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Is immunohistochemistry-based screening for Lynch syndrome in endometrial cancer effective? The consent's the thing

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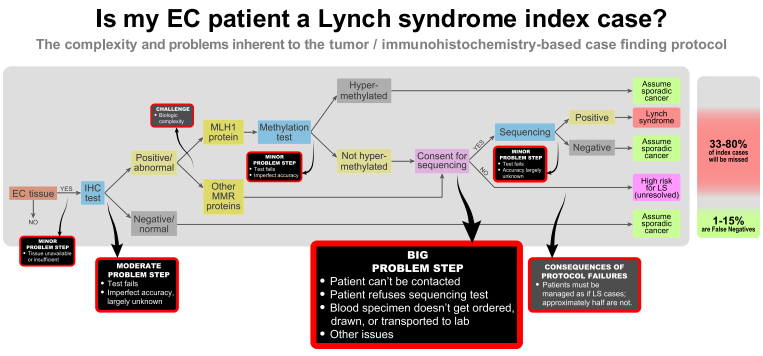
HIGHLIGHTS

- In routine clinical settings, the tumor/immunohistochemistry (IHC)-based Lynch syndrome (LS) screening protocol often fails.
- In endometrial cancer (EC) populations, the protocol will likely fail to identify 33% to 80% of LS index cases.
- The dominant factor in failure of the protocol is non-consent for sequencing among screen positive patients.
- Even under the most optimistic consent scenario (e.g., 80%), this protocol will miss between 29% and 42% of index cases.
- There is considerable evidence suggesting that many LS screening programs in the U.S. have consent rates below 50%.

GRAPHICAL ABSTRACT



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ABSTRACT

Purpose. To investigate the plausible failure rate of the immunohistochemistry (IHC)-based screening protocol to identify Lynch syndrome (LS) index cases among endometrial cancer (EC) patients.

Methods. We developed a simulation model of the IHC protocol in this context. The model was populated from systematic and focused reviews, augmented with local data and expert opinion. The virtual cohort represents the number of women expected to be diagnosed with EC in the U.S. in 2018. The outcomes include protocol failure rates and LS cases missed in a variety of hypothetical scenarios.

Results. The best estimate of failure rate of the IHC protocol is 58%; minimum and maximum estimates are 33% and 80%, respectively. These failure rates are driven primarily by the high rates of failure to obtain consent from patients for sequencing (25% to 80%). The multiple imperfect tests and potential failure points in this protocol, collectively, make up 7% to 20% of the total failure rate. When consent for sequencing was fixed in the model at 25%, 50%, and 80%; the expected ranges for index case identification failure are 78%–82%, 57%–64%, and 29%–42%, respectively.

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Conclusion. The primary driver of failure to identify index cases remains consent for sequencing. Consent rates have shown little improvement since LS screening programs were instituted in the U.S., leaving us to conclude these high failure rates are resistant to substantial improvement. These missed opportunities will be magnified because cascade screening for carrier status among family members will not be pursued.

1. Introduction

Lynch syndrome (LS) is a familial cancer syndrome defined by germline defects in DNA mismatch repair (MMR) genes (i.e., *MLH1, PMS2, MSH2,* and *MSH6*) or the genes that regulate them (e.g., *EPCAM*). The presence of a germline mutation in one of these genes is a risk factor for a variety of cancers, including colorectal, endometrial, ovarian, stomach, pancreatic, renal pelvis and ureter, and brain, with the highest risk being for colorectal (CRC) and endometrial cancers (EC). The risk for these cancers (i.e., penetrance) is highly variable depending on the specific MMR gene mutation. The lifetime risks of developing EC are 64–71% with an *MSH6* mutation and 40–50% with *MSH2* or *MLH1* mutations [1]. The risks associated with *PMS2* and *EPCAM* mutations are substantially lower and estimates continue to evolve. The penetrance of CRC due to pathogenic variants in MMR genes is even higher, with up to 82% lifetime risk, and also vary depending on the gene, and other factors that are not yet fully understood [1].

Early estimates of the prevalence of LS in the general population were about 1 in 440 to 500. This estimate has recently increased to about 1 in 280, based on population exome sequencing in central Pennsylvania and confirmed computationally by Win and colleagues [2,3].

Similarly, early estimates of the prevalence of LS carriers among EC populations varied from about 1.5% to 3% [4–6]. These estimates were based almost entirely on incomplete ascertainment of MMR mutations in this population; that is, only patients whose screening test was positive were tested by the confirmatory test for these mutations, sequencing for the MMR genes. These underestimates are being substantiated by several recent studies among EC populations that applied sequencing on cohorts of unselected EC patients. For example, Ferguson et al. identified a prevalence rate of 5.8% and Ring et al. a rate of 5.9% [7,8]. Egoavil et al. directly measured a rate of 4.6% but "corrected" that rate to 6.6% to account for dropouts and failures in their testing process [9]. Similarly, Goodfellow et al. directly measured a rate of 3.9% but stated this is a "minimum value" [10], for similar reasons as Egoavil. Thus, the true LS prevalence is likely higher that early estimates, within the range of 4–7%, with variability in populations.

Fortunately, risk for LS-related cancers can be substantially reduced by identifying LS carrier status in individuals with a LS-related cancer (i.e., *index cases*) and, subsequently, testing family members for carrier status. Both types of carriers can receive enhanced surveillance and/or prophylactic surgical interventions that can substantially reduce LS-related cancer risks [1].

Formal programs to identify LS index cases are widely but inconsistently implemented in the U.S. and elsewhere, with programs in CRC patient populations instituted earlier and more commonly than in EC patient populations. This lag among EC patients is likely because the association of EC with LS carrier status was made several years later than with CRC, thus the evidence base and medical practice has had less time to develop. Several medical specialty societies now endorse LS screening in EC populations, with additional support from the Lynch Syndrome Screening Network (LSSN).

The most common approach to identifying LS index cases in EC patients is initiated with immunohistochemical (IHC) staining of tumor tissue for the MMR proteins. This is likely due to the fact that the IHC test is widely available and reimburseable, and has been shown in simulation studies to have superior cost effectiveness compared to the alternative, lab-based screening test, microsatellite instability (MSI) testing, at least as extrapolated from studies in CRC patient populations [11]. The IHC-based protocol is also endorsed by the National Comprehensive Cancer Network [12]. Some healthcare systems use the MSI test, either in addition to the IHC test, or as an alternative to it, in spite of its demonstrated poorer cost effectiveness. There are also multiple medical history-based screening tools for LS, but such tools have been repeatedly demonstrated to be quite challenging to administer and have poor sensitivity when compared to tumor-based protocols [13].

In EC patient populations, an IHC-based protocol includes two additional tests; methylation of the *MLH1* promoter as an indicator of a somatic mutation, and sequencing of the MMR genes as the final confirmatory test. The IHC and methylation tests are performed on tumor tissue, which may be performed in different labs, and sequencing performed on blood or cheek cells, prior to which informed consent is universally recommended. There are many steps in this testing protocol, which takes weeks to months to complete. There is evidence that consent for sequencing is low in this context, as summarized by Stewart in 2013 [14] and in a more recent systematic review by Willis [15].

To our knowledge, there are no published analyses of the effectiveness of an IHC-based protocol in EC populations that characterizes the impact of the multiple preanalytic and analytic steps of the protocol, including consent rates. The aim of this study was to conduct analysis to ascertain real-world failure rates of a LS screening protocol for EC.

2. Methods

We developed a simulation model of a virtual cohort of women diagnosed with EC who could be screened for LS using an IHC-based protocol. The cohort size is based on the number of new diagnoses of uterine cancer expected in the U.S. in 2018 (approximately 63,000), multiplied by 0.92 (92%), the percent of uterine cancer that are "endometrial" [16]. This results in a cohort of 58,000 new cases of EC annually. No other exclusion criteria were applied, including age at diagnosis.

The model, depicted in Fig. 1, explicitly included multiple steps involved in clinical testing identified by the authors, beyond test performance, to represent pre-analytic as well as analytic failure of the protocol. We also represented the multiple elements of the consenting process. Quantitative values (*parameters*) for these variables were extracted from the literature, augmented by local data and expert opinion, and the most conservative estimates were used when multiple estimates were available. We assumed a 5% prevalence of LS in the model cohort, which is the midpoint of our estimate of prevalence range. Model parameters and their sources are presented in Table 1. Variable ranges represent minimum to maximum plausible values, unless otherwise noted.

The parameters were fixed for three variables: prevalence of LS in the EC population, the rate of positive IHC screens due to the MLH1 protein (and indicated for methylation testing), and correlation of sensitivity & specificity.

The LS prevalence variable was fixed–to focus modeling output on the performance of the IHC protocol and away from the influence of LS prevalence. The prevalence rate (5%) is the approximate midpoint from our targeted review of literature of studies where all EC cases were sequenced for LS (unpublished).

The rate of positive IHC screens due to the MLH1 protein was fixed because doing otherwise would introduce challenges to calibration of the model related to correlation of variables without adding to its usefulness. The rate is based on a targeted review of the literature and

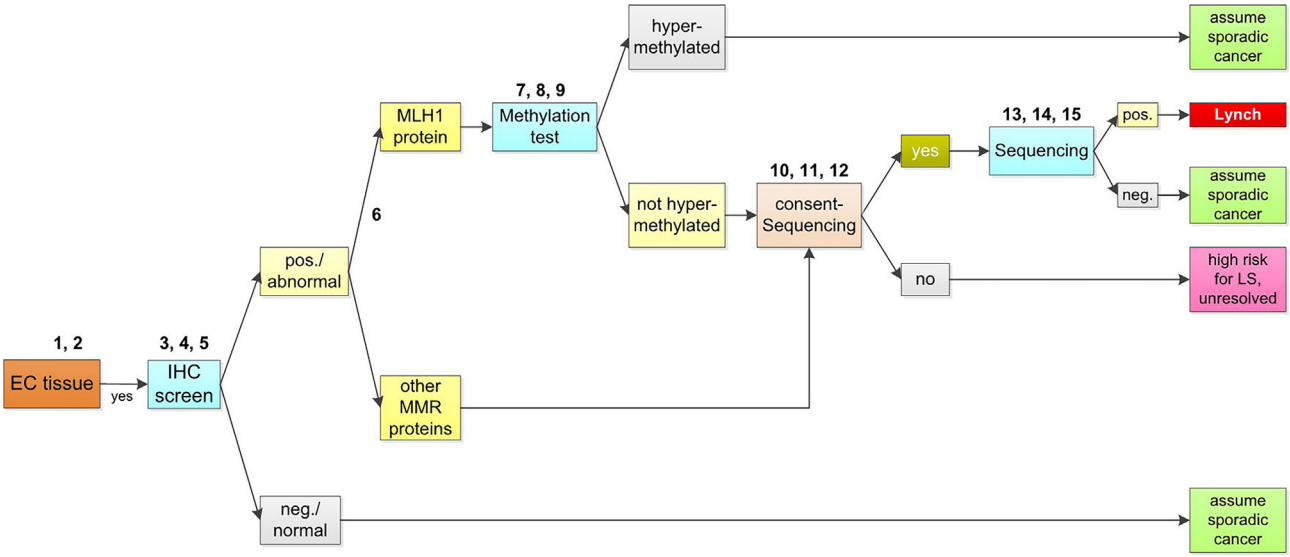

Fig. 1. Illustration of the flow of the IHC-based screening protocol and where model variables fit.

represents a statistical mean of a subset of the studies we believe to be highly representative of institutions in the U.S.

The sensitivity & specificity of tests are widely understood to be inversely correlated. Since we were unable to identify any published data on this issue, we ran a series of simulations, calibrating model output on percent of abnormal (positive) IHC results, for which we had robust empirical evidence from a targeted review of the literature. A value of −0.75 yielded calculations of this outcome that were consistent with the empirical data set, thus assigned to this variable and fixed. We did not repeat this exercise for the other tests since we did not have good empirical data to base simulations; rather, we used the same correlation value for these tests. The primary outcome of our study is percent of LS index cases missed by the IHC protocol, or the *failure rate*, under various conditions. To provide a sense of protocol failure at the critical point in the protocol–the consent process–we also calculated the percent and absolute number of cases missed just prior to this part of the protocol. Informed by one-way sensitivity analysis, we performed a series of 'what-if' analyses examining the impact of the most influential model variable on the primary outcome. For these analyses, we selected three rates to represent the low, mid-range, and high rates of this variable, which were fixed for each analysis, while remaining variables were allowed to vary across their programmed ranges. Secondary outcomes were absolute numbers of LS index cases identified and missed in the cohort.

Results from our modeling, for the sake of simple interpretation, will be referred to as "**best estimate**", and for range of results as "**minimum**" and "**maximum**". All results were generated from two different analytic methods used in the study: *deterministic*, where all variables are fixed for any given 'run' and *probabilistic*, where some or all variables are populated with *distributions* to represent the shape of the variable data. In deterministic models, results are expressed as single outputs. In *probabilistic* models, Monte Carlo simulation is employed to randomly pick data points during 1000s of iterations, as defined by model structure and variable distributions, and data points further constrained by the correlation coefficients between tests. Results are expressed in the form of probability distributions, which software displays as a graph and summarizes as a *mean*, representing the best estimate, and either min./max. values (as with our models) or confidence intervals. We do not report the probability distribution graphs, and have explicitly avoided the multiple other terms used to define modeling output. The failure rates and 'what-if' analyses were calculated using the probabilistic method (sensitivity analysis); cases identified and missed by simple math, and all other outcomes where calculated using deterministic analysis.

The model was constructed in Excel© software (Microsoft, Inc., Redmond, WA). Sensitivity analyses were performed using @Risk software (Palisade, Inc., Ithaca, NY), an Excel "add-on". Models in these formats are available by request.

Table 1
Variables and parameters used in the analysis.

#	Variable	Base-case	Range	Source
1	Prevalence of LS	5.0%	fixed	Unpublished review of literature [7–10]
2	Appropriate EC tissue available for screening	97.5%	95–99%	Consensus of local gyn oncologist and molecular pathologist
3	IHC screens reportable	98%	97–99%	Published evidence [22,23,33]
4	Sensitivity of IHC screen	95%	90–100%	Published evidence [7,14]
5	Specificity of IHC screen	80%	48–86%	Published evidence [7,14]
6	Positive IHC screen due to MLH1	77%	fixed	Unpublished review of literature
7	Methylation tests reportable	98%	97–99%	Published evidence [34,35]
8	Sensitivity of methylation test	97.5%	95–99%	Published evidence [34–36]
9	Specificity of methylation test	90%	87–93%	Published evidence [34–36]
10	Patients successfully contacted	95%	90–100%	Local experience and published evidence [14]
11	Patients consented for sequencing	50%	25–80%	Estimate based on published evidence [14]
12	Blood specimen for sequencing ordered, collected and transported	98%	96–100%	Local pathologist opinion
13	Sequencing successful & reportable	98%	95–99%	Estimate based on expert opinion
14	Sensitivity of Sequencing test	99.0%	98.0–99.9%	Estimate based on published evidence [11,14,37]
15	Specificity of Sequencing test	99.0%	98.0–99.9%	Estimate based on published evidence [11,14,37]

3. Results

The primary analyses yielded a best estimate of failure rate to identify LS index cases of 58%, with minimum to maximum range of 33% to 80%. The calculations of the percent and absolute number of cases missed just prior to the consent step yielded a best estimate of failure rate of 12%, or 347 of the 2900 index cases in the virtual cohort (Fig. 3). The secondary outcomes of absolute number of women in the virtual cohort expected to be missed and identified by the IHC protocol are presented in Table 2.

The tornado graph that represents the one-way sensitivity analysis (Fig. 2) illustrates that the success rate of the consent process for ordering the sequencing test is the dominant factor influencing the primary model outcome. For the 'what-if' analyses to explore the impact of different consent rates on case-finding, we selected assent rates of 25%, 50%, and 80%. This analysis yielded corresponding best estimates of failure rates of 80%, 58%, and 36% (Table 3).

4. Discussion

Our study quantifies several outcomes important to stakeholders concerning the performance of the IHC-based LS case-finding protocol in EC patient populations. The primary outcome of our model suggests the IHC protocol will miss between 33% and 80% of LS index cases, with most of the loss coming after the consenting step.

Our analyses also quantified the absolute numbers of index cases expected to be identified or missed in the virtual cohort by the IHC-based protocol, assuming our best estimates of all the protocol elements we identified and modeled (Table 2). In this population of 58,000 women, 2900 are expected to be LS index cases. Of these, our model predicts that the IHC protocol would miss between 951 and 2320 cases. These women will NOT have the opportunity to consider an enhanced colonoscopy schedule to reduce their CRC risk, nor would they be offered enhanced surveillance or prophylactic procedures to reduce risk for other LS-associated cancers.

The larger missed opportunity at the population level are the multiple family members of each index case who are also LS carriers. LS is an *autosomal dominant* disorder, with first-degree relatives (of index cases) having a 50% chance of being carriers, second-degree relatives a 25% risk, and so on. If index cases are missed, relatives will not be tested and, if LS carriers, not be offered interventions to reduce their risk of LS-associated cancers. In the Goverde et al. study, the reduction in cancer risk from cascade testing in families and appropriate interventions among carriers was about 15 times greater than the reduction in these risks in the index case [17].

The one-way sensitivity analysis established the dominant influence of consent rate for sequencing on the failure rate of the IHC-based protocol. Though the large influence of consent will be no surprise to those who work closely in this domain, this has not been accounted for by previous studies, and may be surprising to gynecologic oncologists.

Awareness of the large influence of consent rate motivated our 'what-if' analyses to illustrate that even when the consent rate is fixed at its most optimistic (80%), the model predicts a failure rate between 29% to 42%. These numbers reflect the many points of potential failure in the real world of this testing protocol, as well as the residual rate of consent failure. These calculations provide stakeholders with estimates of protocol failure based on knowledge of local sequencing consent rates (see Table 2).

Randall and colleagues identified numerous factors contributing to low genetic testing consent and uptake [18]. These include lack of physician awareness of, and/or time, lack of patient acceptance, delays and/or denials by third party payers, variable availability of genetic counselors, lack of reimbursement for genetics professionals, and racially and culturally disparate and/or underinsured populations.

Of the four reported cost effectiveness analyses (CEA) of LS screening in EC patient populations [5,6,17,19], only the Resnick et al. study modeled the effectiveness of different testing protocols for the identification of LS index cases [5]. They reported performing a one-way sensitivity analysis, but no detail was provided other than a brief summary of their focus on the influence of the cost of sequencing. Consent rate was not explicitly included. The other relevant CEAs all modeled life-years saved (LYs) as the outcome of interest which, while important to understand the cost effectiveness of the entire clinical pathway out to long term direct health outcomes, does not directly address the most influential variables affecting LS case-finding. We believe that in order to understand the absolute effectiveness of a LS screening program it is essential to begin with understanding the effectiveness of the index case-finding protocol(s), since no LY will be saved if LS index cases are not identified.

If the consent rate is known, the next most influential factor in the protocol would be the sensitivity of the IHC test, which also has a wide plausible range. Consumers of this test may find it challenging to obtain verifiable estimates of their vendor's test performance because proficiency testing results are generally not available outside the performing lab [20].

Though consent rates are the dominant factor in our model, additional failure accumulates throughout the protocol. Our model included 15 variables, at least 10 of which represent steps where failure to identify index cases can occur. The literature on laboratory error suggests that complex testing protocols may be even more prone to failure than we have represented [21]. Regarding tests sent to external labs, an AHRQ-funded systematic review identified 40 potential factors for diagnostic error in send-out testing [22]. Specific to an IHC-based testing protocol, Plebani states that "a relatively high frequency of analytical errors has been documented for immunoassays…" [21]. Other investigators have confirmed this view, and expanded it to pre- and post-analytic error specific to IHC testing for the MMR proteins [22–24]. As Hampel states "…patients are likely lost at every step in this process …" [25].

Our analyses provide objective evidence to support prior assertions about real-world screening for LS. Brennan and colleagues stated that "while technically accurate, the yield of 'universal' IHC [screening] in detecting new Lynch probands is limited by real-world factors that reduce referrals and genetic testing" [26]. Watkins and colleagues examined the question of barriers and facilitators to successful application of LS screening and follow-up interventions at the system level [27]. They determined that "…the current organization and implementation of health care services are inadequate…" and "…go beyond the individual to the provider and health care system levels." Hall (2010) further stated that [28]:

Poorly addressed issues include the role/timing of informed consent for testing, access and cost barriers associated with genetic counseling

Table 2
Model outcomes.

Outcomes	Best estimate[a]	Minimum	Maximum
Percent of total LS cases ***lost*** in protocol **prior to consenting process steps**	12%	7%	20%
Number of LS cases ***lost*** in protocol **prior to consenting process steps**	347	205	593
Percent Lynch syndrome **cases *lost*** in complete protocol	58.0%	32.8%	80.0%
Total Number Lynch syndrome cases ***lost* in complete protocol**	1682	951	2320
Number Lynch syndrome cases ***identified* by complete protocol**	1218	1949	580

[a] Number of LS cases expected among 58,000 EC patients = 2900.

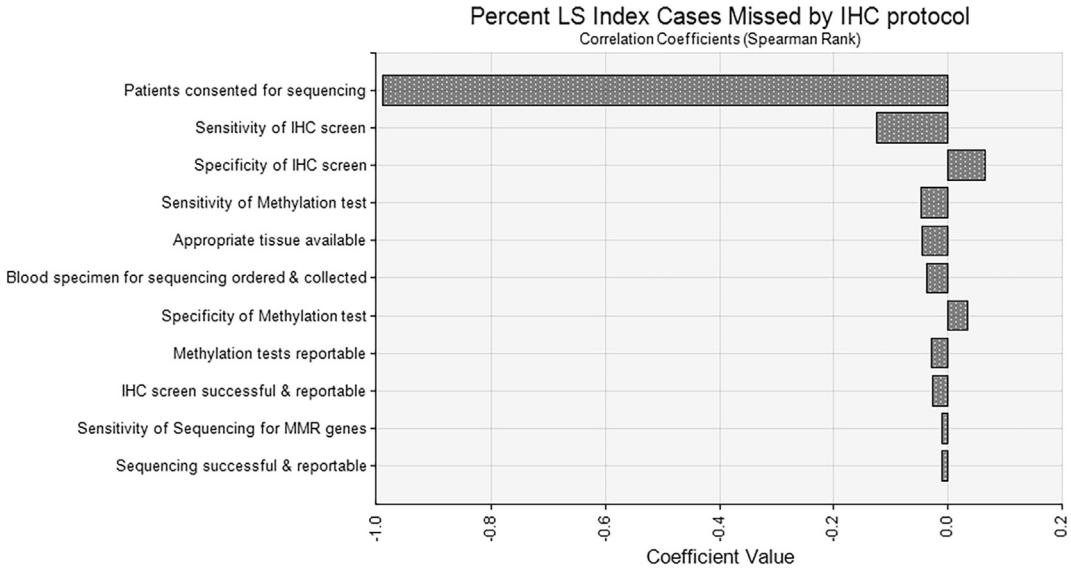

Fig. 2. Tornado graph illustrating variables most influential to primary outcome.

and DNA testing, psychosocial burdens to the thousands of middle-aged and elderly patients with CRC coping with surgical and chemotherapy treatments and poor prognosis, the need for providers to warn third-party relatives of risk for Lynch syndrome, limited effectiveness of screening, and the cost burden to society when poor DNA testing uptake, test limitations, and modest screening compliance are considered. Diverse barriers to the success of a population-based Lynch screening program in the United States remain (e.g., clinical resource needs, financial limitations, clinical expertise gaps, educational deficits). Data supporting clinical efficacy (feasibility) and effectiveness (real-life performance) are critical before important policy changes are adopted, especially where issues of hereditary cancer risk and genetic privacy are involved (p. 606).

Based on current evidence, our study suggests there to be several ways forward. It is tempting to think that if the consent rate for sequencing could be increased to its highest plausible level (e.g., 80%) that the protocol would be "good enough". Even under the most optimistic assumption for consent, the screening protocol still has a significant failure rate, leading to lost opportunities for identification that may raise doubts among stakeholders regarding use of this protocol. Moreover, based on published evidence, poor consent rates seem to be resistant to substantial improvement [26–29], even when formal implementation efforts are applied to improve them [30]. Consent rates vary for a variety of reasons. Irons and colleagues demonstrated that GC compliance was about 36% overall but about 86% among those with a family history of related cancers [31]. And two studies demonstrated that consent rates in this setting are markedly lower in minority (Muller et al) [32] or ethnically diverse cohorts of patients (Lee et al) [33].

There are several alternative approaches to identification of LS patients. The MSI assay as the initial test can be considered as a substitute for or in addition to IHC, as some LS screening programs do. However, both have been demonstrated to be less cost effective than and have the same consent issues as IHC only (as the screening test), thus also resulting in high failure rates [34,35]. Traditionally, the risk assessment ("screen") for LS was performed by collecting clinical information, specifically the Amsterdam Criteria or the Bethesda Guidelines [36]. However, collecting clinical criteria is challenging and these models have relatively poor sensitivity for LS [37]. More recently, an assortment of prediction models have been developed (e.g., MMRpredict, MMRpro, and the PREMM model). These models have higher sensitivity for LS than either Amsterdam or Bethesda models, as high as 90% for the $PREMM_5$ model, but also require collection of clinical information that poses challenges in collection and follow-up. Thus, in spite of the theoretical superiority of the best of these prediction models, implementation for all EC patients would be challenging.

Direct-to-sequencing of the MMR genes is under consideration as the cost of sequencing drops as a consequence of next generation sequencing moving into clinical practice. This approach eliminates screening by directly identifying LS cases but, historically, has been prohibitively expensive thus not cost effective when compared to screening protocols [19,34]. Not only is sequencing the gold standard for diagnosing the presence of LS, a direct-to-sequencing protocol is much simpler, thereby reducing much of the error inherent in complex testing protocols. The steps might include just patient consent (which may be different in this protocol) and test ordering, collection of and transport of blood specimen to performing lab, and running and reporting sequencing results. No information about consent rates for this approach has been published, to our knowledge.

Though the costs of sequencing have dropped dramatically in the past few years, viable market prices have a wide range, between $500 to $2000. Thus it is still not clear whether direct-to-sequencing protocols will be cost effective. However, there is no question this approach is more effective at LS case-finding than any other approach.

Finally, sequencing of the patient's tumor tissue to determine both somatic and germline variation is emerging as an approach to identify treatment targets and hereditary cancer syndromes [38]. This approach would screen the tumor tissue for the prevalence of LS mutations (and other molecular biomarkers). This testing strategy seems to be too new to understand its efficiency in LS case-finding or for consensus to be reached on consent.

Based on this line of thinking and the results of our modeling study, we believe the near-term future for LS case finding in EC (and CRC) populations to be direct-to-sequencing of the germline administered at the time of clinical work-up. The only potentially substantial barriers are its cost and consent prior to ordering. Consent rates in this context are largely unknown. However, we believe it is likely to be much higher when administered by the gynecologic-oncologist at the time of treatment. We will tackle these issues in subsequent work.

Table 3
'What-If' analyses.

Consent rate fixed at:	Best estimate of failure rate	Min./Max. failure rate
80%	36.1%	29.2–41.8%
50%	58.0%	56.8–63.5%
25%	80.0%	77.9–81.9%

Our study has several limitations. As with all simulation models, it is limited by both the model structure accurately portraying the scenario of interest and the data used to populate it. The tumor/IHC-based LS screening protocol is well established, understood, and widely used in the U.S., albeit with several variations. The principal variations are age cut-offs and use of the MSI test either as a substitute for or in addition to IHC. The testing protocol we modeled was IHC-only as the initial test and represents universal screening. Quantitative results from our models will not apply to other screening paradigms.

Regarding valuation of model variables, we believe the data used to populate our models, as plausible ranges, will be directly relevant to virtually all healthcare systems performing IHC-based universal screening, including consent rates. However, for a specific healthcare system to understand the performance of its own IHC-based protocol it would need to measure or model it using local data.

The LS prevalence rate (5%) assumed in our models may be too high. If it is, the absolute number of women who will fail to be identified as LS index cases will be over-estimated by our models. However, we believe this rate is a robust estimate, since it is based on studies with maximum ascertainment of LS index case status, as previously discussed. A different LS prevalence will not change the relative failure rates.

Finally, our sequencing sensitivity and specificity estimates may be overestimates. This is a complex topic due to continued and rapid technological changes, constantly evolving knowledge bases, differences between performing labs, and difficulties in obtaining proficiency testing results in the U.S. Any plausible changes to these estimates would have no influence on our conclusions.

In conclusion, we have calculated best estimates and ranges of failure to identify LS index cases among EC patients and explored the relative and absolute impact of protocol test performance, addition of pre-analytic and analytic steps, and consent rate of sequencing. While the failure rate has contributions from imperfect laboratory tests and the multiple points of potential failure, these have less impact than broader healthcare process issues, particularly consent for sequencing. Our model provides the first estimates of a LS screening program's performance that accounts for healthcare delivery processes in addition to test performance.

Author contributions

Conception and design: J. Gudgeon, M. Varner, M. Hashibe, M. Williams.

Development of methodology: J. Gudgeon, M. Williams.

Acquisition of data: J. Gudgeon.

Analysis and interpretation of data: J. Gudgeon, M. Varner, M. Hashibe, M. Williams.

Writing, review, and/or revision of the manuscript: J. Gudgeon, M. Varner, M. Hashibe, M. Williams.

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Declaration of Competing Interest

The authors have nothing to disclose and no conflict of interest to declare.

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References

[1] H.T. Lynch, C.L. Snyder, T.G. Shaw, C.D. Heinen, M.P. Hitchins, Milestones of Lynch syndrome: 1895–2015, Nat. Rev. Cancer 15 (2015) 181–194.
[2] A.K. Win, M.A. Jenkins, J.G. Dowty, A.C. Antoniou, A. Lee, G.G. Giles, et al., Prevalence and penetrance of major genes and polygenes for colorectal cancer, Cancer Epidemiol. Biomark. Prev. 26 (2017) 404–412.
[3] D.H. Ledbetter, Geisinger MyCode. Genomics and Population Health Action Collaborative Leadership Meeting, Washington, D.C. 2018.
[4] H. Hampel, J. Panescu, J. Lockman, K. Sotamaa, D. Fix, I. Comeras, et al., Comment on: screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients, Cancer Res. 67 (2007) 9603.
[5] K. Resnick, J.M. Straughn Jr., F. Backes, H. Hampel, K.S. Matthews, D.E. Cohn, Lynch syndrome screening strategies among newly diagnosed endometrial cancer patients, Obstet. Gynecol. 114 (2009) 530–536.
[6] J.S. Kwon, J.L. Scott, C.B. Gilks, M.S. Daniels, C.C. Sun, K.H. Lu, Testing women with endometrial cancer to detect Lynch syndrome, J. Clin. Oncol. 29 (2011) 2247–2252.
[7] S.E. Ferguson, M. Aronson, A. Pollett, L.R. Eiriksson, A.M. Oza, S. Gallinger, et al., Performance characteristics of screening strategies for Lynch syndrome in unselected women with newly diagnosed endometrial cancer who have undergone universal germline mutation testing, Cancer 120 (2014) 3932–3939.
[8] K.L. Ring, A.S. Bruegl, B.A. Allen, E.P. Elkin, N. Singh, A.R. Hartman, et al., Germline multi-gene hereditary cancer panel testing in an unselected endometrial cancer cohort, Mod. Pathol. 29 (2016) 1381–1389.
[9] C. Egoavil, C. Alenda, A. Castillejo, A. Paya, G. Peiro, A.B. Sanchez-Heras, et al., Prevalence of Lynch syndrome among patients with newly diagnosed endometrial cancers, PLoS One 8 (2013), e79737.
[10] P.J. Goodfellow, C.C. Billingsley, H.A. Lankes, S. Ali, D.E. Cohn, R.J. Broaddus, et al., Combined microsatellite instability, MLH1 methylation analysis, and immunohistochemistry for Lynch syndrome screening in endometrial cancers from GOG210: an NRG oncology and gynecologic oncology group study, J. Clin. Oncol. 33 (2015) 4301–4308.
[11] T. Snowsill, N. Huxley, M. Hoyle, T. Jones-Hughes, H. Coelho, C. Cooper, et al., A systematic review and economic evaluation of diagnostic strategies for Lynch syndrome, Health Technol. Assess. 18 (2014) 1–406.
[12] National Comprehensive Cancer Network, Guideline-Genetic/Familial High-Risk Assessment: Colorectal. Version 1, 2018 ed., 2018.
[13] V.N. Palter, N.A. Baker, A. Pollett, C. Daly, M. Facey, C. Rotenberg, et al., Learning by example: an international perspective on reflex-testing for Lynch syndrome, Ann. Surg. Oncol. 26 (2) (2019) 425–436, https://doi.org/10.1245/s10434-018-6809-7.
[14] A. Stewart, Genetic testing strategies in newly diagnosed endometrial cancer patients aimed at reducing morbidity or mortality from lynch syndrome in the index case or her relatives, PLoS Curr 5 (2013).
[15] A.M. Willis, S.K. Smith, B. Meiser, M.L. Ballinger, D.M. Thomas, M.A. Young, Sociodemographic, psychosocial and clinical factors associated with uptake of genetic counselling for hereditary cancer: a systematic review, Clin. Genet. 92 (2017) 121–133.
[16] Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov), SEER*Stat Database: cancer stat facts: uterine cancer, https://seer.cancer.gov/statfacts/html/corp.html 2018.
[17] A. Goverde, M.C. Spaander, H.C. van Doorn, H.J. Dubbink, A.M. van den Ouweland, C.M. Tops, et al., Cost-effectiveness of routine screening for Lynch syndrome in endometrial cancer patients up to 70 years of age, Gynecol. Oncol. 143 (2016) 453–459.
[18] L.M. Randall, B. Pothuri, E.M. Swisher, J.P. Diaz, A. Buchanan, C.T. Witkop, et al., Multi-disciplinary summit on genetics services for women with gynecologic cancers: A Society of Gynecologic Oncology White Paper, Gynecol. Oncol. 146 (2) (2017)https://doi.org/10.1016/j.ygyno.2017.06.002.
[19] J.L.D. Dottino, S. Cantor, R. Suidan, K.H. Lu, A cost-effectiveness analysis of universal testing for Lynch syndrome in endometrial carcinoma, Gynecol. Oncol. 147 (2017) 203.
[20] Department of Health and Human Services, Centers for Medicare & Medicaid Services, Clinical Laboratory Improvement Amendments (CLIA), Description of CLIA, https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/index.html?redirect=/CLIA/05_CLIA_Brochures.asp 2017.
[21] M. Plebani, Diagnostic errors and laboratory medicine - causes and strategies, EJIFCC 26 (2015) 7–14.
[22] M. Graber, L.C. Morgan, E. Tant, et al., Proactive Risk Assessment During the Laboratory Testing Process to Reduce Diagnostic Error: Literature Review, Agency for Healthcare Research and Quality, Washington, D.C., 2012
[23] B. Djordjevic, R.R. Broaddus, Laboratory assays in evaluation of Lynch syndrome in patients with endometrial carcinoma, Surg Pathol Clin 9 (2016) 289–299.
[24] N. Buza, J. Ziai, P. Hui, Mismatch repair deficiency testing in clinical practice, Expert. Rev. Mol. Diagn. 16 (2016) 591–604.
[25] H. Hampel, Genetic counseling and cascade genetic testing in Lynch syndrome, Familial Cancer 15 (2016) 423–427 Jul.
[26] B. Brennan, C.T. Hemmings, I. Clark, D. Yip, M. Fadia, D.R. Taupin, Universal molecular screening does not effectively detect Lynch syndrome in clinical practice, Ther. Adv. Gastroenterol. 10 (2017) 361–371.
[27] K.E. Watkins, C.Y. Way, J.J. Fiander, R.J. Meadus, M.J. Esplen, J.S. Green, et al., Lynch syndrome: barriers to and facilitators of screening and disease management, Hered Cancer Clin Pract 9 (8) (2011).
[28] M.J. Hall, Counterpoint: implementing population genetic screening for Lynch Syndrome among newly diagnosed colorectal cancer patients–will the ends justify the means? J. Natl. Compr. Cancer Netw. 8 (2010) 606–611.

[29] Z. Loh, D.S. Williams, L. Salmon, E. Dow, T. John, The impact of universal immunohistochemistry on Lynch syndrome diagnosis in an Australian colorectal cancer cohort, J. Natl. Compr. Canc. Netw. 8 (5) (2010) 606–611.
[30] J.C. Long, D. Debono, R. Williams, E. Salisbury, S. O'Neill, E. Eykman, et al., Using behaviour change and implementation science to address low referral rates in oncology, BMC Health Serv. Res. 18 (2018) 904.
[31] R.F. Irons, K.M. Contino, J.J. Horte, B. Levin, K.D. Mattie, M. Wight, et al., Success of referral to genetic counseling after positive lynch syndrome screening test, Int. J. Color. Dis. 32 (9) (2017) 1345–1348, https://doi.org/10.1007/s00384-017-2849-x.
[32] C. Muller, S.M. Lee, W. Barge, S.M. Siddique, S. Berera, G. Wideroff, et al., Low referral rate for genetic testing in racially and ethnically diverse patients despite universal colorectal cancer screening, Clin. Gastroenterol. Hepatol. 16 (2018) 1911–1918 (e2).
[33] J. Lee, L.R. Gubernick, A.L. Brodsky, J.E. Fehniger, D.A. Levine, D. Gerber, et al., Missed opportunities: genetic counseling and testing among an ethnically diverse cohort of women with endometrial cancer, Gynecol. Oncol. 151 (2018) 153–158.
[34] S.D. Grosse, Is universal tumor testing for Lynch syndrome cost-effective? It depends! Genetics in Medicine (2018) 1–4 in press.
[35] A. Guglielmo, N. Staropoli, M. Giancotti, M. Mauro, Personalized medicine in colorectal cancer diagnosis and treatment: a systematic review of health economic evaluations, Cost Eff Resour Alloc 16 (2) (2018).
[36] A. Tognetto, M.B. Michelazzo, G.E. Calabro, B. Unim, M. Di Marco, W. Ricciardi, et al., A systematic review on the existing screening pathways for Lynch syndrome identification, Front. Public Health 5 (2017) 243.
[37] H. Hampel, W.L. Frankel, E. Martin, M. Arnold, K. Khanduja, P. Kuebler, et al., Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer), N. Engl. J. Med. 352 (2005) 1851–1860.
[38] H. Hampel, R. Pearlman, M. Beightol, W. Zhao, D. Jones, W.L. Frankel, et al., Assessment of tumor sequencing as a replacement for Lynch syndrome screening and current molecular tests for patients with colorectal cancer, JAMA oncology 4 (6) (2018) 806–813, https://doi.org/10.1001/jamaoncol.2018.0104.