Annual Outpatient Hysteroscopy and Endometrial Sampling (OHES) in HNPCC/ Lynch Syndrome (LS)

Introduction and background

Lynch Syndrome (LS) or HNPCC is a heritable syndrome caused by a mutation in one of the DNA mismatch repair (MMR) genes: MLH1, MSH2, MSH6, and PMS2.[1] Strict family history based diagnostic criteria (Amsterdam Criteria (AC)-II)[2] are used to identify people at increased risk. LS women have a 40-60% life time risk for endometrial cancer (EC). Some data suggest EC risk may be higher than that of colorectal cancer (CRC)[3,4] and greater for MSH2 and MSH6 than for MLH1 carriers.[5,4,6,3] Gynaecological surveillance with endometrial sampling has been recommended from 30-35 years age in women who wish to delay/avoid risk-reducing surgery.[5,7] Unlike screening for CRC, the efficacy of EC screening remains unproven with only a handful of published series evaluating different modalities for EC screening in LS. These include: (a) Transvaginal (TVS) and/or transabdominal ultrasound;[8-10]; (b) TVS and endometrial sampling;[11,9] and (c) Hysteroscopy.[12,13]

The possibility of using hysteroscopy as a screening modality in LS was initially suggested in 2002.[14] However, no screen detected cancers have been reported in the published literature In this paper we report on our initial experience of annual outpatient hysteroscopy and endometrial sampling (OHES) as a screening strategy for EC in women with LS and compare it with TVS alone.
Methods

The familial gynaecological cancer clinic at University College London Hospital is a tertiary clinic for managing women at ‘high-risk’ of gynaecological cancers. All women attending the clinic are managed by a multidisciplinary team, which includes gynaecological oncologists, clinical geneticist, clinical psychologist, radiologist, clinical nurse specialist, minimal access gynaecologist and pathologist. All women attending the clinic undergo detailed pedigree based risk-assessment and counselling. They receive comprehensive advice on advantages and disadvantages of risk-reducing surgery and screening, as well as reproductive and life style issues.

High-risk women with LS are identified on the basis of the diagnostic AC-II criteria[2] or the presence of a MMR gene mutation. LS women >35-40 years, who have completed their family, are offered risk-reducing surgery: total laparoscopic hysterectomy (TLH), bilateral salpingo-oophorectomy (BSO) and peritoneal washings. Women >35 years are offered screening for ovarian cancer within the United Kingdom Familial Ovarian Cancer Screening Study (UKFOCSS).

Since October-2007 eligible LS women attending the clinic are offered EC screening with TVS and OHES. Inclusion criteria are: LS women >30 years age. Exclusion criteria are: current pregnancy, prior hysterectomy, women opting for risk-reducing surgery or those negative on predictive testing for a known familial mutation. Women keep a menstrual calendar and undergo annual TVS with endometrial thickness (ET) measurement and OHES. TVS is performed by experienced ultrasonographers with over 10 years scanning experience. Hysteroscopy is carried out by one of a team of 3 experienced hysteroscopists, in an office setting, as described previously.[15]
Endometrial sampling is undertaken in all women using guided biopsies/polypectomy where indicated and/or a Pipelle device (Laboratoire C.C.D, France) at the end of the procedure.[15] Any irregular, heavy or unscheduled vaginal bleeding reported is investigated by bringing forward the annual OHES. Histological specimens are processed in total by a dedicated senior pathologist (EB). All data are stored on a bespoke database.

The current analysis includes all OHES procedures between October-2007 and March-2010. The primary outcome was EC and atypical endometrial hyperplasia (AEH). Inclusion of AEH as an outcome was based on it being an established premalignant lesion, lack of concordance in the diagnosis of AEH or EC between pathologists,[16] and 42.6% of AEH having concurrent EC.[17] Wherever necessary, case notes, histopathology and TVS/biochemistry reports were reviewed. A screen positive on TVS is defined as (a) ET >5mm (measuring both endometrial layers): postmenopausal women,[18] (b) ET >12mm (measuring both endometrial layers in the second week of the cycle): premenopausal women,[10] (c) presence/suspicion of a polyp, or (d) irregular endometrium with fluid in the cavity. Although a cut off of 4 mm for endometrial thickness has been used by some to triage symptomatic postmenopausal women for hysteroscopy, we use a cut off of 5mm in our practice, which is consistent with that advocated by a number of other institutions, a meta-analysis[18] and systematic review.[19] In addition, our experience of modelling endometrial thickness in asymptomatic post-menopausal women indicates the best performance characteristic may be obtained at a cut off of 5.1mm.[20] Cut-offs for endometrial thickness in asymptomatic premenopausal women unlike those for postmenopausal women are not well-defined. The 12mm cut off was chosen on the
basis of an earlier published report in the literature which evaluated TVS for screening in these women.[10] A screen positive at OHES is defined by the histology report.

Statistical analysis was undertaken using SPSS 12.0.1. The Mann-Whitney test was used to compare age distributions between groups after reviewing histograms. Fisher’s test was used to calculate the difference between proportions. Kaplan Meier curves and the Log Rank Test was used to evaluate any difference in time to diagnosis. Two sided p values are reported for all statistical tests. Confidence intervals for a single proportion were calculated using the statistics package ‘Measuring Usability’ (J Sauro) LLC, Denver, Colorado, USA. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), negative likelihood ratio (NLR) and positive likelihood ratio (PLR) were used to compare the screening performance for TVS and OHES.

**Results**

Between March-2004 and March-2010, 69 women with LS (fulfilling AC-II )[2] attended the clinic, of whom 25 were known mutation carriers (12MLH1, 12MSH2, 1PMS2). Thirteen women preferred risk-reducing surgery, five moved away, two were <30 years age and two tested negative for a familial mutation. The remaining 47 women opted for EC screening. Four eventually declined OHES and two have an appointment scheduled (Figure-1). The characteristics of the remaining cohort (41) are described in Table-1.
Between October-2007 and March 2010, 41 prevalent and 28 incident screens (49.2 women screen years) were performed. The screening outcomes of the cohort are described in Figure-1. There was one failed outpatient hysteroscopy procedure which was completed under a general anaesthetic.

At prevalence screen, 39 were asymptomatic and 2 complained of irregular periods with intermenstrual bleeding. One of the symptomatic women (benign polyp) and 6 of the 39 (15.4%) asymptomatic women had an abnormal TVS (2 endometrial polyps, 3 ET>12mm, 1 irregular endometrium/fluid in the cavity) of whom 1 (polyp) had EC at histology. Three asymptomatic women were detected to have EC/AEH on OHES. Two of these three women with EC/AEH, underwent hysterectomy because of prevalence screen detected EC/AEH and 6 women opted for risk-reducing surgery after their initial prevalent screen. TVS and OHES in these 6 women were normal.

22 eligible women underwent incident screen-1 (Figure-1). Of these, 3 (13.6%) presented with abnormal bleeding at 9-11 months from last screen. One had an in-situ levonorgestrel intrauterine system (LNG-IUS, Mirena® Schering Health Care). None had EC/AEH. All three had a normal TVS. Of the 19(86.4%) asymptomatic women, TVS was abnormal in 5(26.3%) which included the one woman with EC. This woman underwent hysterectomy following incident screen detected EC. Six of the 22 incident screen-1 women, underwent a subsequent second incident screen (incident screen-2) during the study time-period (Figure-1). Of these five were asymptomatic with normal TVS. However, endometrial hyperplasia (EH) was found at OHES in two of these cases.
Overall 3 women (2 prevalence, 1 incident screen) had EC and one woman had AEH (prevalence screen) (Table-2). The latter includes one woman initially diagnosed as AEH who had a stage1a, grade1 EC at TLH BSO. Three of four women with screen detected endometrial pathology were MMR mutation carriers. No adjuvant treatment was needed for any of the three EC cases who remain recurrence free at 10, 16 and 22 months follow-up respectively. The woman with AEH opted for conservative management with a LNG-IUS (Mirena®, Schering Health Care, UK). Follow-up OHES 4 months later was normal with no histological evidence of atypia/EC. No interval cancers have occurred in the cohort to date.

Combining incident and prevalent screens, for EC/AEH, OHES had similar specificity 89.8%(95%CI 79.2%, 96.2%) but higher PLR 9.8(95%CI 4.6,21) and lower NLR (0 ) compared to TVS: specificity 84.75%(95%CI 73%, 92.8%), PLR 3.28(95%CI 1.04,10.35) and NLR 0.59(95%CI 0.22,1.58) (Table-3).

The median age of women with EC/AEH was 40.9 years (IQR 5.3 years) and the median age for the rest of the screened cohort was 43 years (IQR 12.3 years). EC/AEH cases were not found to differ from the rest of the cohort with respect to mutation status (p=0.64), number of screens (p=0.70) or age (Mann Whitney, p=0.40). However, mutation carriers undergoing screening had a significantly younger median age (39.7years, IQR 6.3) than the remaining AC-II positive women (44.2years, IQR12.8) (p=0.022). Kaplan Meier and Log Rank analysis of time to diagnosis for EC/AEH did not show any difference between known MMR carriers and those fulfilling AC-II alone (p=0.85).
Additional pathology found in this cohort included 6 endometrial polyps (3 prevalent and 3 incident). The annual incidence rate was 3.57% (95% CI 0.1, 18.35) for EC/AEH, 10.71% (95% CI 2.27, 28.23) for polyps, 7.1% (95% CI 0.88, 23.5) for EH (Table-4).

Discussion

This is to our knowledge the first series reporting the diagnosis of early stage EC in asymptomatic LS women using hysteroscopy based screening. Our findings indicate that OHES has high specificity and PLR and low NLR. The higher annual incidence rate of EC in our cohort (3.57%; 95% CI 0.09, 18.35) compared to the expected rate of approximately 1% in this population suggests that there may be lead time. OHES seems superior to TVS alone as a test for detecting EC/AEH in these women (Table-3), with all four women with EC/AEH being detected by OHES compared to TVS which only detected two. The data further support the current guidelines that recommend that endometrial sampling should be undertaken in all LS women.[21,7]

TVS alone had a PLR of 3.28 for EC/AEH and NLR of 0.59 for EC/AEH. In contrast, OHES achieved an overall PLR of 9.8 for EC/AEH and a NLR of 0. Likelihood ratio combines information about sensitivity and specificity to assess test performance. It is independent of prevalence and permits comparisons across different types of tests. A value close to 1 has little clinical significance as the post-test probability (odds) of disease is little different from the pre-test probability. A PLR of >5 indicates moderate and >10 strong probability for disease being present when a test is positive.
A PLR of >5 is generally a pre-requisite for adoption of a clinical test or procedure A
NLR of <0.2 is moderately and <0.1 strongly indicative of the disease being absent.
The results suggest that TVS alone as a screening test has poor ability to detect or rule
out disease and is not very helpful in clinical decision making, while OHES has good
diagnostic ability to detect disease and excellent ability to rule out disease in LS,
making it an effective test for endometrial screening. For comparison the PLR and
NLR for mammography are 9.4 and 0.19[22], and for CT pulmonary angiogram in
diagnosing pulmonary embolism are 8.6 and 0.06 respectively.[23] OHES
performance in asymptomatic women in our series is consistent with the previously
reported performance in symptomatic women (PLR 60.9, NLR 0.15) for diagnosing
EC,[24] with a negative hysteroscopy reducing EC probability to 0.6%.[24]

TVS alone would have missed 2 of 4 cases of EC/AEH, and did not seem to add to
the performance of OHES. Our findings are consistent with a previous series using
TVS and endometrial sampling which found that 55%(6/11) of the screen detected
EC, 50%(2/4) AEH and 50% of complex hyperplasia (4/8) cases had a normal
TVS.[11] A majority of these false negative cases were post-menopausal. An increase
in screen detected EC/AEH cases has been reported when TVS guided screening was
replaced by a combined approach of TVS and endometrial sampling.[9] Overall our
findings and these data suggest that in contrast to symptomatic postmenopausal
general population women, the performance of TVS for detecting endometrial
pathology in asymptomatic (pre and postmenopausal) LS women undergoing
screening is poor. Reliable cut-offs for pre-menopausal and asymptomatic post-
menopausal LS women are unknown. Two previous series have compared TVS alone
to a combination of TVS and endometrial sampling and found the latter to be better at
detecting EC/AEH.[9,11]

There are limitations to use of Pipelle endometrial sampling alone, including
variability in its performance with menopausal status and type of pathology.[25]
Reliable performance characteristics in asymptomatic LS women (significant
proportion of who are premenopausal) are not available. Cancers have been missed on
Pipelle alone, with three of the 11 cases of EC not detected on endometrial sampling
in a previous LS series.[11]

The median age of our cohort was 42.9 (95%CI 39.4, 49.7) years. Known MMR
carriers undergoing screening were significantly younger than those fulfilling AC-II
alone. This may reflect increased screening awareness among gene carriers and
increased uptake of preventative surgery by older mutation carriers. The median age
(40.9 years, IQR38.3, 43.5) of women diagnosed with EC/AEH in our study is
significantly lower than that reported in two previous series using TVS and
endometrial sampling: (a) 51.5 years (IQR 47,54, p=0.016),[11] median age of the
entire cohort not reported and (b) 51 years (IQR 46,55, p=0.008 Mann-Whitney) with
median age of cohort 46 years.[9] Our median age at diagnosis is also lower than that
reported in epidemiological data (48-50 years),[3,26] though it lies within the
expected range (27-72 years) found in LS.[26,3] The small size of our data set makes
it difficult to draw conclusions. If confirmed, age differences could be related to a
combination of age and risk factor profile of our cohort and the lead time of
screening.
The strengths of our study include a strict screening protocol with prospectively collected data, longitudinal follow-up and description of outcomes for both prevalence and incident screens. The main weakness is the small size of the cohort and the need for validation in larger multicentre studies. It is to be noted that previous reports on LS screening using hysteroscopy have involved up to 62 patients and no EC were detected. Although we have compared TVS and OHES, the standard practice in a number of institutions is screening using a combination of TVS and endometrial sampling. TVS alone has a poor detection rate for endometrial pathology. A limitation of our study is a lack of ability to directly compare OHES with TVS and endometrial sampling. Future research in this area should be directed towards addressing this issue. Given the small sample size in most centres, this will necessitate a multicentre international study.

We are not aware of any reports on incidence rate of endometrial pathology in LS women. We found that 1 in 5 (incidence rate 21.4%; 95% CI 8.3, 40.1) LS women had endometrial pathology with an annual incidence of polyps of 11%. This included one women with adenocarcinoma in a polyp. Our findings are in keeping with randomised trials in the general population which have shown that TVS and Pipelle have lower sensitivity for focal lesions like polyps compared to hysteroscopy.[27,28] Hysteroscopy detects lesions which may be missed by both TVS and Pipelle[28,27,15] and also permits directed biopsy from a focal lesion.

In the general population, up to 5% of non-atypical EH and 30% of AEH progress to EC.[29] It is unknown if the rates of progression are greater in the LS population with a much higher a priori risk for EC. We can speculate that subsequent EC may have
been prevented or delayed in some of the cases of polyps/hyperplasia treated in our series. EC/AEH was found in 9.76% (95%CI 2.7, 23.1) cases which is consistent with reports in the literature,[11,10] though higher than rates reported in some series.[8,12,13]

Both Hysteroscopy and Pipelle endometrial sampling are effective, easy to perform and well tolerated as outpatient procedures. Our failure rate of 1.45% (95%CI 0.04, 7.81) is consistent with those in systematic reviews (4.2%, 95%CI 3.9, 4.5).[24] The compliance with annual screening suggests that this is acceptable. A recent study evaluating acceptability of screening, found no significant difference in pain scores between either hysteroscopy or endometrial sampling.[30] The efficacy and patient acceptability of OHES is similar to the in-patient procedure and the former is more cost-effective.[31,32] Preliminary data suggest that hysteroscopy-based endometrial screening in LS does not appear to be associated with any psychological morbidity.[33]

**Conclusion**

LS women have a high incidence of polyps, premalignant lesions and EC. Initial data suggest that an OHES based surveillance strategy has good performance characteristics for detecting early stage EC/AEH in LS and may be superior to that of TVS alone. However, definitive data would require a larger international study. The challenges are the relatively small numbers of LS women at individual centres, the rates of risk reducing surgery and differences in use of routine outpatient hysteroscopy between countries.
Acknowledgements

We are particularly grateful to all our patients. We acknowledge the support and help provided by Ms S.Chamberlain (secretary), Ms S.Parker and Ms E.Palmer (nurse hysteroscopists) as well as The Eve Appeal. A large portion of this work was done at UCLH/UCL within the “Women’s Health Theme” of the National Institute for Health Research UCLH/UCL CBRC supported by the Department of Health.
Disclosure of interests

IJ has consultancy arrangements with Becton Dickinson, who have an interest in tumour markers and ovarian cancer. IJ and UM have a financial interest in Abcodia, Ltd., a company formed to develop academic and commercial development of biomarkers for screening and risk prediction. IJ is a member of the board of Abcodia Ltd and Women’s Health Specialists Ltd. ANR has received honoraria from Fujirebio Diagnostics for giving lectures and attending meetings on the use of biomarkers in ovarian cancer management, but none were directly related to this work. ES received honoraria from Ethicon for provision of training to healthcare professionals; this was not related to this work. The other authors declare no conflict of interest.

Contribution to authorship

RM, AA and MJ were involved in initial data collection. RM, UM were involved in analysis, drafting and writing of the paper. IJ, ANR, LS, ES, AA, SG, MJ, CB, EB contributed to writing of the manuscript. UM, IJ, ANR, RM, CB, LS, SG, ES, were responsible for the clinical care of the patients. EB reviewed the histological specimens and contributed to the histopathological sections of the manuscript. RM and UM performed the statistical analysis and contributed to writing the statistical sections of the manuscript. The final draft was prepared by RM, UM, IJ and approved by the others.

Details of ethics approval

The project was referred to the Chair of the Research Ethics committee (University College London Hospital, Research Deanery). Under the Research Governance
Framework the project was deemed to be a clinical audit, and permission for data analysis and submission for publication was given on 26/04/2010.

**Funding**

This work has not been directly funded by any commercial organisation, charity, or other sources. A large portion of this work was done at UCLH/UCL within the “women’s health theme” of the NIHR UCLH/UCL comprehensive biomedical research centre (CBRC) supported by the Department of Health.

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References

**Table 1: Characteristics and outcome of Lynch Syndrome (LS) women undergoing OHES screening for endometrial cancer**

*One patient had a MLH1 VUS (variant of uncertain significance)*

AC–Amsterdam Criteria, AEH-atypical endometrial hyperplasia, OHES-Outpatient Hysteroscopy and Endometrial Sampling, IQR-Interquartile range

**Table 2- Screening details in cases of screen detected abnormal endometrial pathology**

* Benign endometrial polyps were diagnosed at OHES alone on 2 consecutive screens

** Diagnosis confirmed following total laparoscopic hysterectomy and bilateral salpingo-oophorectomy

AEH-atypical endometrial hyperplasia, BSO-Bilateral salpingo-oophrectomy; EC-Endometrial cancer; EP-endometrial polyp; ECxP-endocervical polyp; Gr-Grade, OHES -Outpatient Hysteroscopy and Endometrial Sampling; P-prevalent screen; I-incident screen; MMR-mismatch repair gene, TLH-Total Laparoscopic Hysterectomy, VUS-variant of uncertain significance
Table-3: Performance Characteristics of OHES and TVS

CI-confidence interval, FN-false negative, FP-false positive, I-incident, NLR-negative likelihood ratio, NPV-negative predictive value, OHES-Outpatient Hysteroscopy and Endometrial Sampling, P-prevalent, PLR-positive likelihood ratio, PPV-positive predictive value, TN-true negative, TP-true positive, TVS-transvaginal scan

Table 4- Abnormal pathology rates (per patient) at Prevalent and Incident screening in the cohort

* Endometrial polyps diagnosed on 2 consecutive screens in one case
** One case with endometrial cancer also had a malignant endometrial polyp
# Includes 22 women, 6 of whom underwent a third round of annual screening

Figure 1 legend: Consort flow chart for the cohort