Screening and risk reducing surgery for endometrial or ovarian cancers in Lynch syndrome: a systematic review

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HIGHLIGHTS

- Endometrial and ovarian cancer rates were highest in MLH1 and MSH2 carriers, respectively
- Endometrial biopsy had a sensitivity of 57.1% and the number needed to screen was 23–380 (median 78)
- Risk reducing surgery could be offered based on genetic pathogenic variant

ABSTRACT

Objective Lynch syndrome is a hereditary cancer syndrome caused by mismatch repair gene mutations, and female carriers are at an increased risk of endometrial and ovarian cancer. The best approach to screening is not yet clear and practice varies across countries and centers. We aimed to provide evidence to inform the best approach to screening and risk reduction.

Methods A systematic search of the literature was conducted (Medline, Embase, PubMed). Studies evaluating the following were included: women with Lynch syndrome (by mismatch repair mutation or Amsterdam II criteria), screening methods for endometrial and/or ovarian cancer, intervention included endometrial biopsy, transvaginal ultrasound, or serum cancer antigen 125 (CA-125), outcomes evaluated were number of cancers and/or endometrial hyperplasia.

Results A total of 18 studies of Lynch syndrome carriers which screened for endometrial cancer using transvaginal ultrasound and/or hysteroscopy/endometrial biopsy revealed an incidence of 3.9% at the time of screening. Most (64.1%) endometrial cancers detected were from screening, with the balance detected in symptomatic women at the first screening visit, regular review, or between screening intervals. In mismatch repair carriers, the overall sensitivity of endometrial screening was 66.7%, and the number needed to screen ranged between 4 and 38 (median 7). The sensitivity of endometrial biopsy was 57.1% and the number needed to screen was 23–380 (median 78). The sensitivity of transvaginal ultrasound was 34.4% and the number needed to screen was 35–973 (median 170). Fourteen studies which screened for ovarian cancer using transvaginal ultrasound and/or CA-125 revealed an incidence of 1.3% at the time of screening and 42.9% of ovarian cancers were detected at asymptomatic screening. The sensitivity of ovarian screening was 54.6%, and the number needed to screen was 9–191 (median 23) in mismatch repair carriers. Thirteen studies reported 5.8% incident endometrial cancers and 0.5% ovarian cancers at time of risk reducing surgery.

Conclusions There is limited evidence to support screening for endometrial and ovarian cancer in Lynch syndrome and data on mortality reduction are not available. Further prospective, randomized trials comparing targeted screening methods are needed. Risk reducing surgery remains the most reliable way to reduce endometrial and ovarian cancer risk in Lynch syndrome.

INTRODUCTION

Lynch syndrome is an autosomal dominant hereditary cancer syndrome caused by mutations in mismatch repair genes (MLH1, MSH2, MSH6, PMS2). Epithelial cell adhesion molecule (EPCAM) can also predispose to MSH2 deficient cancers. Women have 13–47% lifetime risk of endometrial cancer, and 3–17% risk of ovarian cancer, depending on the mismatch repair gene. The management of gynecological cancer risk involves screening and/or risk reducing hysterectomy and salpingo-oophorectomy. The Manchester International Consensus Guidelines and Mallorca Group recommend risk reducing surgery in all mismatch repair carriers except for PMS2 carriers, without screening, while the US Multi-Society Task Force recommends screening for endometrial cancer by annual endometrial biopsy from 35 to 40 years.

Only one previous systematic review has evaluated the benefits of gynecological cancer screening in Lynch syndrome. This included five studies and concluded there was insufficient evidence to support screening for either endometrial or ovarian cancer. Our review provides an updated analysis of the evidence for the effectiveness of endometrial and ovarian cancer screening and risk reducing hysterectomy and bilateral salpingo-oophorectomy in cancer prevention.
METHODS

Endometrial and Ovarian Cancer Screening
Medline (Ovid), Embase, and PubMed databases were searched in August 2020 using relevant medical subject headings and keywords (Online Supplemental Table 1). Reference lists were searched for relevant articles. The same search was updated in November 2021. Articles meeting all of the following criteria were included: women with Lynch syndrome (by mismatch repair mutation or Amsterdam II criteria), screening methods for endometrial and/or ovarian cancer, intervention included endometrial biopsy, transvaginal ultrasound, or serum cancer antigen 125 (CA-125), and those where outcomes were number of cancers and/or endometrial hyperplasia.

Articles meeting any of the following criteria were excluded: personal history of endometrial or ovarian cancer, non-Lynch syndrome hereditary cancer syndromes, other screening methods, screening was not for gynecological cancers, outcomes were cost effectiveness of screening or patient perception of screening, those not published in English, or did not contain patient data.

Risk Reducing Surgery
Medline (Ovid), Embase, and PubMed databases were searched in August 2020 using relevant medical subject headings and keywords. Reference lists were searched for relevant articles. The same search was updated in November 2021. Similar inclusion and exclusion criteria to the above were applied detailed in Online Supplemental Table 1).

RESULTS

Endometrial and Ovarian Cancer Screening
The search from August 2020 to November 2021 identified 338 studies meeting the inclusion criteria. After removing 95 duplicates and excluding 194 results through abstract screening, 43 full text articles were assessed for eligibility and six abstracts were included. Eleven full text articles were included, and four additional articles were added through reference searching. Hence 21 articles were included in the analysis (PRISMA diagram presented in Online Supplemental Figure 1).

Of the 21 studies included, nine were retrospective and 12 were prospective (Online Supplemental Table 2). Eleven studies screened for both endometrial and ovarian cancers (Table 1). The age of patients screened ranged from 18 to 84 years. All studies used transvaginal ultrasound, and additional screening methods included CA-125 (12 studies), routine endometrial sampling (14 studies), and routine hysteroscopy (four studies) (Table 1).

Rates of Endometrial Cancer Detected by Screening
Eighteen studies of endometrial cancer screening detected a total of 104 cancers among 2688 women (3.9%), diagnosed between ages 36–72 years (Figure 1).16–27 A total of 1193 of 2688 (44.4%) patients had confirmed mismatch repair/EPCAM mutations and 1495 of 2688 (55.6%) were identified through Amsterdam criteria (Figure 1).

A total of 78 of 1193 (6.5%) mismatch repair/EPCAM carriers were diagnosed with endometrial cancer (Figure 1), representing 75% of all endometrial cancers found. Fifty of 78 (64.1%) patients were detected through asymptomatic screening at their first or subsequent screening visit, while the remainder were diagnosed due to symptoms at or between screening intervals (Figure 1). Twenty-six of 1495 women (1.7%) with Lynch syndrome diagnosed through Amsterdam criteria (or where genetic testing information was not published) were diagnosed with endometrial cancers, representing 25% of all endometrial cancers. Fourteen of 26 (53.8%) of these were through screening, while the remainder were diagnosed due to symptoms, or between screening intervals (Figure 1).

Fifty of 57 (70.2%) cases of endometrial hyperplasias were found in mismatch repair carriers, 50% of which were in MLH1 carriers (Online Supplemental Table 3). The number needed to screen, defined as the number of people needed to be screened for a diagnosis of cancer or hyperplasia, ranged between 4 and 135 (median 13) (Online Supplemental Table 4). This reduced to between 4 and 38 (median 7) when only mismatch repair carriers were included. In the mismatch repair carrier population, the sensitivity of screening to detect endometrial cancer (excluding hyperplasia) was 66.7%.

Combining studies from Table 2 where sufficient data were provided to inform the number of cancers or hyperplasia detected by each screening method, endometrial biopsy found 20 of 64 endometrial cancers detected via screening in total (65% stage I, 15% stage II, 5% stage III, remainder unreported) and 29 hyperplasias of 36 detected via screening in total. The sensitivity and specificity of endometrial biopsy in detecting cancer (excluding hyperplasia) were 57.1% and 97.7%, respectively. Number needed to screen, defined as the number of endometrial biopsies required to detect cancer or hyperplasia, ranged between 12 and 380 (median 19) (Online Supplemental Table 4), and between 23 and 380 (median 78) in detecting cancer only. Transvaginal ultrasound detected 11 endometrial cancers (81.1% stage I, remainder unreported) and seven cases of hyperplasia in total. Sensitivity and specificity in detecting endometrial cancer was 34.4% and 87.1%, respectively. The number needed to screen to detect either endometrial cancer or hyperplasia by transvaginal ultrasound ranged between 35 and 973 (median 89); this range remained the same to detect cancer only, however, the median increased to 170 (Online Supplemental Table 4). In studies of mismatch repair carriers only, two8,9 studies provided sufficient data to inform sensitivity. The sensitivity of endometrial biopsy and transvaginal ultrasound were 79.3% and 53.8%, respectively. In three studies13,19,22 which specified cancers detected by hysteroscopy, no additional cancers were detected when hysteroscopy was performed with endometrial biopsy.

Rates of Ovarian Cancer Detected by Screening
Fourteen studies detected 29 cancers among 2224 women (1.3%), diagnosed between 35 and 83 years old (Online Supplemental Figure 3).11,12,14,15,20,21,23–30 Twenty-eight of 1458 (1.9%) mismatch repair/EPCAM carriers were diagnosed with ovarian cancer, representing 96.6% of all ovarian cancers found. Twelve of 28 (42.9%) were in asymptomatic women detected through screening at their first or subsequent screening visit (Online Supplemental Figure 3). Of these, 11 of 12 (91.7%) were detected through transvaginal ultrasound and 3 of 12 (25%) were detected through CA-125. A total of 58.3% of ovarian cancers detected in gene carriers through screening were stage I, 25% were stage II, 8.3% were stage III, and 8.3% did not have staging reported. Eight of 28 were interval ovarian cancers which presented between regular screening visits; 50.0% were stage I, 12.5% stage II (12.5%), 25.0% stage III,

Lim N, et al. Int J Gynecol Cancer 2022;0:646–655. doi:10.1136/ijgc-2021-003132
and 12.5% had no reported staging. Interval ovarian cancers are defined as ovarian cancers which present clinically between regular screening visits. They are either cancers missed by screening tests defined as ovarian cancers which present clinically between regular screening visits, or cancers which rapidly developed between screening intervals. Two incident ovarian cancers were added through reference searching. Hence 13 articles were included, and two additional articles were added through abstract screening, 48 full text articles were assessed for eligibility. Eleven articles were included, and two additional articles were added through reference searching. Hence 13 articles were

Table 1  Screening programs for gynecological cancer and participant characteristics across studies which studied outcomes of gynecological cancer screening in female Lynch syndrome carriers

| Authors                     | Recommended age to commence screening (years) | Age (mean or median [range]) (years) | MMR mutation carrier status (%) | Cancers screened for | Screening method | Screening interval |
|-----------------------------|---------------------------------------------|--------------------------------------|---------------------------------|---------------------|-----------------|-------------------|
| Dove-Edwin et al10           | 30–35                                       | UK: 40 (24–64) Netherlands: 42 (23–68) | AC: 171 (Non-AC/AC-II: 98)      | EC                  | TVUS            | 1–2 years         |
| Rijken et al11              | 30–35                                       | 37 (27–60)                           | MMR: 11 (27%)                   | EC + OC             | GE + TVUS + CA-125; curettage if positive TVUS | Annually          |
| Renkonen-Sinisalo et al12    | 30–35                                       | MMR: 175 (100%)                      | EC + OC                         | Varied between institutions. GE + TVUS + CA-125 + EB | 2–3 years |
| Lecuru et al13              | Not provided                               | 42                                   | MMR: 13 (21%)                   | EC                  | EB + hysteroscopy | Annually          |
| Gerritzen et al14           | 30                                          | 46 (23–72)                           | MMR: 67 (87%)                   | EC + OC             | GE + TVUS + CA-125 + ES if indicated; routine ES from 2006 | Annually          |
| Jarvinen et al15            | 35                                          | MMR carriers: 36 (18–72) Non-carriers: 42 (18–72) | MMR: 103 (100%)                | EC + OC             | TVUS + EB        | 2–3 years         |
| Lecuru et al16              | 30                                          | 42.5                                 | MMR: 14 (24%)                   | EC + OC             | TVUS + EB        | Not provided      |
| Guillen-Ponce et al17       | Not provided                               | Not provided                         | Not provided                    | EC                  | GE + TVUS + EB   | Not provided      |
| Bats et al18                | Not provided                               | 41                                   | Not provided                    | EC                  | GE + pelvic US + hysteroscopy; EB reference standard | Not provided      |
| Arts-De Jong et al16        | 30                                          | Not provided                         | MMR: 123 (87.9%)                | OC                  | TVUS + CA-125    | Annually          |
| Manchanda et al19           | 30                                          | 43                                   | MMR: 16 (39%)                   | EC                  | TVUS + EB + hysteroscopy | Annually | |
| Stuckless et al14           | Not provided                               | 36                                   | MSH2: 54 (100%)                 | EC + OC             | TVUS + CA-125 + EB | Not provided      |
| Helder-Woolderink et al17   | 30                                          | Period I: 38 (26–61)  Period II: 41 (23–67) | MMR: 44 (69%)                   | EC + OC             | TVUS + CA-125, ES and hysterectomy if indicated, routine ES from 2008 | Annually          |
| Douay-Hauser et al12        | 30                                          | Not provided                         | Not provided                    | EC                  | GE + TVUS + EB   | Not provided      |
| Ketabi et al15              | 25                                          | 39 (19–78)                           | LS (family with confirmed MMR): 236 (27%) | EC + OC             | GE + TVUS; EB + CA-125 if TVUS abnormal | 2 years |
| Tzortzatos et al14          | 30                                          | 50 (24–84)                           | MMR: 45 (100%)                  | EC + OC             | TVUS + CA-125 + EB | Annually          |
| Gosset et al25              | Not provided                               | 51                                   | MMR: 191 (100%)                 | EC + OC             | GE + pelvic US + hysteroscopy | Annually       |
| Nebgen et al26              | Not provided                               | 39.2 (25.5–73.7)                     | MMR: 56 (70%)                   | EC + OC             | GE + EB for EC; TVUS + CA-125 for OC | Annually         |
| Rosenthal et al23           | 35                                          | Nil age range provided for MMR carriers only | MMR: 65 (100%)                 | OC                  | TVUS + CA-125    | Annually          |
| Rosenthal et al23           | 35                                          | Nil age range provided for MMR carriers only | MMR: 120 (100%)                | OC                  | TVUS annually + CA-125 every 4 months | Annually and every 4 months |
| Eikenboom et al27           | 30–35 prior to 2016 40 from 2016            | 46 (21.5–75) prior to 2016 53.8 (30–71.3) after 2016 | MMR: 164 (100%)                | EC + OC             | ES + TVUS +/- CA-125 | Annually          |

AC, Amsterdam criteria; AC-II, Amsterdam II criteria; CA-125, cancer antigen 125; EB, endometrial biopsy; EC, endometrial cancer; ES, endometrial sampling (curettage or biopsy); GE, gynecological examination; LS, Lynch syndrome; MMR, mismatch repair; OC, ovarian cancer; TVUS, transvaginal ultrasound; US, ultrasound.

Risk Reducing Surgery

The initial search identified 438 results and the updated search in November 2021 identified 171 results, resulting in 609 total results. After removing 135 duplicates and excluding 424 reports through abstract screening, 48 full text articles were assessed for eligibility. Eleven articles were included, and two additional articles were added through reference searching. Hence 13 articles were

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648  Lim N, et al. Int J Gynecol Cancer 2022;0:646–655. doi:10.1136/ijgc-2021-003132

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DISCUSSION

Summary of Main Results

Endometrial Cancer Screening

In the 18 studies, the incidence of endometrial cancer was 3.9% over a median screening duration of 4.5 years, compared with 13–47% lifetime incidence reported in the Prospective Lynch Syndrome Database. In addition to cancer, both atypical and non-atypical hyperplasia were included as pre-malignant lesions. Atypical hyperplasia has a 27.5% cumulative risk of progression to cancer in the general population, compared with 4.6% for non-atypical hyperplasia. Despite the lower cumulative risk, we included non-atypical hyperplasia as it occurs with mismatch repair protein deficiency, and represents an opportunity for risk reduction.

More cancers were detected by screening in mismatch repair carriers compared with those who were clinically diagnosed. This may be due to a lower prevalence, more targeted screening or frequent medical reviews in carriers, or a higher index of suspicion in use of screening. Also, mismatch repair carriers were older on average than those who were not genetically tested (51 years vs 44.5 years). No endometrial cancers were diagnosed in PMS2 carriers, consistent with findings from the Prospective Lynch Syndrome Database (12.8%), suggesting that screening is not indicated in this population.

Interval cancers, defined as cancers which present between regular screening visits, are either cancers missed by a screening method at a previous screening visit or cancers which rapidly develop between screening visits and hence were not detected previously. Interval cancers were detected in 14.3% of studies of annual screening with transvaginal ultrasound and biopsy, and most (80%) were stage I. No interval cancers occurred where screening included hysteroscopy. However, these studies had low cancer detection rates overall. The specificity of hysteroscopy alone as a screening tool cannot be determined because it was always combined with other methods.

The false negative rate for endometrial cancer screening is uncertain because there is no gold standard. However, interval cancers may indicate a false negative rate. Interval cancers could overestimate false negatives, as they will include rapidly growing de novo cancers. However, most studies reporting interval cancers used annual screening, making this less likely. Cancers presenting after missed screening visits were excluded in this sensitivity analysis. We found a 57.1% sensitivity rate for endometrial biopsy with most cancers detected early (65% stage I). However, this rate was no better than staging information available on interval cancers (72.2%), suggesting that asymptomatic screening does not detect endometrial cancers at an earlier stage than symptomatic presentations. However, not all studies reported staging. The low transvaginal ultrasound sensitivity could be influenced by a higher proportion of premenopausal women included.

Although more invasive, the specificity of endometrial biopsy is substantially higher than transvaginal ultrasound, especially in premenopausal women. False positives are more likely in premenopausal women which may lead to anxiety and over treatment. Only a small number of studies have reported sufficient data to inform sensitivity and specificity. Whether screening impacts on endometrial cancer mortality in Lynch syndrome is unknown. The 5
| Authors                          | Sample size (screening visits) | No of cancers detected on final pathology (% of sample size) and age at diagnosis | No of cancers detected by screening (% of confirmed cancers) | No of interval cancers detected (% of sample size) | No of symptomatic cancers detected at screening or prevalent visit |
|---------------------------------|--------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------|---------------------------------------------------|---------------------------------------------------------------|
| Dove-Edwin et al\(^1\)           | 269 (522)                      | 2 (0.74%) EC Both in AC population                                              | 0 (0%) EC                                                      | 2 (0.74%) EC (2 stage I)                               |                                                               |
| Rijcken et al\(^1\)             | 41 (179)                       | 1 (2.4%) EC at 61 years, MMR status not provided.                               | 0 (0%) EC                                                      | 0 (0%) OC                                            | 1 (2%) EC (stage I)                                            |
| Renkonen-Sinisalo et al\(^2\)   | 175 (503)                      | 13 (7.4%) EC at 36-71 years, all MMR carriers (1 additional EC was not screened). | 11 (78.6%) EC (9 stage I, 1 stage II, 1 stage III)              | 2 (1.1%) EC (2 stage I)                               |                                                               |
|                                 |                                | 4 (2.3%) OC at 41-50 years, all MMR carriers                                     | 0 (0%) OC                                                      | 2 (1.1%) OC (stage I, stage III).                     |                                                               |
|                                 |                                |                                                                                | 2 (1.1%) OC (stage I, stage III).                               | 2 (1.1%) OC incidental finding from EC surgery (both stage II) |                                                               |
| Lecuru et al\(^3\)              | 62                             | 3 (4.8%) EC at 37-50 years, MMR status not provided.                            | 0 (0%) EC                                                      | 0 (0%) EC                                            | 3 EC presented with symptoms (3 stage I)                      |
| Gerritzen et al\(^4\)           | 100 (285)                      | Period I: 2 (2%) EC at 52-55 years, in MMR carriers. Period II: 1 (1%) EC at 51 years, in MSH6 carrier. Unknown period: 1 (1%) OC at 50 years, MSH2 | Period I: 1 (50%) EC Period II: 1 (100%) EC (both stage I) 1 (100%) OC (stage III)                  | 0 (0%) EC                                            | 1 EC symptomatic at prevalent visit (stage III)               |
| Jarvinen et al\(^5\)            | 103 MMR carriers               | 19 (18%) EC at 36-72 years 6 (5.8%) OC                                           | 17 (89.5%) EC (13 stage I, 2 stage II, 2 stage III) 3 (50%) OC (2 stage I, 1 stage II) |                                                               | 2 EC symptomatic: 1 during screening visit; one after prolonged interval. (2 stage I) 3 OC symptomatic (2 stage I, 1 stage III) |
| Lecuru et al\(^6\)              | 58 (96)                        | 2 (3.4%) EC age and MMR status not provided.                                    | 0 (0%) EC                                                      | 0 (0%) EC                                            | 2 EC at regular review. Stages not provided                  |
| Guillen-Ponce et al\(^7\)       | 91                             | 2 (2.2%) EC                                                                       | 2 (0%) EC. Stages not provided                                  |                                                               |                                                               |
| Bats et al\(^8\)                | 140 (533)                      | 7 (6.3%) EC                                                                       | 7 (100%) EC. Stages not provided                                | 0 (0%) EC                                            |                                                               |
| Arts-De Jong et al\(^9\)        | 41 (69)                        | 3 (7.3%) EC (2 MLH1 carriers, 1 unknown status). Ages 40-44 years                | 3 (100%) EC (3 stage I)                                         | 0 (0%) EC                                            |                                                               |
| Manchanda et al\(^10\)          | 54                             | 9 (16.7%) EC at 37-54 years, in MSH2 carriers. 6 (11.1%) OC at 37-82 years, in MSH2 carriers. | 5 (55.6%) EC (4 stage I, 1 stage III) 1 (16.7%) OC (stage II)   | 4 (7.4%) EC (3 stage I, 1 stage not provided) 2 (3.7%) OC (1 stage II, 1 not reported) | 3 OC where reason for diagnosis was not reported (1 stage I, 1 stage II, 1 unreported) |
| Helder-Woolderink et al\(^11\)  | Total: 75 (266)                | Period I: 1 (2.3%) EC at 42 years, in MSH6 (stage I) Period II: 0 (0%) EC 0 (0%) OC | 0 (0%) EC                                                      | 0 (0%) EC                                            | 1 EC with symptoms (stage I)                                  |
| Stuckless et al\(^12\)          | 157 (504)                      | 6 (3.8%) EC                                                                       | 2 (50%) EC stages not provided                                  | 2 ECs at regular review. 2 ECs after 5 years disrupted follow-up |                                                               |
| Ketabi et al\(^13\)             | 871 (1945)                     | 13 (1.5%) EC at 40-70 years, all in MMR carriers. 4 (0.46%) OC at 37-42 years, in MMR carriers | 3 (23.1%) EC (2 stage I, 1 stage not provided) 1 (25%) OC (stage II) | 5 (0.57%) EC (2 stage I, 2 stage II, 1 stage III) 2 (0.23%) OC (1 stage I, 1 stage III) | 4 EC at regular review (all stage I); 1 EC after prolonged interval (stage IV) 1 OC (stage I) |
| Tzortzatos et al\(^14\)         | 45                             | 7 (15.6%) EC at 40-80 years, all MMR carriers. 2 (4.4%) OC, at 38-45 years, in MSH2 carriers | 3 (42.9%) EC (1 stage I, 2 stage II) 2 (100%) OC (2 stage I)  | 4 (8.9%) EC (3 stage I, 1 stage 1)                    |                                                               |
| Gosset et al\(^15\)             | 191 (620)                      | 5 (2.6%) EC in MMR carriers. 1 (0.52%) OC Ages not provided                      | 5 (100%) EC 1 (100%) OC stages not provided                   |                                                               |                                                               |
| Nebgen et al\(^16\)             | 80 (215)                       | MMR status and ages not provided                                                  | 2 (7.4%) EC. Stages not provided                                | 0 (0%) EC                                            |                                                               |

Continued
false positives are common one of which had no false positives or ovarian cancers, questioning trial and 5% ovarian cancers in controls.31 This study has formed carriers, consistent with ovarian cancer risk of 17%.3 Screening shown (Table 3). These findings are consistent with those from other endometrial cancer, and one misdiagnosed hyperplasia as cancer (Table 3). These findings are consistent with those from other studies of endometrial biopsy was less than with transvaginal ultrasounds, however, this could only be calculated for six studies, and sample sizes were small.

Ovarian Cancer Screening
The overall incidence of ovarian cancer was low (1.3%) and was higher among mismatch repair carriers (Online Supplemental Table 5).

The false negative rate for screening is unknown, but one-third of ovarian cancers in mismatch repair carriers were interval cancers (Online Supplemental Figure 3). In some of these cases, the cancers were diagnosed within a year after a negative screening test which would lead to false reassurance for patients, assuming they were missed at the previous screening test.20 Transvaginal ultrasound and CA-125 had high specificity rates based on three11 14 21 studies, one of which had no false positives or ovarian cancers, questioning the selection of patients for screening. False positives are common with CA-12514,21 which may increase the rates of unnecessary oophorectomy.28

Risk Reducing Surgery
Risk reducing surgery for Lynch syndrome includes hysterectomy with bilateral salpingo-oophorectomy. A multicenter study of 315 women (aged 20–63 years)35 found no endometrial or ovarian cancers after risk reducing surgery, compared with 33% endometrial and 5% ovarian cancers in controls.35 This study has formed the basis of guidelines recommending hysterectomy and bilateral salpingo-oophorectomy from 35 to 40 years of age or completion of childbearing.4 6,7 Eighteen of 26 (69.2%) endometrial cancers identified at hysterectomy and salpingo-oophorectomy were early stage (stage I), with only two stage II cancers (Figure 2). The earliest endometrial cancer was 38 years old,31 consistent with recommended age for risk reducing surgery.4 From the Prospective Lynch Syndrome Database, we would expect 59–212 endometrial cancers to be diagnosed by 70 years in 450 patients, based on a 13–47% lifetime risk, depending on pathogenic variant.3 A number needed to treat between 2 and 8 is calculated. This means that two surgeries are needed to diagnose one endometrial cancer in MSH2 carriers, compared with eight surgeries in PMS2 carriers.

In some studies, preoperative diagnoses prior to risk reducing surgery were obtained using transvaginal ultrasound or biopsy, and then compared with histopathological findings post-hysterectomy. Only 7 of 9 studies used biopsy and one study used ultrasound, limiting generalizability. Three biopsies did not correctly diagnose endometrial cancer, and one misdiagnosed hyperplasia as cancer (Table 3). These findings are consistent with those from other studies of endometrial biopsy and transvaginal ultrasound.

Ovarian cancer is much less common than endometrial cancer in Lynch syndrome (0.5% vs 5.8%), consistent with the Prospective Lynch Syndrome Database (3–17%).3 Based on these rates, 12–70 ovarian cancers would be expected to occur by 70 years in 413 women depending on pathogenic variant, with an estimated number needed to treat of 6–34.3 Six surgeries would need to occur in MSH2 carriers to detect one ovarian cancer, compared with 34 surgeries needed in PMS2 carriers. This aligns with recent recommendations for risk reducing salpingo-oophorectomy for MSH2 and MLH1 carriers and not for MSH6 and PMS2 carriers. In the Prospective Lynch Syndrome Database, ovarian cancer had a high 5 year and 10 year survival rate of 84%3 compared with 46% in the general Australian population.4 4 This could be due to the highly screened targeted population and younger age group included in the former, or the specific phenotype of Lynch syndrome cancers.

Results in the Context of Published Literature
We found limited evidence to support ovarian cancer screening in Lynch syndrome. Similarly, the general population showed no reduction in deaths due to ovarian cancer in screened women compared with those who were not screened.45 Although ovarian cancer screening showed a stage shift in those at elevated risk,30
### Table 3: Incidence of endometrial cancer or hyperplasia and ovarian cancer in female mismatch repair carriers undergoing risk reducing surgery

| Author            | Sample size | Participant characteristics (%) | Women with personal history of cancer (%) | Age (years) (median (range)) at RRS | RR type | Preoperative evaluation | No of cancers (% of sample size) | No of hyperplasias (% of sample size) |
|-------------------|-------------|---------------------------------|------------------------------------------|-------------------------------------|---------|-------------------------|----------------------------------|--------------------------------------|
| Schmeler et al     | 61          | MMR: 61 (100%)                  | Not provided                             | 47 (22%)                            | TH-BSO  | Not performed           | 3 (4.9%) EC, Genes not provided, 38, 48, 58 years, 0 (0%) OC | 1 (2.3%) |
| Lachiewicz et al  | 24          | MMR: 20 (83%) AC I/AC II/AC-like: 4 (17 %) | Not provided                             | 47 (32-61)                          | TH-BSO  | ES: 3/24 results available; 1 EC misdiagnosed as CAH, 2 correctly diagnosed as normal. TVUS: OC patient had ovarian cysts on preoperatively | 3 (12.5%) EC, Ages not provided, MLH1, MSH2, MSH6, 1 (4.2%) OC | 0 (0%) |
| Karamzin et al     | 25          | MMR: 20 (80%) AC-II: 5 (20%)    | Not provided                             | 48 (36-61)                          | BSO    | ES: 9/24 results available; all negative for CAH or EC. TVUS: 1 patient with EC had abnormal findings. | 2 (8%) EC in 58 years MLH1; 54 years MSH2, 44 years MSH2, 1 (4%) OC | 3 (12%) |
| Downes et al      | 25          | MMR: 23 (92%) EPCAM: 1 (4%)     | Not provided                             | 47 (34-59)                          | BSO    | EB: 3/25 available; 2 correctly detected CAH | 2 (8%) EC in 42 years, MSH2; 59 years MSH6, 0 (0%) OC | 6 (24%) |
| Tzortzatos et al  | 41          | MMR: 41 (100%)                  | Not provided                             | 50 (40-77)                          | BSO    | EB: 1/41 available which missed EC        | 3 (7.3%) EC in 49 years MLH1, 46 years MSH2, 0 (0%) OC | 1 (2.4%) |
| Bartosch et al    | 39          | MMR: 39 (100%)                  | Not provided                             | 45 (32-73)                          | TH-BSO  | EB: 10/39 available; 3 EH, 1 of which was actually EC | 3 (7.7%) EC, all asymptomatic, in 50 years MLH1, 44 years MSH1, 47 years MSH2, 0 (0%) OC | 6 (15.4%)|
| Wong et al        | 27          | MMR: 22 (81%) Basal of LS diagnosis unspecified: 5 (19%) | Not provided                             | 49 (36-61)                          | TH-BSO  | EB: 12/27 available; 1 CH +11 normal IOE gross; 15/27 available; 8 abnormal including EC IOE histology: 14/27 available; 6 malignancies found (specimen with EC was not sent) | 1 (3.7%) EC in 57 years, mutation positive, specific gene unavailable | 2 (7.4%)|
| Fedda et al       | 29          | MMR: 28 (97%) Unavailable: 1 (3%) | Not provided                             | 50 (34-69)                          | TH-BSO  | EB: 11/29 available; 3 correctly diagnosed CAH, 1 EH misdiagnosed as EC | 0 (0%) EC | 5 (17.2%) |
| Pistorius et al   | 4           | MMR: 3 (75%) AC: 1 (25%)        | Not provided                             | 4 (100%)                            | TH-BSO  | TVUS: 4/4 normal        | 2 (50%) EC, 49 years, AC-II; 47 years, MSH2 | 0 (0%) |
| Piedimonte et al  | 41          | MMR: 41 (100%)                  | Not provided                             | 47 (38-68)                          | BSO    | Not reported            | 0 (0%) EC | 3 (20%); all atypical | 0 (0%) |
| Rush et al        | 15          | MMR: 15 (100%)                  | Not provided                             | 47 (38-68)                          | TH-BSO  | Not reported            | 0 (0%) EC | 0 (0%) OC | 0 (%) |
| Duenas et al      | 66          | MMR: 66 (100%)                  | Not provided                             | 49 (36-72)                          | TH-BSO  | All 6 women diagnosed with cancer had normal screening prior to RRS | 6 (9.1%) EC, all asymptomatic | 0 (0%) OC |
| Ekenboom et al    | 53          | MMR: 53 (100%)                  | Not provided                             | 51                                  | TH-BSO  | TH +salpingectomy       | 1 (1.5%) EC in MSH6 | 1 (2.6%) |

Number needed to treat is defined as the number of patients needed to undergo risk reducing surgery to detect endometrial/ovarian cancer or hyperplasia.

AC, Amsterdam criteria; BS, bilateral salpingectomy; BSO, bilateral salpingo-oophorectomy; CAH, complex atypical hyperplasia; CH, complex hyperplasia without atypia; EB, endometrial biopsy; EC, endometrial cancer; EH, endometrial hyperplasia; ES, endometrial sampling; IOE, intraoperative evaluation; LEEP, loop electrosurgical excision procedure; LS, Lynch syndrome; MMR, mismatch repair; OC, ovarian cancer; RRS, risk reducing surgery; SC, supraovarian hysterectomy; SH, simple hyperplasia; TH, total hysterectomy; TVUS, transvaginal ultrasound; UBO, unilateral salpingo-oophorectomy.
these were mostly BRCA1/2 carriers who have a higher penetrance for ovarian cancer compared with mismatch repair carriers.

In Lynch syndrome, risk reducing hysterectomy and salpingooophorectomy is cost effective at age 40\(^2\) and prevents 13–45% of endometrial cancers if performed at age 40 years, and 4–18% if performed at age 60.\(^3\) Despite this, uptake by 50 years of age of endometrial cancers if performed at age 40 years, and 4–18% of which were atypical. Germline mutations and cancer stages are listed where specified.

Figure 2  Rate of endometrial cancer (EC) or endometrial hyperplasia (EH) in prophylactic specimens from risk reducing surgery in Lynch syndrome carriers according to mismatch repair (MMR) carrier status. Of 450 participants, 433 had germline mutations, of whom 5.7% had EC at the time of risk reducing surgery; 6.0% had EH, 84.6% of which were atypical. Germline mutations and cancer stages are listed where specified.

**Strengths and Weaknesses**

This is the first systematic review to report number needed to screen in endometrial and ovarian cancers associated with Lynch syndrome. Limitations include that approximately half the studies were retrospective, and the median sample size was only 29 women. Most had no control groups. Inclusion criteria for participants were heterogeneous with respect to family history, inclusion of mismatch repair carriers, age of onset of screening, period of follow-up, and sample size. Reduction in mortality due to screening could not be determined.

**Implications for Practice and Future Research**

Adequately powered randomized controlled trials are needed to evaluate the sensitivity and specificity of each potential screening method and their effect on morbidity and mortality. The relative risks and benefits of surgery versus screening are also uncertain.

**CONCLUSION**

Transvaginal ultrasound for the detection of endometrial or ovarian cancers does not appear to reduce morbidity in Lynch syndrome. Endometrial biopsy is more sensitive and specific with a lower number needed to screen. However, endometrial biopsy is invasive. Risk reducing hysterectomy and bilateral salpingo-oophorectomy is the mainstay of prevention in Lynch syndrome but recommendations vary depending on the mismatch repair gene.

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**Acknowledgements** The authors thank Dr David H Wrede for his advice and review of the work.

**Contributors** NL, guarantor, completed the literature search and drafted the manuscript. Titles and abstracts of articles identified through the search strategy were evaluated against inclusion and exclusion criteria by the primary authors NL and FAM. Full text articles were further excluded by NL against the same criteria. CK, FAM, MH, and GPY reviewed and edited the manuscript.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial, or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent for publication** Not applicable.

**Ethics approval** Not applicable.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request. Data used to calculate sensitivity, specificity, or number needed to screen are available on request.

Lim N, et al. Int J Gynecol Cancer 2022;32:646–655. doi:10.1136/ijgc-2021-003132
Original research

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REFERENCES

1. Vassen HF, Watson P, Mecklin JP, et al. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPPC, Lynch syndrome) proposed by the International Collaborative Group on HNPPC. Gastroenterology 1999;116:1453–6.
2. Dominguez-Valentin M, Sampson JR, Seppälä TT, et al. Cancer risks by gene, age, and gender in 6330 carriers of pathogenic mismatch repair variants: findings from the prospective Lynch syndrome database. Genet Med 2020;22:15–25.
3. Dominguez-Valentin M, Crosbie EJ, Engel C, et al. Risk-reducing hysterectomy and bilateral salpingo-oophorectomy in female heterozygotes of pathogenic mismatch repair variants: a prospective Lynch syndrome database report. Genet Med 2021;23.
4. Crosbie EJ, Ryan NAJ, Arends MJ, Evans DG, et al. The Manchester International Consensus Group recommendations for the management of gynaecological cancers in Lynch syndrome. Genet Med 2019;21:S10:2390–400.
5. Seppälä TT, Latchford A, Negoi I, et al. European guidelines from the EHTG and ESCP for Lynch syndrome: an updated third edition of the Mallorca guidelines based on gene and gender. Br J Surg 2021;108:484–98.
6. Giardiello FM, Allen J, Axilbund JE, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-Society Task Force on Colorectal Cancer. Dis Colon Rectum 2014;57:1025–48.
7. Vassen HFA, Blanco I, Aktan-Collan K, et al. Revised guidelines for the clinical management of Lynch syndrome (HNPPC): recommendations by a group of European experts. Gut 2013;62:812–23.
8. Auranen A, Joutsiniemi T. A systematic review of gynaecological cancer surveillance in women belonging to hereditary nonpolyposis colorectal cancer (Lynch syndrome) families. Acta Obstet Gynecol Scand 2011;90:437–44.
9. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71.
10. Dove-Edwin I, Bardou D, Goff S, et al. The outcome of endometrial carcinoma surveillance by ultrasound scan in women at risk of hereditary nonpolyposis colorectal carcinoma and familial colorectal carcinoma. Cancer 2002;94:1708–12.
11. Rijken FEM, Mounts MJ, Kleibeuker JH, et al. Gynecologic screening in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2003;91:74–80.
12. Renkonen-Siniluoto L, Bützow R, Leminen A, et al. Surveillance for endometrial cancer in hereditary nonpolyposis colorectal cancer syndrome. Int J Cancer 2007;120:821–4.
13. Lecuru F, Le Frédiné MA, Bats AS, et al. Performance of office hysteroscopy and endometrial biopsy for detecting endometrial disease in women at risk of human non-polyposis colon cancer: a prospective study. Int J Gynecol Cancer 2008;18:1326–31.
14. Gerritsen LHM, Hoogerbrugge N, Oei ALM, et al. Improvement of endometrial biopsy over transvaginal ultrasound alone for endometrial surveillance in women with Lynch syndrome. Fam Cancer 2009;8:391–7.
15. Järvinen HJ, Renkonen-Siniluoto L, Aktän-Collan K, et al. Ten years after mutation testing for Lynch syndrome: cancer incidence and outcome in mutation-positive and mutation-negative family members. J Clin Oncol 2009;27:4793–7.
16. Lecuru F, Huchon C, Metzger U, et al. Contribution of ultrasonography to endometrial cancer screening in patients with hereditary nonpolyposis colorectal cancer/Lynch syndrome. Int J Gynecol Cancer 2010;20:583–7.
17. Guillet-Poncet C, Martinez-Sevilla C, Perea R, et al. Gynecologic cancer screening in women at high risk of Lynch syndrome. JCO 2011;29:1559.
18. Bats AS, Bouquier J, Le Frere-Belda MA. Endometrial cancer screening in patients with Lynch syndrome. Int J Gynecol Cancer 2011;21:S1060.
19. Manchanda R, Sardigano E, Abdelrahema A, et al. Annual outpatient hysteroscopy and endometrial sampling (OHES) in HNPPC/Lynch syndrome (LS). Arch Gynecol Obstet 2012;286:1555–62.
20. Stuckless S, Green J, Dawson L, et al. Impact of gynecological screening in Lynch syndrome carriers with an MSH2 mutation. Clin Genet 2013;83:359–64.
21. Helder-Woolderink JM, De Bock GH, Sijmons RH, et al. The additional value of endometrial sampling in the early detection of endometrial cancer in women with Lynch syndrome. Gynecol Oncol 2013;131:304–8.
22. Douay-Hauser NDH, Bats ASB, Bensaid GB. Which strategy for the gynaecological screening in Lynch syndrome? A retrospective comparison of clinical examination, transvaginal ultrasound, and diagnostic hysteroscopy. Int J Gynecol Cancer 2014;24:1520.
23. Ketasl Z, Geres M, Maugard B, et al. The results of gynecologic surveillance in families with hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2014;133:526–30.
24. Tzortzatos G, Andersson E, Soller M, et al. The gynecological surveillance of women with Lynch syndrome in Sweden. Gynecol Oncol 2015;138:717–22.
25. Goss M, Rossi L, Cormou C. Impact of gynecologic screening in Lynch Syndrome. Int J Gynecol Cancer 2017;27:221.
26. Nebgen DR, KL, Chisholm GB. Lynch syndrome-combined endometrial and colon cancer screening results. Familial Cancer 2019;18:552.
27. Eikenboom EL, van Doorn HC, Diniens WNM, et al. Gynecological surveillance and surgery outcomes in Dutch Lynch syndrome carriers. Cancers 2021;13. doi:10.3390/cancers13030459. [Epub ahead of print: 26 01 2021].
28. Arts-De Jong M, Van Huis MA, Massuger LF. Efficacy of gynecological surveillance on ovarian cancer in women with Lynch syndrome. Int J Gynecol Cancer 2012;22:E264.
29. Rosenthal AN, Fraser L, Manchanda R, et al. Results of annual screening in phase I of the United Kingdom familial ovarian cancer screening study highlighting the need for strict adherence to screening schedule. J Clin Oncol 2013;31:49–57.
30. Rosenthal AN, Fraser LSM, Philip S, et al. Evidence of stage shift in women diagnosed with ovarian cancer during phase II of the United Kingdom familial ovarian cancer screening study. J Clin Oncol 2017;35:1411–20.
31. Schmelker MM, Lynch HT, Chen L-may, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. N Engl J Med 2006;354:261–9.
32. Pinedo MV, Zia A, Fergusson S. Baseline clinical outcomes of Lynch syndrome patients undergoing annual surveillance versus risk-reducing surgery in a prospective cohort study. Int J Gynecol Cancer 2021;31:A78.
33. Rush SK, Swisher EM, Garcia RL, et al. Pathologic findings and clinical outcomes in women undergoing risk-reducing surgery to prevent ovarian and fallopian tube carcinoma: a large prospective single institution experience. Gynecol Oncol 2020;157:514–20.
34. Lachiewicz MP, Kravchuck SE, O’Malley MM, et al. Prevalence of occult gynecologic malignancy at the time of risk reducing and non-ophthalmic surgery in patients with Lynch Syndrome. Gynecol Oncol 2014;132:434–7.
35. Karamurzin Y, Soslow RA, Garg K. Histologic evaluation of prophylactic hysterectomy and oophorectomy in Lynch syndrome. Am J Surg Pathol 2013;37:579–85.
36. Downes MR, Ali G, McCluggage WG, et al. Review of findings in prophylactic gynaecological specimens in Lynch syndrome with literature review and recommendations for grossing. Histopathology 2014;65:228–39.
37. Bartosch C, Pires-Luis AS, Meireles C, et al. Pathologic findings in prophylactic and nonprophylactic hysterectomy specimens of patients with Lynch syndrome. Am J Surg Pathol 2016;40:1177–91.
38. Wong S, Ratner E, Buzza N. Intra-operative evaluation of prophylactic hysterectomy and salpingo-oophorectomy specimens in hereditary gynaecological cancer syndromes. Histopathology 2018;73:109–23.
39. Perdida FA, Euscher ED, Malagon-Fuentes V, et al. Prophylactic risk reducing hysterectomies and bilateral salpingo-oophorectomies in patients with Lynch syndrome: a clinicopathologic study.
of 29 cases and review of the literature. Int J Gynecol Pathol 2020;39:313–20.
40 Pistorius S, Kruger S, Hohl R, et al. Occult endometrial cancer and decision making for prophylactic hysterectomy in hereditary nonpolyposis colorectal cancer patients. Gynecol Oncol 2006;102:189–94.
41 Dueñas N, Navarro M, Teulé Àlex, et al. Assessing effectiveness of colonic and gynecological risk reducing surgery in Lynch syndrome individuals. Cancers 2020;12:3419.
42 Lacey JV, Sherman ME, Rush BB, et al. Absolute risk of endometrial carcinoma during 20-year follow-up among women with endometrial hyperplasia. J Clin Oncol 2010;28:788–92.
43 Nieminen TT, Gylling A, Abdel-Rahman WM, et al. Molecular analysis of endometrial tumorigenesis: importance of complex hyperplasia regardless of atypia. Clin Cancer Res 2009;15.
44 Welfare AloHa. Cancer in Australia. Canberra: AIHW, 2019.
45 Menon U, Gentry-Maharaj A, Burnell M, et al. Ovarian cancer population screening and mortality after long-term follow-up in the UK Collaborative trial of ovarian cancer screening (UKCTOCS): a randomised controlled trial. Lancet 2021;397:2182–83.
46 Kwon JS, Sun CC, Peterson SK, et al. Cost-effectiveness analysis of prevention strategies for gynecologic cancers in Lynch syndrome. Cancer 2008;113:326–35.
47 Seppälä TT, Domínguez-Valentín M, Crosbie EJ, et al. Uptake of hysterectomy and bilateral salpingo-oophorectomy in carriers of pathogenic mismatch repair variants: a prospective Lynch syndrome database report. Eur J Cancer 2021;148:124–33.
48 Domínguez-Valentín M, Seppälä TT, Engel C, et al. Risk-reducing gynecological surgery in Lynch syndrome: results of an international survey from the prospective Lynch syndrome database. J Clin Med 2020;9. doi:10.3390/jcm9072290. [Epub ahead of print: 18 07 2020].
49 Ryan N, Nobes M, Sedgewick D, et al. A mismatch in care: results of a United Kingdom-wide patient and clinician survey of gynaecological services for women with Lynch syndrome. BJOG 2021;128.
50 Burn J, Sheth H, Elliott F, et al. Cancer prevention with aspirin in hereditary colorectal cancer (Lynch syndrome), 10-year follow-up and registry-based 20-year data in the CAPP2 study: a double-blind, randomised, placebo-controlled trial. Lancet 2020;395:1855–63.
51 Frias-Gomez J, Benavente Y, Ponce J, et al. Sensitivity of cervico-vaginal cytology in endometrial carcinoma: a systematic review and meta-analysis. Cancer Cytopathol 2020;128:792–802.
52 O’Flynn H, Ryan NAJ, Narine N, et al. Diagnostic accuracy of cytology for the detection of endometrial cancer in urine and vaginal samples. Nat Commun 2021;12:952.
53 Njoku K, Chiasserini D, Jones ER, et al. Urinary biomarkers and their potential for the non-invasive detection of endometrial cancer. Front Oncol 2020;10:559016.