.2)	182 (17.7)	98 (14.5)
Smoking in pregnancy[a]			
No	11,129 (88.1)	899 (87.7)	587 (87.1)
Yes	1500 (11.9)	126 (12.3)	87 (12.9)

[a]Missing values not included: born in Denmark ($n = 34$), parity ($n = 42$), BMI ($n = 50$), and smoking ($n = 12$).
BMI, body mass index.

Percentile levels for CysC and creatinine were established among all women and stratified by week of pregnancy (Table 2). For both markers of renal function, a decreasing trend for all percentile levels with increasing week of pregnancy was found, and results were to a large extent comparable when evaluated in the study cohort and in the reference cohort specifically (Table 2).

Considering the longitudinal changes in women with repeated blood sampling in early pregnancy (Table 3), these findings corroborated the dynamics observed in the established reference intervals. Thus, CysC and creatinine levels were lower in sample 2 compared with sample 1, and similarly maternal TSH decreased, whereas fT4 slightly increased (Table 3). No associations between levels of CysC and creatinine and the magnitude of a change in TSH and fT4 were found (data not shown).

When evaluating the association between levels of CysC and creatinine and maternal thyroid function parameters (Table 4) in the study cohort from single blood samples, higher percentile levels of CysC and creatinine associated with higher TSH among all pregnancies and live births specifically. On the other hand, no association with maternal fT4 was seen (Table 4). The associations were substantiated when

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Table 2 Percentiles (PC) with 90% confidence interval (CI) established in early pregnancy for cystatin C and creatinine within the study cohort and the reference cohort among all participants and stratified by weeks of pregnancy.

	Cystatin C (mg/L)								**Creatinine** (µmol/L)							
	All		Weeks 4–8		Weeks 9–11		Weeks 12–15		All		Weeks 4–8		Weeks 9–11		Weeks 12–15	
	PC	90% CI	PC	90% CI	PC	90% CI	PC	90% CI	PC	90% CI	PC	90% CI	PC	90% CI	PC	90% CI
Study cohort (*n*)	1112		121		846		145		1,112		121		846		145	
PC																
2.5th	0.54	0.53–0.55	0.59	0.57–0.62	0.54	0.53–0.55	0.52	0.49–0.55	42.3	41.3–43.1	46.7	46.1–48.7	42.3	41.1–43.4	40.6	38.5–42.4
5th	0.57	0.56–0.57	0.61	0.59–0.64	0.56	0.56–0.57	0.55	0.52–0.57	44.0	43.5–44.7	47.6	46.6–48.8	43.9	43.5–44.6	42.4	40.6–43.5
10th	0.60	0.59–0.61	0.65	0.63–0.67	0.60	0.59–0.61	0.58	0.57–0.59	46.4	45.7–46.9	49.1	48.1–50.8	46.0	45.5-46.9	44.8	42.7–46.4
25th	0.65	0.64–0.65	0.70	0.69–0.71	0.65	0.64–0.65	0.62	0.61–0.64	50.2	49.6–50.6	53.5	52.0–55.3	50.1	49.5–50.5	48.5	47.7–50.1
50th	0.71	0.70–0.71	0.76	0.74–0.77	0.70	0.69–0.71	0.68	0.67–0.69	54.7	54.1–55.1	58.3	56.8–59.4	54.4	53.9–54.9	52.8	51.9–54.1
75th	0.77	0.77–0.78	0.85	0.82–0.86	0.77	0.76–0.78	0.73	0.72–0.77	59.2	58.8–59.6	62.7	61.9–65.5	59.0	58.4–59.3	56.9	55.7–58.9
90th	0.85	0.84–0.86	0.90	0.87–0.91	0.84	0.83–0.85	0.82	0.79–0.86	64.5	63.3–65.3	69.3	66.3–71.1	64.1	63.1–64.9	61.5	60.2–65.1
95th	0.89	0.88–0.91	0.91	0.90–0.97	0.88	0.87–0.90	0.86	0.83–0.91	67.6	66.4–68.7	71.2	69.8–75.9	66.6	65.7–68.1	66.5	63.0–68.7
97.5th	0.93	0.91–0.95	0.96	0.91–1.09	0.93	0.91–0.96	0.91	0.86–0.97	70.6	69.4–71.2	75.6	71.2–88.3	69.5	68.4–70.8	68.7	66.5–73.7
Reference cohort (*n*)	675		64		530		81		675		64		530		81	
PC																
2.5th[a]	0.53	0.52–0.55	0.58	0.57–0.63	0.54	0.52–0.55	0.52	0.48–0.55[b]	41.6	40.8–43.1	46.9	46.6–48.1	42.0	40.8–43.5	38.8	38.4–41.3
5th	0.56	0.55–0.57	0.60	0.58–0.65	0.56	0.55–0.57	0.52	0.50–0.57	43.7	43.3–45.0	47.4	46.7–48.5	44.0	43.4–45.1	41.3	38.6–42.7
10th	0.59	0.58–0.60	0.64	0.59–0.68	0.59	0.58–0.60	0.57	0.53–0.59	46.3	45.5–47.1	48.4	47.4–50.8	46.4	45.6–47.1	42.8	42.3–45.4
25th	0.64	0.63–0.65	0.69	0.67–0.71	0.64	0.64–0.65	0.61	0.60–0.62	50.0	49.3–50.6	53.4	50.8–55.8	50.1	49.4–50.7	47.8	45.5–49.1
50th	0.70	0.69–0.70	0.75	0.72–0.78	0.70	0.69–0.70	0.66	0.64–0.68	54.2	53.8–54.9	58.6	56.5–60.3	54.2	53.8–54.9	51.9	50.7–52.8
75th	0.75	0.75–0.76	0.83	0.80–0.86	0.75	0.74–0.76	0.71	0.70–0.73	58.8	58.2–59.3	63.0	61.5–65.9	58.7	58.0–59.2	55.7	54.4–57.3
90th	0.83	0.82–0.84	0.89	0.86–0.91	0.82	0.80–0.83	0.77	0.73–0.81	63.2	62.6–64.6	68.4	65.9–71.2	63.0	62.0–64.2	61.0	58.0–63.0
95th	0.87	0.86–0.89	0.91	0.88–0.93	0.87	0.84–0.88	0.82	0.77–0.88	66.3	65.6–67.6	71.1	68.2–75.4	65.9	65.1–67.3	63.0	61.2–66.9
97.5th[a]	0.91	0.89–0.92	0.92	0.90–0.93	0.91	0.88–0.95	0.86	0.80–0.90[b]	69.0	67.7–71.2	73.0	70.1–75.9	68.4	67.2–71.2	66.4	62.8–67.3

[a]Lower and upper confidence limit held at minimum and maximum of the sample in the group 'Weeks 4–8' and 'Weeks 12–15'.

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Table 3 Maternal levels of cystatin C, creatinine, and thyroid function parameters in pregnant women ($n = 68$) with repeated blood sample in early pregnancy and no known thyroid disease. Data are presented as median (95 % CI).

	Sample 1	Sample 2	*P*[b]	Difference[a]
Pregnancy week of blood sampling	8 (8–8)	11 (11–12))	<0.01	3 (3–4)
Cystatin C (mg/L)	0.73 (0.70–0.77)	0.69 (0.68–0.71)	<0.01	−0.06 (−0.07; −0.04)
Creatinine (µmol/L)	57.0 (54.5–58.9)	53.0 (51.8–55.3)	<0.01	−3.1 (−4.4; −2.5)
TSH (mIU/L)	1.53 (1.23–1.80)	1.09 (0.90–1.34)	<0.01	−0.32 (−0.45; −0.10)
Free T4 (pmol/L)	15.9 (15.4–16.2)	16.1 (15.7–16.8)	0.02	0.4 (−0.08; 0.8)

[a]Calculated as the difference between result of sample 2 minus result of sample 1; [b]*P*-value is the result of comparison of sample 1 and sample 2 using Wilcoxon signed rank test.
TSH, thyroid-stimulating hormone.

CysC and creatinine were included as continuous independent variables (TSH and CysC: aβ 2.29 (95% CI: 1.44–3.62), TSH and creatinine: aβ 1.01 (95% CI: 1.01–1.02), fT4 and CysC: aβ 1.04 (95% CI: 0.97–1.11), fT4 and creatinine: aβ 1.001 (95% CI: 1.000–1.002). No nonlinear association was seen (TSH and CysC: $P = 0.2$; TSH and creatinine: $P = 0.1$; fT4 and CysC: $P = 0.3$; fT4 and creatinine: $P = 0.3$).

Finally, when evaluating the outcome of maternal biochemical thyroid autoantibody status and hypothyroidism in early pregnancy (Table 5), the frequency of maternal TPO-Ab and Tg-Ab positivity as well as autoimmune hypothyroidism increased with increasing percentile levels of CysC and creatinine, whereas no associations with nonautoimmune hypothyroidism were seen.

Discussion

This study was a large, regional investigation of Danish pregnant women performed to establish reference intervals for maternal CysC and creatinine within the early pregnancy and to evaluate the association between levels of these renal markers and maternal hypothyroidism. The principal findings revealed dynamics in the reference intervals for CysC and creatinine with a decreasing trend for increasing

Table 4 The association between maternal percentile (PC) levels of cystatin C and creatinine and thyroid-stimulating hormone (TSH) as well as free thyroxine (fT4) in the early pregnancy when evaluated among pregnant women with no known thyroid disease.

	All pregnancies ($n = 1079$)					**Live births** ($n = 994$)				
	n	Mean[a]	95% CI	aβ[b]	95% CI	*n*	Mean[a]	95% CI	aβ[c]	95% CI
TSH levels										
Cystatin C										
<25th PC	233	0.93	0.84–1.02	Ref.		218	0.91	0.83–1.01	Ref.	
25th–75th PC	509	1.01	1.01–1.15	1.14	1.02–1.28	470	1.05	0.99–1.13	1.12	1.00–1.26
>75th PC	337	1.27	1.18–1.37	1.31	1.16–1.47	306	1.25	1.15–1.35	1.25	1.10–1.43
Creatinine										
<25th PC	269	0.94	0.86–1.03	Ref.		249	0.93	0.85–1.02	Ref.	
25th–75th PC	524	1.13	1.06–1.20	1.17	1.05–1.30	488	1.11	1.04–1.18	1.14	1.02–1.28
>75th PC	286	1.22	1.11–1.31	1.22	1.09–1.37	257	1.17	1.07–1.27	1.17	1.03–1.32
fT4 levels										
Cystatin C										
<25th PC	233	16.3	16.1–16.5	Ref.		218	16.3	16.1–16.5	Ref.	
25th–75th PC	509	16.3	16.1–16.4	1.00	0.99–1.02	470	16.3	16.2–16.5	1.01	0.99–1.03
>75th PC	337	16.3	16.1–16.5	1.00	0.99–1.02	306	16.3	16.1–16.5	1.02	1.00–1.04
Creatinine										
<25th PC	269	16.1	15.9–16.3	Ref.		249	16.1	15.9–16.3	Ref.	
25th–75th PC	524	16.3	16.2–16.5	1.02	1.00–1.03	488	16.4	16.2–16.5	1.02	1.00–1.03
>75th PC	286	16.4	16.2–16.6	1.02	1.00–1.04	257	16.4	16.2–16.7	1.02	1.00–1.04

[a]Geometric mean; [b]Adjusted model included maternal age, pregnancy week of blood sampling, origin, diabetes, and thyroid autoantibody status; [c]Adjusted model included maternal age, pregnancy week of blood sampling, origin, diabetes, thyroid autoantibody status, body mass index, parity, and smoking status in the pregnancy.
aβ, adjusted exponentiated beta coefficient.

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Table 5 Frequency of maternal thyroid autoantibody positivity and hypothyroidism according to percentile (PC) levels of Cystatin C and creatinine in early pregnancy. Data are presented as *n* (%).

	All, *n*	Thyroid autoantibody positivity			Hypothyroidism	
		TPO-Ab[a]	Tg-Ab[a]	TPO-Ab and/or Tg-Ab[a]	AI[b]	Non-AI[c]
Cystatin C						
<25th PC	239	28 (11.7)	28 (11.7)	33 (13.8)	4 (1.7)[d]	7 (2.9)
25th–75th PC	523	53 (10.1)	69 (13.2)	81 (15.5)	20 (3.8)	18 (3.4)
>75th PC	350	51 (14.6)	58 (16.6)	68 (19.4)	26 (7.4)	10 (2.9)
Creatinine						
<25th PC	277	26 (9.4)	26 (9.4)[d]	33 (11.9)[d]	7 (2.5)[d]	4 (1.4)
25th–75th PC	539	64 (11.9)	78 (14.5)	92 (17.1)	22 (4.1)	20 (3.7)
>75th PC	296	42 (14.2)	51 (17.2)	57 (19.3)	21 (7.1)	11 (3.7)

[a]Cutoff for autoantibody positivity; TPO-Ab >60 U/mL, Tg-Ab >33 U/mL; [b]Autoimmune hypothyroidism; TSH above the pregnancy week-specific upper reference limit and TPO- and/or Tg-Ab positive; [c]Defined by TSH above the pregnancy week-specific upper reference limit and TPO- and Tg-Ab negative; [d]*P*-value < 0.05 for comparison between percentile groups using Chi-squared test.
AI, autoimmune; Tg-Ab, thyroglobulin antibodies; TPO-Ab, thyroid peroxidase antibodies.

gestational age. Considering the association with maternal thyroid disease, biochemically assessed maternal autoimmune hypothyroidism was more frequently observed with increasing levels of CysC and creatinine. The findings corroborate a link between renal markers and thyroid disease and add to the discussion regarding underlying mechanisms.

Pregnancy is a certain clinical situation and awareness on maternal as well as fetal health is important. Thus, close monitoring is critical, particularly in women with chronic disorders, but is challenged by the physiological changes in a normal pregnancy that may itself affect biochemical test results (1). Furthermore, the pregnant state may have an impact on the course of chronic diseases for example autoimmune diseases via the effect of pregnancy on the maternal immune system (11). These pregnancy-associated alterations necessitate the use of pregnancy-specific reference intervals for many biochemical analyses to avoid any misclassification of disease status in pregnant women. It is well-established that thyroid function test results show considerable alterations in pregnancy which is predominantly caused by the thyroid-stimulatory effect of hCG. Thus, maternal TSH physiologically decreases in the first trimester of pregnancy, and trimester-specific reference intervals are recommended (20). It has, however, also been shown that the alterations in maternal TSH show considerable dynamics within the first trimester of pregnancy with higher levels in the early pregnancy weeks 4–6 as compared to weeks 9–11 (3, 12). In the present study, we aimed to investigate the association between maternal hypothyroidism and markers of renal function in the first trimester of pregnancy. Considering the known dynamics in maternal TSH in early pregnancy, we first considered the reference intervals for CysC and creatinine within the first trimester of pregnancy. Previous studies on these renal markers primarily focused on each trimester of pregnancy and/or levels in pregnancy as compared to nonpregnant individuals. A substantial number of studies exist on the alterations in maternal creatinine in pregnancy and gathering of results in a systematic review showed 15–20% lower levels in each trimester of pregnancy as compared to nonpregnant controls (4). Less comprehensive data exist on the pregnancy-mediated alterations in CysC, but results indicate a similar trend as to that of creatinine with lower levels in pregnancy (6, 21). We found a dynamic trend in the reference intervals for CysC and creatinine within the first trimester of pregnancy with decreasing levels for increasing gestational age. The main underlying mechanism for lower levels of creatinine in pregnancy is considered the increase in creatinine clearance, which is seen already from week 5 to week 7 of pregnancy and thereby aligns with our results (1). The underlying mechanisms for pregnancy alterations in levels of CysC are less clear, and whereas the lower levels of creatinine are persistent throughout pregnancy, results have indicated that CysC may increase in later pregnancy (6, 21).

CysC is a small-molecule protein and an inhibitor of cysteine proteases, and it is produced at a constant speed in all nucleated cells (22). It is recommended as an alternative for estimation of the glomerular filtration rate when creatinine is deemed uncertain, e.g. in patients with reduced muscle mass (5). However, uncertainties also apply to CysC as a renal marker since levels of CysC may be altered for non-thyroidal reasons and guidelines on the use of CysC as a renal marker list the association with inflammation (elevated C-reactive

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protein), glucocorticoid treatment, and thyroid disease (5). This link between thyroid disease and CysC in the guidelines inspired the present study because we speculated on the underlying mechanisms. Previous studies were all performed in nonpregnant individuals and were from 2003 and onward. The initial study included 13 patients with hyperthyroidism and 9 patients with hypothyroidism and evaluated CysC prior to and after treatment (7). On the other hand, no thyroid-healthy control group was investigated. Gathering of 11 studies up until 2019 in a meta-analysis led to the conclusion that hyperthyroidism associates with higher levels of CysC whereas hypothyroidism associates with lower levels (10). However, the studies included showed considerable heterogeneity and small sample sizes, which led the authors to interpret with caution and call for further studies (10).

In more recent years, an increasing focus has been on levels of CysC in autoimmune thyroid disease. Thus, a study in children and adolescents (9) as well as in female nonpregnant adults (8) showed higher levels of CysC in patients with Hashimoto's thyroiditis irrespective of thyroid function status. In consistence with these findings, we here report among pregnant women that higher levels of CysC associated with a higher frequency of maternal biochemical hypothyroidism among women positive for thyroid autoantibodies. On the other hand, no such association was seen in thyroid antibody-negative women. Our study differs in that we assessed maternal thyroid function and thyroid autoantibody status in biobank samples drawn for non-thyroidal reasons, thus, including measures of unidentified thyroid function abnormalities (13). The fact that an association was seen only among thyroid autoantibody-positive women supports a link between CysC and thyroid autoimmunity. The predominant cause of hypothyroidism in women of reproductive age is autoimmune hypothyroidism (23), and smaller alterations in thyroid function among pregnant women who are thyroid autoantibody negative may reflect biochemical variation rather than actual thyroid disease (24).

We can only speculate on the underlying mechanism for an association between CysC and autoimmune hypothyroidism. First, we found a similar association with creatinine which may suggest a shared link with renal function. Evidence from nonpregnant adults suggests lower eGFR (and thereby higher creatinine) with increasing TSH as well as in individuals positive for TPO-Ab (25). Secondly, one may speculate on the role of CysC in the immune system and the link to autoimmune thyroid disease. Notably, increased levels of CysC have been described to shift the immune response toward a predominant type 1 T-helper (Th1) response, and decreased levels toward a type 2 T-helper (Th2) response (22). Following this line of thought, Hashimoto's thyroiditis (autoimmune hypothyroidism) has been considered a predominant Th1 response, whereas Graves' disease (autoimmune hyperthyroidism) has been linked to a Th2 response (26). Our findings in pregnant women and previous findings in children (9) and nonpregnant female adults (8) with higher levels of CysC in autoimmune hypothyroidism support this hypothesis, but further studies are needed to explore and substantiate the underlying mechanisms. Pregnant women with low TSH in early pregnancy were not included in our study, because their biobank samples were not available, thus, we could not examine the association between maternal hyperthyroidism and renal markers. Furthermore, evaluation of the subtype of maternal hypothyroidism (overt or subclinical) was not possible due small numbers in the stratified groups.

We studied an unselected cohort of Danish pregnant women in the North Denmark Region. The samples were drawn as part of the prenatal nationwide screening program for chromosomal anomalies and the rate of participation is high (27). We consecutively included more than 1000 biobank samples from the full cohort. However, numbers were small in some of the stratified analyses which may have influenced the robustness of our findings. The samples were stored at −80°C for up to 8 years until analyses, but thyroid hormones and thyroid autoantibodies as well as the renal markers investigated are shown to be stable for long-term frozen storage (28, 29). Furthermore, we measured CysC and creatinine in duplicate. Linkage to the Danish nationwide registers, enabled obtaining information on diseases and pregnancy complications used for the identification of a 'healthy' reference population. The validity of the Danish nationwide registers is considered high (30); however, it should be acknowledged that these data sources are indirect measures and that no contact with the patient or the responsible physician was part of the investigation.

Conclusion

In conclusion, reference intervals for CysC and creatinine are dynamic in the first trimester of

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pregnancy and decrease with increasing gestational age. High levels of both renal markers in early pregnancy associated with a higher frequency of biochemical autoimmune hypothyroidism in Danish pregnant women. The results corroborate a link between autoimmune hypothyroidism and higher levels of CysC and add to the discussion on the underlying mechanisms.

Declaration of interest

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the study reported.

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Author contribution statement

SLA and LH conceptualized the study, performed data analyses, and drafted the manuscript. All authors participated in the interpretation of data, critically reviewed the manuscript, and approved the final draft.

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