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CHAPTER 8

Prevalence and risk factors for asymptomatic bacteriuria in pregnancy: a prospective cohort study

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ABSTRACT

Objective
Support for a routine screen- and treat-policy for asymptomatic bacteriuria (ASB) in pregnancy is scarce and based on old data. To build the evidence for decision-making, we investigated prevalence, uropathogens and risk factors for ASB in pregnancy. In addition, we tested the predictive ability of risk factors to assess the feasibility of a targeted screening-program.

Design
Prospective cohort

Setting
We collected data between October 2011 and August 2013 in a cohort study in 13 hospitals and ultrasound centers in the Netherlands.

Population
Women with a singleton pregnancy between 16 and 22 weeks of gestation, were screened for the presence of ASB with urine dipslides. The dipslide was considered positive when ≥10e5 colony forming units (CFU)/mL of a microorganism were found.

Methods
Characteristics and risk factors were collected using a questionnaire. Univariable and multivariable logistic regression analysis were used to assess associations between risk factors and ASB.

Results
Data of 5,136 participating women were analyzed. ASB was present in 255 women (5%), while 13% of the dipslides were considered contaminated and excluded from further analysis. *Escherichia coli* was the most prevalent species (71%). Three risk factors were significantly associated with ASB in the multivariable analysis: pre-pregnancy body mass index (BMI) (OR 1.03, 95%CI1.01-1.1), a history of three or more previous urinary tract infections (UTI) (OR 1.3, 95%CI1.02-1.8) and conception with assisted reproductive technology (ART) (OR 2.0, 95%CI1.05-3.8).

Conclusions
Although pre-pregnancy BMI, three or more previous UTI and conception with ART are risk factors for ASB, their predictive ability is insufficient to recommend a targeted screening-program.

INTRODUCTION

Asymptomatic bacteriuria (ASB) is defined as significant bacteriuria without symptoms of a urinary tract infection (UTI) [1]. The term clinically significant bacteriuria is used to differentiate assumed bacteriuria of bladder origin (monoculture) from bacteriuria resulting from contamination during micturition (mixed culture)[2]. ASB occurs in 2-10% of pregnant women[3] although frequencies up to 40% have also been reported [4;5].

ASB in pregnancy is thought to be a risk factor for serious complications in both mother and child, such as pyelonephritis, preterm birth[6] and possibly low birth weight[7;8]. Therefore, many countries routinely screen all pregnant women for ASB in pregnancy and treat them with antibiotics[8-10]. The last few years, costs of healthcare are rapidly increasing[11], leading to re-evaluation of the cost-effectiveness of standard treatments in different subspecialties[12;13]. Whether a targeted screening program based on risk factors is a possible, thereby possibly reducing costs of healthcare, has not been evaluated before.

The feasibility and effectiveness of any screening program rely on valid estimates of ASB prevalence and risk factors [14;15 10]. Unfortunately, epidemiological data on incidence, risk factors and causative uropathogens are limited or outdated. Current knowledge is largely based on studies performed in the 60ies, 70ies and 80ies[16-19]. More recently published research was predominantly performed in non-western countries[4;5;20-22].

As a result, there is paucity of data on current prevalence and risk factors in modern western obstetric populations. We set out to obtain valid estimates of the prevalence, causing uropathogens and risk factors for ASB in pregnancy in a prospective, national cohort study. In addition, we tested the predictive ability of demographic risk factors to assess whether they could be used in a targeted screening-program.

METHODS

Study design and participants

This prospective multicenter cohort study was performed in eight hospitals and five ultrasound centers across the Netherlands. Women with a singleton pregnancy without symptoms of a urinary tract infection (UTI) were offered the possibility to be screened for ASB with the dipslide technique between 16 and 22 weeks of gestation. Excluded from participation in the study were women with a history of preterm delivery before 34 weeks, threatening preterm delivery, major fetal congenital malformations, use of antibiotics at time of screening, known G6PD deficiency, hypersensitivity to nitrofurantoin or risk factors for complicated UTI (pre-gestational diabetes mellitus, immunosuppressive medication, functional or structural abnormalities of the urinary tract) . The protocol was approved by the Ethics Committee of the Academic Medical Centre in Amsterdam (MEC 08/374) and the boards of all participating hospitals. All participants gave written informed consent.

Procedures
A single dipslide (UricultW, Orion Diagnostica) with two media (green cysteine lactose electrolyte deficient (CLED) medium on one side and red MacConkey medium on the other side) was used to diagnose ASB. The dipslide was inoculated with midstream urine and sent to the Laboratory for Infectious diseases in Groningen, the Netherlands.

Laboratory technicians read the dipslide after incubation for 2 to 3 days at room temperature (incubated while transported by mail). If on arrival at the laboratory no colonies were formed, the dipslide was incubated for another 24 hours at 35° Celsius. Dipslides were considered positive when the colony concentration was ≥ 10e5 CFU/mL of a single uropathogen or when two different colonies were present but one had a concentration of ≥10e5 CFU/mL. When more than two species were present, the urine was considered contaminated. The isolates of positive dipslides were determined by VITEK MS maldi-tof technology (BioMerieux).

Because of previously found associations with adverse neonatal outcomes and Group B S*treptococcus* (GBS) in pregnancy, all dipslides possibly containing GBS received further attention to identify GBS independent of growth density or possible contamination.

All participating women filled out a questionnaire containing questions about age, ethnicity, marital status, length, weight, education, smoking, alcohol- and drugs use, previous UTI, possible co-morbidity, obstetric history, inter-pregnancy interval (if applicable) and exclusion criteria.

Statistical analysis
Primary outcomes of interest in this study were prevalence, causing uropathogens and risk factors for ASB. Prevalence of ASB was estimated by counting all positive dipslides in the cohort. For the risk factor analyses, all cases with assumed contaminated urine were excluded. Univariable logistic regression was used to identify possible risk factors associated with the presence of ASB. Factors investigated were maternal age, non-European ethnicity, primigravida, short inter-pregnancy interval (<1 year), not living with a partner, body mass index (BMI), low education, smoking, alcohol use, drugs use, history of three or more previous UTI and conception with assisted reproductive technology (ART).

In the multivariable logistic regression analysis, all variables reaching a 0.1 significance level in the univariable logistic regression were retained in the model to evaluate conditional associations between the characteristics and ASB. We used backwards elimination to construct the final multivariable regression model, a p value <0.05 was used to indicate statistical significance. The associations are expressed as adjusted odds ratios (OR) with 95% confidence intervals (CI). To estimate the predictive ability of the full model, a receiver operating curve was constructed and the area under the curve was calculated. All analyses were performed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

RESULTS

Between October 2011 and August 2013, a total of 5,621 women participated in the ASB screening study of which 78.5% were included in ultrasound centers. A total of 372 women had to be excluded because of symptoms of a UTI at the time of screening (n=163) or antibiotic use within 2 weeks before screening (n=103), history of preterm birth before 34 weeks (n=72), diabetes mellitus (n=23), a twin pregnancy (n=7) or structural abnormality of the urinary tract (n=4). Furthermore, 113 women did not correctly hand in an inoculated dipslide and they were therefore excluded from analysis, leaving a final study group of 5,136 women.

Baseline characteristics of the cohort are presented in Table 1. The majority of the participants had their origins in Europe (82%). Women were on average 31 years old, with an average pre-pregnancy BMI of 22.8. Forty percent of the women were pregnant for the first time.

Table 1 | Baseline characteristics of the study population (n=5,139).

Characteristics		**N**	**(%)#**
Maternal age, mean (SD)		31.2	(4.6)
Ethnicity-	European	4230	(82.3)
	Surinamese	148	(2.9)
	Moroccan	124	(2.4)
	Asian	105	(2.0)
	Turkish	76	(1.5)
	Mixed*	263	(5.1)
	Other	193	(3.8)
Primigravida		2050	(40.2)
If multiparous; inter pregnancy interval ≤12 months		723	(23.7)
Not living with a partner		434	(8.5)
Pre-pregnancy BMI, median (IQR)		22.8	(20.8-25.5)
Morbid obesity (BMI ≥30)		405	(8.0)
Low education		407	(8.0)
Smoking		336	(6.6)
Alcohol use		69	(1.4)
Drugs use		11	(0.2)
Previous UTI	None	1959	(38.6)
	1-2 times	1701	(33.5)
	More than 3 times	1413	(27.9)
Comorbidity	Thyroid disease	119	(2.3)
	Lung disease	115	(2.2)

Table 1 | Continued

Characteristics		N	(%)[#]
Comorbidity	Psychiatric condition	93	(1.8)
	Bowel disease	77	(1.5)
	High blood pressure (pre-existing)	52	(1.0)
Conception	Spontaneously	4787	(93.9)
	IVF/ICSI[$]	135	(2.6)
	IUI[&]	92	(1.8)
	Other	85	(1.7)

[#] unless indicated otherwise

* Parents from different origins

[$] IVF= in vitro fertilization, ICSI= intracytoplasmic sperm injection

[&] IUI= intra uterine insemination

ASB was present in 255 women (5.0%), the dipslide was contaminated in 688 women (13%). *Escherichia coli* was the most prevalent species (182 women, 71%), followed by *Staphylococcus* species (24 women 9.4%) and *Enterococcus* species (19 women, 7.5%), Table 2.

Table 2 | Detected uropathogens of ≥10e5 CFU/mL.

Uropathogen	N	%
Escherichia Coli	182	71,4
Staphyloccus species	24	9,4
Enterococcus species	19	7,5
Klebsiella species	6	2,4
Citrobacter species	5	2,0
Proteus species	5	2,0
Enterobacter species	4	1,6
Pantoeae species	3	1,2
Acinetobacter species	3	1,2
Other[#]	4	1,6
Total	255[*]	100%

[#]= *Comamonas testosteroni*, *Sereatia marcescens* and two gram negative rod-shaped bacteria not otherwise specified.

*= none of the dipslides had more than one strain of ≥10e5 CFU/mL, therefore the number of positive dipslides matches the number of detected uropathogens.

Because of previously found associations with adverse neonatal outcomes and Group B S*treptococcus* (GBS) in pregnancy, all dipslides possibly containing GBS received further attention to identify GBS independent of growth density. GBS was present in 77 women (1.5%), but none had GBS count ≥10e5 CFU/ml. GBS was found as an isolated uropathogen (<10e5 CFU/mL) in 58 women and on dipslides with mixed flora in 19 women.

Univariable logistic regression analyses showed a significant association ($p<0.1$) between ASB and maternal age, not living with a partner, BMI, low education, smoking, three or more previous UTI and conception with ART (Table 3).

Table 3 | Univariable logistic regression analysis of possible risk factors for the development of ASB in pregnancy.

Possible risk factors	ASB positive (N=255)	ASB negative (N=4196)	OR	95% CI	*p*-value
Mean maternal age, (±SD)	30.5 (±4.8)	31.2 (±4.6)	0.97	0.94-0.997	0.03
Non-european origine	45 (18	712 (17)	1.05	0.75-1.5	0.78
Primigravida	110 (43)	1700 (41)	1.11	0.86-1.4	0.41
Interpregnancy interval < 1 year	29 (21)	582 (24)	0.83	0.54-1.3	0.37
Not living with a partner	28 (11)	338 (8.2)	1.41	0.94-2.1	0.097
Mean pre-pregnancy BMI (±SD)	24.1 (±4.2)	23.5 (±4.0)	1.03	1.01-1.07	0.022
Low education	27 (11)	311 (7.5)	1.48	0.98-2.2	0.065
Smoking	26 (10)	251 (6.0)	1.79	1.17-2.7	0.007
Alcohol use	2 (0.8)	58 (1.4)	0.56	0.14-2.3	0.43
Drugs use	0 (0.0)	7 (0.2)	0.12	0.00-13.3	0.34
3 or more previous UTI	84 (33)	1145 (28)	1.30	0.99-1.7	0.057
Conception with ART	11 (4.3)	107 (2.6)	1.72	0.91-3.2	0.095

In multivariate logistic regression analysis only three risk factors were significantly associated with ASB in pregnancy ($p<0.05$): BMI (OR 1.03, 95%CI 1.01-1.1), three or more previous urinary tract infections (OR 1.3, 95%CI 1.02-1.8) and conception with ART (OR 2.0, 95% CI 1.1-3.8, Table 4).

Table 4 | Multivariable logistic regression analysis of risk factors for ASB.

Full model	OR	95% CI	*p*-value
Maternal age	0.98	0.95-1.01	0.14
Not living with a partner	1.3	0.98-2.0	0.19
Pre-pregnancy BMI	**1.03**	**1.01-1.1**	**0.03**
Low education	1.1	0.72-1.8	0.57
Smoking	1.5	0.95-2.4	0.08
Three or more previous UTI	**1.3**	**1.02-1.8**	**0.04**
Conception with ART	**2.0**	**1.1-3.8**	**0.04**

Bold risk factors were significantly associated with ASB.

Prediction of ASB with the full model based on baseline characteristics was poor (area under the ROC curve of 0.57 (95%CI 0.54-0.61)). Of the 109 women with one or more risk factors for ASB, 6.0% were found to actually have ASB, which was higher than the ASB prevalence in the 146 women without risk factors (4.4%, p=0.007). However, still the majority of women with ASB did not have any of the risk factors present (146 women vs 109 women).

DISCUSSION

Main findings

We found that in a prospective contemporary cohort, 5% of pregnant women between 16 and 22 weeks of gestation have asymptomatic bacteriuria. *Escherichia coli* was the most prevalent species. Risk factors associated with ASB were BMI, a history of three or more previous UTI and conception with ART. Despite associations between ASB and risk factors, the prediction of ASB with these risk factors was poor and the majority of women with ASB had no risk factors. A targeted screening strategy based on risk factors for ASB is therefore not feasible.

Strengths and limitations

Strengths of this study are the design with prospectively collected data and the relatively large sample size. In the Netherlands around 70% of pregnant women receive antenatal care at a midwifery practice. We chose to use a dipslide with two different media as a convenient alternative for the conventional culture (98.0% sensitivity and 99.6% specificity for detecting ASB in pregnancy)[23]. An advantage of the dipslide is that it could be used in all settings e.g. hospitals, ultrasound centers and midwifery practices, thereby creating a representative sample of the Dutch obstetric population. Found prevalence of ASB and causing uropathogens are consistent with previous research[24].

All dipslides were sent to one laboratory with experience in interpreting dipslides. Consistency in interpretation of the dipslides and diagnosis of ASB was therefore guaranteed.

Contamination of dipslides can contribute to both under- and over diagnosis of ASB. We chose to use midstream urine instead of clean catch midstream urine as it was shown that midstream samples were equivocal to clean catch midstream samples in pregnant women[25]. The 13% contamination found in our study is lower than the proportion of contaminated dipslides in a previous study (27%)[23] and lower than in previous studies using ordinary cultures, 22%[23] and 17%[26].

Baseline characteristics, exclusion criteria and possible risk factors used in this study were self-reported by the women before they handed in the dipslide. Recall bias may have occurred for instance for the history of previous UTI. Although the women were able to fill out the questionnaire in privacy, true prevalence of smoking, alcohol use and drugs use during pregnancy could be underestimated. Since not all caregivers ask about current use of antibiotics or symptoms of a UTI at the time of screening, we decided to add these items to the questionnaire. A total of 163 women reported to have complaints of a UTI and 103 women reported to have used antibiotics in the last two weeks prior to screening. However, women's own knowledge about UTI and treatment have shown to be limited[27], possibly antifungal treatment or antiviral treatment may have been confused by the women for antibiotic treatment leading to over estimation of antibiotic use

Interpretation

A relationship between BMI and symptomatic UTI has been described before in various patient groups [28-30]. In pregnancy, a BMI of 35 or higher was associated with an increased risk of symptomatic UTI[31]. The same association was shown in this study between ASB and increasing BMI. This finding is in contrast with the findings of a small Iranian study where no significant relation with BMI and ASB was found[32]. Differences with our study is the time of screening (all gestational ages) and the sample size. In pregnant Nigerian women with HIV, previous UTI was associated with an increased risk of ASB in pregnancy [33] as was in our study.

Our data show that ART was independently associated with ASB. An association between fertility treatments and ASB was reported before by Sheiner *et al*.[34] who studied 4,890 women with ASB from a single center in Israel. They found that women with ASB underwent fertility treatments (not otherwise specified) more often (OR 1.7, 95%CI 1.4-2.0). The reason for this association is not clear, possibly the procedure with ovum pick up and embryo transfer back in to the womb affects migration of bacteria towards the urinary tract or women with fertility problems have different micro biomes than women without fertility problems[35] but this is all speculative.

Conclusion

Our data indicate that ASB is present in 5% of pregnant women between 16 and 22 weeks of gestation in the Netherlands. Although some maternal factors are associated with ASB, the prediction of ASB with baseline characteristics is poor. It is therefore difficult to clearly point out a high-risk group to use for a targeted screening program.

The feasibility of a universal screening program in modern time depends not only on the current prevalence of ASB but also on the rate and severity of the associated consequences. A cost evaluation study reported that screening for pyelonephritis is appropriate when the prevalence of asymptomatic bacteriuria is greater than 2%, and the incidence of pyelonephritis is above 13%[11]. Associations between ASB and preterm birth and low birth weight were all reported in literature[10] before introduction of ultrasound in dating pregnancies. Whether the alleged consequences of ASB are still associated with and common enough in modern time to justify universal screening programs needs to be further evaluated.

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