SHORT COMMUNICATION

BJC

British Journal of Cancer (2016) 115, 588–591 | doi: 10.1038/bjc.2016.150

Keywords: breast cancer; conisation; cohort study; epidemiology; human papilloma virus; incidence; standardised incidence ratio

Conisation as a marker of persistent human papilloma virus infection and risk of breast cancer

Mette Søgaard[1], Dora K Farkas[1], Anne G Ording[1], Henrik T Sørensen[1] and Deirdre P Cronin-Fenton*[,1]

[1]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus N, 8200 Aarhus N, Denmark

Background: Human papillomavirus (HPV) infection may increase breast cancer (BC) risk.

Methods: To examine this, we used nationwide medical registries to identify all Danish women who underwent conisation to remove HPV-associated cervical precancerous lesions ($n = 87\,782$) from 1978 to 2013. We computed the absolute risk of BC and standardised incidence ratios (SIRs) and 95% confidence intervals (95% CIs) for breast cancer, based on national breast cancer incidence rates.

Results: Conisation was associated with slightly increased BC incidence (SIR = 1.1, 95% CI = 1.0–1.1), and an absolute BC risk of 7.7% (95% CI = 7.3–8.1%) in 35.9 years of follow-up. BC risk was elevated throughout follow-up, especially in the first 5 years (<1 year: SIR = 1.2, 95% CI = 0.92–1.5; 1–5 years: SIR = 1.2, 95% CI = 1.1–1.3; ⩾5 years: SIR = 1.1, 95% CI = 1.0–1.1). Women who underwent conisation and had autoimmune disease had elevated BC risk after 5 years of follow-up (SIR = 1.4, 95% CI = 1.0–1.8).

Conclusions: BC risk is slightly elevated in women with persistent HPV infection, possibly due to detection bias.

Conisation is a surgery that removes abnormal cervical lesions—cervical intraepithelial neoplasia. Human papilloma virus (HPV) infection is associated with almost all cervical cancers; conisation is therefore a definitive marker of HPV infection (Gosvig *et al*, 2015). HPV DNA (oncogenic subtypes 16 and 18) can transform normal breast cells into a growth factor-independent phenotype (Dimri *et al*, 2005). HPV DNA and koilocytes—hallmarks of HPV infection—have been identified in breast tumours (Lawson *et al*, 2009, 2016). HPV may therefore contribute to breast carcinogenesis, although the underlying biology is poorly understood (Ohba *et al*, 2014; Vieira *et al*, 2014). Most HPV cervical infections resolve untreated (Jaisamrarn *et al*, 2013), but persistent infection warranting treatment intervention may signify impaired immune function (Bosch and Munoz, 2002).

Three meta-analyses have investigated the association between HPV infection and breast cancer (BC), including 9, 10, and 16 case–control studies, respectively (Li *et al*, 2011; Simoes *et al*, 2012; Zhou *et al*, 2015). The summary effect estimates suggested three- to six-fold increased BC incidence among women with HPV infection. Each called for large studies on HPV and BC incidence. The existing research has limited follow-up, small sample size, and no information on comorbid diseases, which may compromise immune function (de Visser *et al*, 2006). A large Norwegian study suggested a 10% increased risk of *in situ* and localised but not metastatic breast tumours among women with precancerous cervical lesions (Hansen *et al*, 2012).

Given the high incidence of BC in developed countries, any association between HPV and BC risk requires confirmation. We therefore conducted a large nationwide population-based study using prospectively collected data from Danish registries to investigate the association between cervical conisation as a marker of chronic HPV infection and risk of BC.

MATERIALS AND METHODS

The Danish National Health Service guarantees tax-supported health care for all residents. Health service utilisation is recorded in nationwide registries using each resident's unique personal

*Correspondence: Dr DP Cronin-Fenton; E-mail: dc@clin.au.dk

Received 1 March 2016; revised 18 April 2016; accepted 30 April 2016; published online 2 June 2016

identifier, which permits linkage across registries (Schmidt *et al*, 2014). We used the Danish National Patient Registry (DNPR), covering all Danish hospitals (Schmidt *et al*, 2015), to identify all women aged ⩾18 years who underwent cervical conisation during the study period (Supplementary Appendix 1). All surgical procedures are registered in the DNPR according to the 'Danish Classification of Surgical Procedures and Therapy'. Inpatient diagnoses were available from 1978 to 1994 and both in and outpatient diagnoses thereafter. Through linkage to the Civil Registration System, which contains data on vital status and emigration (Schmidt *et al*, 2014), and to the Danish Cancer Registry (DCR), which records information on all incident malignancies (Storm, 1988), we followed women from their conisation date to incident BC, death, emigration, or 30 November 2013, whichever came first. We also retrieved information on cancer stage (localised *vs* non-localised disease). We used the Danish Pathology Registry (Erichsen *et al*, 2010) to retrieve data on oestrogen receptor (ER) status at BC diagnosis among women who underwent conisation (1997 onwards when pathology data was available). We retrieved information from the DNPR on comorbidities diagnosed before conisation, and classified these using the Charlson Comorbidity Index (CCI) (Charlson *et al*, 1987). We used the DNPR to retrieve information on clinical diagnoses of autoimmune disease, obesity and alcohol-related disease (Supplementary Appendix 1).

We computed the expected number of BCs using national incidence rates by age (1-year bands) and diagnosis year (1-year periods) multiplied by person-years of follow-up. We computed standardised incidence ratios (SIRs) for BC as the ratio of observed to expected cancers, overall, and at 1, 1–5, and >5 years (Rothman *et al*, 2008). We stratified results by age at conisation, year of conisation, comorbid diseases, and CCI (excluding cancer diagnoses) (Supplementary Appendix 2). In a subgroup analysis, we computed BC risk according to ER status, and BC stage at diagnosis. This study was approved by the Danish Data Protection Agency (#1-16-02-1-08).

RESULTS

Overall, 87 782 women underwent conisation in 1978–2013; 90% were aged <50 years at conisation (median age = 33.8 years, interquartile range: 28.3–41.1 years). Most patients had no comorbidities (CCI = 0); 3% had autoimmune disease or obesity, and 2% had alcohol-related disease (Table 1).

Women who underwent conisation had increased BC risk (SIR = 1.1, 95% CI = 1.0–1.1, and absolute risk of 7.7%, 95% CI = 7.3–8.1%) over a maximum follow-up of 35.9 years (median = 15.5, interquartile range: 7.9–24.1 years). BC risk increased with age at conisation up to age 70 years, with highest SIRs among women aged 50–69 years (SIR = 1.2, 95% CI = 1.1–1.3). BC risk was elevated in the first 5 years of follow-up (<1 year of follow-up: SIR = 1.2, 95% CI = 0.92–1.5; 1–5 years of follow-up: SIR = 1.2, 95% CI = 1.1–1.3), attenuating thereafter (⩾5 years follow-up: SIR = 1.1, 95% CI = 1.0–1.1). Stratified analyses revealed no effect modification by CCI, obesity, or alcohol diagnoses, but increased BC risk associated with autoimmune disease especially after 5 years of follow-up (SIR = 1.4, 95% CI = 1.0–1.8) (Table 2). The risk of localised BC remained slightly elevated throughout follow-up; that of non-localised disease was elevated only up to 5 years after conisation. The results of analyses stratified by ER status (1997–2013) indicated increased risk of ER positive BC, but not ER negative disease, or, of BCs with unknown ER status, regardless of follow-up time. We observed similar findings in sensitivity analyses including inpatients and outpatients (Supplementary Table 1).

Table 1. Baseline characteristics of the cohort of women who underwent conisation in Denmark between 1978 through 2013

Characteristic	*n*	%
Total study population	87 782	100.0
Age at conisation (years)		
18–29	28 786	33
30–49	50 638	58
50–69	7652	9
⩾70	706	0.8
Year of conisation		
1978–1982	11 727	13
1983–1987	10 803	12
1988–1992	11 973	14
1993–1997	12 677	14
1998–2002	13 584	15
2003–2007	12 354	14
2008–2013	14 664	17
Charlson Comorbidity Index score		
Low (0)	81 523	93
Moderate (1–2)	5727	7
Severe (⩾3)	532	0.6
Autoimmune disease		
No	84 882	97
Yes	2900	3
Obesity diagnosis		
No	85 341	97
Yes	2441	3
Alcohol-related disease		
No	85 871	98
Yes	1911	2

DISCUSSION

Our large population-based cohort study suggests slightly elevated BC risk associated with a history of cervical conisation. Among women diagnosed with local stage breast tumours, risk remained elevated regardless of follow-up time. Our findings may therefore be partly attributable to more intense disease surveillance, that is, women who undergo conisation are more likely to avail themselves of other cancer screening procedures, such as mammography (Hansen *et al*, 2012; Corkum *et al*, 2013). In addition, BC risk increased with longer follow-up, particularly among women with a history of autoimmune disease.

The increased BC risk among women with a history of autoimmune disease is intriguing, and counters the perception that autoimmune disease correlates with decreased BC incidence (Hemminki *et al*, 2012). However, our finding may reflect reports of an increased risk of cervical dysplasia/cancer among individuals with autoimmune disease (Kim *et al*, 2015). The mechanisms underlying this association are unclear but could involve increased healthcare contact among patients with autoimmune conditions. The observed association also may reflect use of immunosuppressive drugs or steroids—indicated for autoimmune disease—which may facilitate HPV immunoevasion. However, our previous research suggests no association between glucocorticoid use and BC risk (Sorensen *et al*, 2005, 2012). Our findings provide important rationale to promote uptake of the HPV vaccine among women with autoimmunities (Kim *et al*, 2015).

The increased BC risk among those younger at conisation may be due to heightened screening. However, it may also reflect findings by Lawson *et al* (2016), where BC patients with a history of cervical cancer were on average 10 years younger at BC diagnosis than those without cervical cancer.

Table 2. Standardised incidence ratios of breast cancer among women who underwent conisation in Denmark between 1978 through 2013 according to follow-up time

	Overall		<1 year		1–5 years		>5 years	
	n	SIR	*n*	SIR	*n*	SIR	*n*	SIR
Overall	2694	1.1 (1.0, 1.1)	71	1.2 (0.92, 1.5)	334	1.2 (1.1, 1.3)	2289	1.1 (1.0, 1.1)
Age at conisation (years)								
0–29	401	1.0 (0.94, 1.2)	4	2.5 (0.68, 6.4)	18	1.4 (0.84, 2.2)	379	1.0 (0.92, 1.1)
30–49	1870	1.1 (1.0, 1.1)	45	1.3 (0.92, 1.7)	208	1.2 (1.0, 1.3)	1617	1.0 (0.99, 1.1)
50–69	405	1.2 (1.0, 1.3)	21	1.0 (0.63, 1.6)	99	1.3 (1.0, 1.5)	285	1.1 (1.0, 1.3)
70+	18	0.89 (0.53, 1.4)	1	0.45 (0.01, 2.5)	9	1.2 (0.57, 2.4)	8	0.74 (0.32, 1.5)
Year of conisation								
1978–1982	819	1.1 (1.0, 1.2)	7	1.0 (0.42, 2.1)	38	1.1 (0.78, 1.5)	774	1.1 (1.0, 1.2)
1983–1987	571	1.0 (0.94, 1.1)	8	1.2 (0.52, 2.4)	37	1.1 (0.76, 1.5)	526	1.0 (0.93, 1.1)
1988–1992	491	1.0 (0.92, 1.1)	10	1.1 (0.54, 2.1)	47	1.1 (0.81, 1.5)	434	1.0 (0.90, 1.1)
1993–1997	379	1.1 (0.96, 1.2)	8	0.86 (0.37, 1.7)	50	1.1 (0.80, 1.4)	321	1.1 (0.95, 1.2)
1998–2002	248	1.1 (0.94, 1.2)	14	1.4 (0.79, 2.4)	60	1.3 (0.95, 1.6)	174	1.0 (0.86, 1.2)
2003–2007	136	1.3 (1.1, 1.5)	10	1.2 (0.59, 2.3)	67	1.5 (1.2, 2.0)	59	1.1 (0.85, 1.5)
2008–2013	50	1.2 (0.92, 1.6)	14	1.3 (0.68, 2.1)	35	1.3 (0.87, 1.7)	1	0.78 (0.02, 4.3)
Charlson Comorbidity Index								
Low (CCI = 0)	2560	1.1 (1.0, 1.1)	61	1.2 (0.88, 1.5)	304	1.2 (1.1, 1.4)	2195	1.1 (1.0, 1.1)
Medium (CCI = 1–2)	126	1.1 (0.91, 1.3)	10	1.5 (0.72, 2.8)	28	1.1 (0.74, 1.6)	88	1.1 (0.85, 1.3)
High (⩾3)	8	0.93 (0.40, 1.8)	0		2	0.70 (0.1, 2.5)	6	1.2 (0.46, 2.7)
Autoimmune disease								
No	2629	1.1 (1.0, 1.1)	65	1.1 (0.87, 1.4)	323	1.2 (1.1, 1.4)	2241	1.0 (1.0, 1.1)
Yes	65	1.3 (1.0, 1.7)	6	2.2 (0.80, 4.7)	11	1.0 (0.51, 1.8)	48	1.4 (1.0, 1.8)
Obesity diagnosis								
No	2674	1.1 (1.0, 1.1)	70	1.2 (0.92, 1.5)	329	1.2 (1.1, 1.3)	2275	1.1 (1.0, 1.1)
Yes	20	0.88 (0.54, 1.4)	1	0.51 (0.01, 2.9)	5	1.9 (0.93, 3.6)	14	1.1 (0.65, 1.7)
Alcohol-related disease								
No	2667	1.1 (1.0, 1.1)	70	1.2 (0.92, 1.5)	324	1.2 (1.1, 1.3)	2270	1.1 (1.0, 1.1)
Yes	30	1.3 (0.84, 1.8)	1	0.76 (0.02, 4.2)	10	1.9 (0.93, 3.6)	19	1.1 (0.65, 1.7)
Oestrogen receptor positive	377	1.2 (1.1, 1.3)	27	1.4 (0.90, 2.0)	121	1.4 (1.2, 1.7)	229	1.1 (0.99, 1.3)
Oestrogen receptor negative	75	0.94 (0.74, 1.2)	3	0.49 (0.10, 1.4)	29	1.1 (0.76, 1.6)	43	0.89 (0.64, 1.2)
Localised breast cancer	1414	1.1 (1.1, 1.2)	35	1.2 (0.84, 1.7)	170	1.3 (1.1, 1.5)	1209	1.1 (1.1, 1.2)
Non-localised breast cancer	1144	1.0 (0.94, 1.1)	32	1.1 (0.78, 1.6)	155	1.2 (1.0, 1.4)	957	0.97 (0.91, 1.0)

Abbreviations: CCI = Charlson Comorbidity Index; SIR = standardised incidence ratio.

Strengths of our study include its population-based design in a country with unfettered access to healthcare and complete follow-up. Data were prospectively collected for administrative purposes, minimising bias. The completeness of BC diagnoses in the DCR and the Danish Pathology Registry approaches 100% (Storm *et al*, 1997; Erichsen *et al*, 2010). Use of personal identity numbers facilitated individual-level data linkage across the registries (Son *et al*, 2014). We observed little difference after stratifying by alcohol-related diagnoses and obesity, so confounding due to such factors seems unlikely.

The Norwegian registry-based study (Hansen *et al*, 2012), observed a 10–50% increased BC risk in women with precancerous cervical lesions, which they attributed to detection bias, as they observed no increased risk of metastatic BC. Our study extends this research incorporating information on comorbidities and hormone receptor status.

The validity of our estimates depends on several factors. We had information on outpatient conisation from 1995, thus our study is prone to non-differential misclassification of conisation between 1978 and 1995. However, a sensitivity analysis restricted to the cohort who underwent conisation from 1995 onwards yielded similar findings. We had no information on HPV subtype, which may modify BC risk (Dimri *et al*, 2005). However, the oncogenic HPV subtypes (16, 18, 33, and 35) are those most likely to lead to persistent infection, for which conisation is indicated in Denmark. We lacked information on parity. Multi-parity correlates with increased cervical cancer risk (Jensen *et al*, 2013), but with decreased BC risk (depending on age at first birth) (Rosner *et al*, 1994). Thus adjusting for parity may attenuate our findings.

BC imposes a substantial burden on health and health services. Contrary to the three- to six-fold increased risk of BC associated with HPV infection observed in meta-analyses (Li *et al*, 2011; Simoes *et al*, 2012; Zhou *et al*, 2015), our findings suggest a long-term slight increase in BC risk among women with a history of chronic HPV infection. Priorities for future research involve evaluating the association of HPV subtypes with BC risk and investigating the incidence of BC among individuals vaccinated for HPV.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Danish Cancer Society (R73-A4284-13-S17); Aarhus University Research Foundation; and Program for Clinical Research Infrastructure (PROCRIN) established by the Lundbeck Foundation and the Novo Nordisk Foundation (HTS). The funding agencies had no role in the study design, collection, analysis, or interpretation of the data.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Bosch FX, Munoz N (2002) The viral etiology of cervical cancer. *Virus Res* **89**(2): 183–190.

Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* **40**(5): 373–383.

Corkum M, Hayden JA, Kephart G, Urquhart R, Schlievert C, Porter G (2013) Screening for new primary cancers in cancer survivors compared to non-cancer controls: a systematic review and meta-analysis. *J Cancer Surviv* **7**(3): 455–463.

de Visser KE, Eichten A, Coussens LM (2006) Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* **6**(1): 24–37.

Dimri G, Band H, Band V (2005) Mammary epithelial cell transformation: insights from cell culture and mouse models. *Breast Cancer Res* **7**(4): 171–179.

Erichsen R, Lash TL, Hamilton-Dutoit S, Bjerregaard B, Vyberg M, Pedersen L (2010) Existing data sources for clinical epidemiology: the Danish National Pathology Registry and Data Bank. *Clin Epidemiol* **2**: 51–56.

Gosvig CF, Huusom LD, Deltour I, Andersen KK, Duun-Henriksen AK, Madsen EM, Petersen LK, Elving L, Schouenbourg L, Iftner A, Svare E, Iftner T, Kjaer SK (2015) Role of human papillomavirus testing and cytology in follow-up after conization. *Acta Obstet Gynecol Scand* **94**(4): 405–411.

Hansen BT, Nygard M, Falk RS, Hofvind S (2012) Breast cancer and ductal carcinoma *in situ* among women with prior squamous or glandular precancer in the cervix: a register-based study. *Br J Cancer* **107**(9): 1451–1453.

Hemminki K, Liu X, Ji J, Forsti A, Sundquist J, Sundquist K (2012) Effect of autoimmune diseases on risk and survival in female cancers. *Gynecol Oncol* **127**(1): 180–185.

Jaisamrarn U, Castellsague X, Garland SM, Naud P, Palmroth J, Del Rosario-Raymundo MR, Wheeler CM, Salmeron J, Chow SN, Apter D, Teixeira JC, Skinner SR, Hedrick J, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, Bosch FX, de Carvalho NS, Germar MJ, Peters K, Paavonen J, Bozonnat MC, Descamps D, Struyf F, Dubin GO, Rosillon D, Baril L. HPV PATRICIA Study Group (2013) Natural history of progression of HPV infection to cervical lesion or clearance: analysis of the control arm of the large, randomised PATRICIA study. *PloS One* **8**(11): e79260.

Jensen KE, Schmiedel S, Norrild B, Frederiksen K, Iftner T, Kjaer SK (2013) Parity as a cofactor for high-grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up. *Br J Cancer* **108**(1): 234–239.

Kim SC, Glynn RJ, Giovannucci E, Hernandez-Diaz S, Liu J, Feldman S, Karlson EW, Schneeweiss S, Solomon DH (2015) Risk of high-grade cervical dysplasia and cervical cancer in women with systemic inflammatory diseases: a population-based cohort study. *Ann Rheum Dis* **74**(7): 1360–1367.

Lawson JS, Glenn WK, Heng B, Ye Y, Tran B, Lutze-Mann L, Whitaker NJ (2009) Koilocytes indicate a role for human papilloma virus in breast cancer. *Br J Cancer* **101**(8): 1351–1356.

Lawson JS, Glenn WK, Salyakina D, Clay R, Delprado W, Cheerala B, Tran DD, Ngan CC, Miyauchi S, Karim M, Antonsson A, Whitaker NJ (2016) Human papilloma virus identification in breast cancer patients with previous cervical neoplasia. *Front Oncol* **5**: 298.

Li N, Bi X, Zhang Y, Zhao P, Zheng T, Dai M (2011) Human papillomavirus infection and sporadic breast carcinoma risk: a meta-analysis. *Breast Cancer Res Treat* **126**(2): 515–520.

Ohba K, Ichiyama K, Yajima M, Gemma N, Nikaido M, Wu Q, Chong P, Mori S, Yamamoto R, Wong JE, Yamamoto N (2014) *In vivo* and *in vitro* studies suggest a possible involvement of HPV infection in the early stage of breast carcinogenesis via APOBEC3B induction. *PloS One* **9**(5): e97787.

Rosner B, Colditz GA, Willett WC (1994) Reproductive risk factors in a prospective study of breast cancer: the Nurses' Health Study. *Am J Epidemiol* **139**(8): 819–835.

Rothman KJ, Greenland S, Lash TL (2008) *Modern Epidemiology,* 3rd edn. Lippincott Williams & Wilkins: Philadelphia.

Schmidt M, Pedersen L, Sorensen HT (2014) The Danish Civil Registration System as a tool in epidemiology. *Eur J Epidemiol* **29**(8): 541–549.

Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT (2015) The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol* **7**: 449–490.

Simoes PW, Medeiros LR, Simoes Pires PD, Edelweiss MI, Rosa DD, Silva FR, Silva BR, Rosa MI (2012) Prevalence of human papillomavirus in breast cancer: a systematic review. *Int J Gynecol Cancer* **22**(3): 343–347.

Son J, Park JW, Lambert PF, Chung SH (2014) Requirement of estrogen receptor alpha DNA-binding domain for HPV oncogene-induced cervical carcinogenesis in mice. *Carcinogenesis* **35**(2): 489–496.

Sorensen GV, Cronin-Fenton DP, Sorensen HT, Ulrichsen SP, Pedersen L, Lash TL (2012) Use of glucocorticoids and risk of breast cancer: a Danish population-based case-control study. *Breast Cancer Research* **14**(1): R21.

Sorensen HT, Mellemkjaer L, Skriver MV, Lash TL, Olsen JH, Baron JA (2005) NO excess risk of breast cancer among female users of systemic glucocorticoids. *Cancer Epidemiol Biomarker Prev* **14**(4): 1022–1023.

Storm HH (1988) Completeness of cancer registration in Denmark 1943–1966 and efficacy of record linkage procedures. *Int J Epidemiol* **17**(1): 44–49.

Storm HH, Michelsen EV, Clemmensen IH, Pihl J (1997) The Danish Cancer Registry–history, content, quality and use. *Dan Med Bull* **44**(5): 535–539.

Vieira VC, Leonard B, White EA, Starrett GJ, Temiz NA, Lorenz LD, Lee D, Soares MA, Lambert PF, Howley PM, Harris RS (2014) Human papillomavirus E6 triggers upregulation of the antiviral and cancer genomic DNA deaminase APOBEC3B. *MBio* **5**(6): pii: e02234-14.

Zhou Y, Li J, Ji Y, Ren M, Pang B, Chu M, Wei L (2015) Inconclusive role of human papillomavirus infection in breast cancer. *Infect Agent Cancer* **10**: 36.

Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)