PATHOLOGICA 2024;116:32-45;
DOI: 10.32074/1591-951X-926

Original Article

Biomarker characterization in endometrial cancer in Europe: first survey data analysis from 69 pathological academic and hospital labs

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Summary

Introduction. Endometrial carcinoma (EC) is the commonest gynecological cancer affecting women in Western populations. To predict patient risk, the 2020 edition of the World Health Organization (WHO) Classification of Tumors of the Female Genital Tract stressed the importance of integrated histo-molecular classification of the disease. This survey analysis poses attention on the most frequently used immunohistochemical and molecular markers adopted in daily categorization of ECs in European laboratories.

Methods. We analyzed data collected through questionnaires administered to 40 Italian, 20 Spanish, 3 Swiss and 6 United Kingdom (UK) laboratories. We collected information regarding daily practice in EC evaluation, specifically concerning mismatch repair status (MMR) and microsatellite instability (MSI). Summary and descriptive statistical analyses were carried out to evaluate the current practice of each laboratory.

Results. The results show that MMR status is mainly evaluated by using immunohistochemistry (IHC) on most EC samples. The most frequent approach for the analysis of MMR status is IHC of four proteins (PMS2, MSH6, MSH2, MLH1). MSI analysis by molecular methods is uncommon but useful as a supplemental tool in specific conditions. MLH1 promoter hypermethylation and BRAF V600 mutations analysis are performed in case of negative expression of MLH1/PMS2. Other markers (mainly p53 followed by POLE and PTEN) are investigated in particular in Spain and Switzerland in a consistent number of cases.

Conclusion. Guidelines consultation and standardization of laboratory procedures are efficient means for EC prognostic risk stratification and improving the quality of care.

Key words: endometrial cancer, biomarkers testing, European Survey, risk stratification

Received: September 30, 2023
Accepted: December 21, 2023

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How to cite this article: Santoro A, Bragantini E, Castiglione F, et al. Biomarker characterization in endometrial cancer in Europe: first survey data analysis from 69 pathological academic and hospital lab. Pathologica 2024;116:32-45. https://doi.org/10.32074/1591-951X-926

Introduction

In 2013, the Cancer Genome Atlas (TCGA) research network proposed four molecular subtypes of EC based on genomic abnormalities, with survival and prognostic differences: POLE/ultra-mutated, MSI/ hypermutated, copy-number low/endometrioid and copy-number high/serous-like [1]. Encouraged by these results, the Proactive Molecular Risk Classifier for Endometrial Cancer [2-4] validated a more practical, simplified molecular classifier, identifying four molecular subtypes that are similar but not identical to those proposed in TCGA. In detail these are: mismatch repair deficient (MMR-D) corresponding to the hypermutated subtype; DNA polymerase

epsilon (POLE)-mutated corresponding to the ultramutated subtype; p53 abnormal (p53abn) corresponding to the copy number high subtype; and p53 wild-type (p53wt) corresponding to the copy number low subtype. The important point consisted in the reproducibility and viability of this classification based on the use of MMR and p53 immunohistochemical staining, methods easily adopted in laboratories [2]. Similar analyses have been carried out by the Leiden/ (Post Operative Radiation Therapy in Endometrial Carcinoma) PORTEC group.
Moreover, this molecular approach appeared to be essential to stratify patients into low, intermediate, and high-risk groups.
Prognostically speaking, considering high-grade ECs, the POLE-mutated cases exhibit an excellent prognosis, differently from the p53-abn group showing the poorest prognosis [5-8].
Given the importance of patient risk stratification for therapeutic purposes, the 2020 EC ESGO/ESTRO/ESP guidelines defined new prognostic risk groups incorporating these markers [5]. This means that, considering EC morphology, molecular profiling should always be taken into consideration [9-12]. With these premises, we performed a survey of 69 laboratories from 4 countries to explore the daily-based approach to EC diagnostic and management pathways.

Materials and methods

Using Diaceutics' Data Repository, a global multisource database including commercial claims and laboratory data, we analyzed the endometrial cancer testing behavior in a cohort of European labs who are part of the Diaceutic's DXRX: Diagnostic Network.

Table I. Typologies of the pathological labs participants.

	Academic	Hospital
Italy	23	17
Spain	20	0
Switzerland	1	2
UK	1	5

We collected information through a 12 questionnaire-based survey from January 2020 to March 2021. Summary and descriptive statistical analyses were used to assess the laboratory routine.
The survey was focused on the immunohistochemical and molecular analysis in EC, the various assessment methods used in laboratories, and the technology used in molecular evaluation. We selected 69 European pathological labs: 40 Italian, 20 Spanish, 3 Swiss and 6 UK labs.
Considering the labs typology, 45 were academic and 24 public hospital pathology laboratories (Tab. I).
More detailed analysis on the testing behavior for EC in the different European countries can be accessed through an interactive dashboard at the following link: https://app.dxrx.io/dashboards/endometrial-cancer-european-biomarker-characterization

Results

MMR analysis

How do you perform MMR analysis?

Do you routinely use IHC markers or molecular assessment?
Answers to the above-mentioned questions were provided by 69 responders.

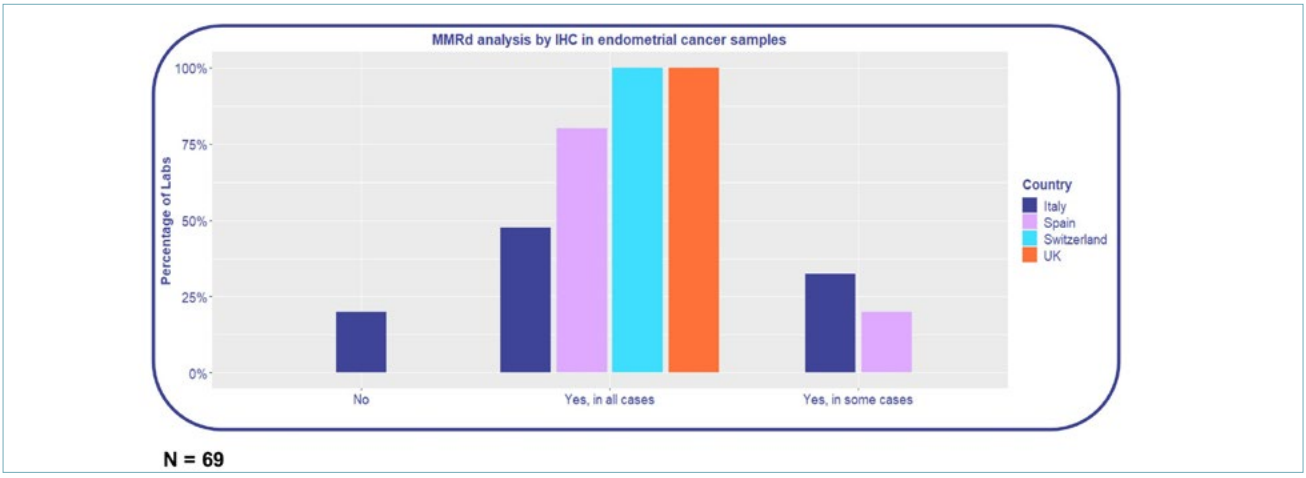

Figure 1. Analysis of MMR deficiency (MMRd) by IHC in Endometrial Cancer. Percentage of laboratories by countries performing IHC testing in all or under specific conditions to reveal a deficiency of the mismatch repair (MMRd) machinery in endometrial cancer. N: total number of laboratories from all the countries.

Spanish laboratories: all responders (100%) perform MMR analysis by IHC. Among them, 80% of laboratories perform MMR analysis by IHC in all EC samples, and 20% choose selected cases (suspicion of Lynch Syndrome, early-onset cancers, and positive family history).
Italian laboratories: nearly all responders (80%) perform MMR analysis by IHC. Among them, 47.5% of laboratories perform MMR analysis by IHC in all EC samples, 32% choose selected cases (suspicion of Lynch Syndrome, early-onset cancers, and positive family history) and the remaining 20% of laboratories do not perform MMR analysis. Among the latter, 50% (represented by molecular pathology labs) directly perform MSI analysis (Fig. 1).
Swiss and UK laboratories: All responders (100%) perform MMR analysis by IHC in all EC samples.

IHC analysis: antibodies

In EC do you use an IHC panel? If yes, which clones do you prefer?
Answers to the above-mentioned questions have been provided by 69 responders.
There is a certain variability concerning the antibodies used for MMR analysis by IHC.
Spanish laboratories: nearly half of the cases (47.4%) being Dako, specifically: MLH1 clone ES05, MSH2 clone FE11, MSH6 clone EP49, PMS2 clone EP51. The second choice (42.1%) is represented by Ventana with the clones MLH1-M1, MSH2-G219-119, MSH6-SP93 and PMS2-A16-4 (Fig. 2A).
Italian laboratories: most of the cases (68.8%) being Ventana with the clones MLH1-M1, MSH2-G219-119, MSH6-SP93 and PMS2-A16-4. The second choice (18.8%) is represented by Leica, specifically: MLH1 clone ES05, MSH2 clone FE11, MSH6 clone EP49, PMS2 clone EP51. (Fig. 2).
Swiss and UK laboratories: all the cases (100%) being Ventana with the clones MLH1-M1, MSH2-G219-119, MSH6-SP93 and PMS2-A16-4.

IHC analysis: platforms

What kind of IHC platform do you routinely use?
Answers to the above-mentioned question were provided by 57 responders.
Regarding platforms (Fig. 2B), some labs use multiple platforms.
The Ventana platform with Ventana clones was the most adopted by the majority of Italian (69%) and Swiss laboratories (66%) testing MMR. Leica (Leica BOND-MAX and Leica BOND-III) was mostly chosen in by 66% of Swiss laboratories, and DAKO platform (Dako Omnis and Dako Link 48) was preferred by 47% of Spanish labs.

IHC analysis: waiting time

How many days does your laboratory take to give IHC results?
Answers to the above-mentioned question were provided by 55 responders.
Spanish laboratories: In 82.4% of the cases, MMR IHC results are reported within 1-3 days, demonstrating a rapid turnaround time (Fig. 2C) while 11.8% of cases require 4-6 days and 5.8% of cases 7-10 days.
Italian laboratories: In 62.5% of cases, MMR IHC results are reported within 1-3 days, demonstrating a rapid turnaround time (Fig. 2C), while 25% of cases require 4-6 days and 12.5% of cases 7-10 days.
Swiss and UK laboratories: In 100% of cases, MMR IHC results are reported within 1-3 days, demonstrating a fast laboratory turnaround time.

Molecular analysis

Molecular analysis: yes, or no? When is it used and how?

Do you perform a molecular MSI analysis? If yes, in which cases?
Answers to the above-mentioned questions were provided by 55 responders.
Responders have provided different type of answers:

- option 1: Yes, when no expression of MMR;
- option 2: Yes, when MMR results unclear;
- option 3: Yes, according to family history even though IHC is positive;
- option 1 and 2;
- option 2 and 3;
- all options;
- other, including after discussion in Multidisciplinary Molecular Tumor Board / as reflex test in all cases / only on specific request / in other Laboratories / to confirm IHC results / depending on family history/ selected cases;
- no.

MSI analysis by molecular methods appears to be infrequent; indeed, in Italy, Spain, Switzerland and UK, respectively, 32.5% - 45% - 33.3% - 66.7% of laboratories do not perform it. However, it appears to be a useful supplement in case of ambiguous MMR IHC results, in case of positive Lynch Syndrome (LS) family history or upon clinician request (Fig. 3A). In particular, Italian labs mostly provided Option 2 (22.5%), Spanish labs opted for Other (30%), Swiss labs selected Options 1+2 (33.3%) and Other (33.3%), UK labs choose Option 2 (16.7%) and Option 1+2 (16.7%)

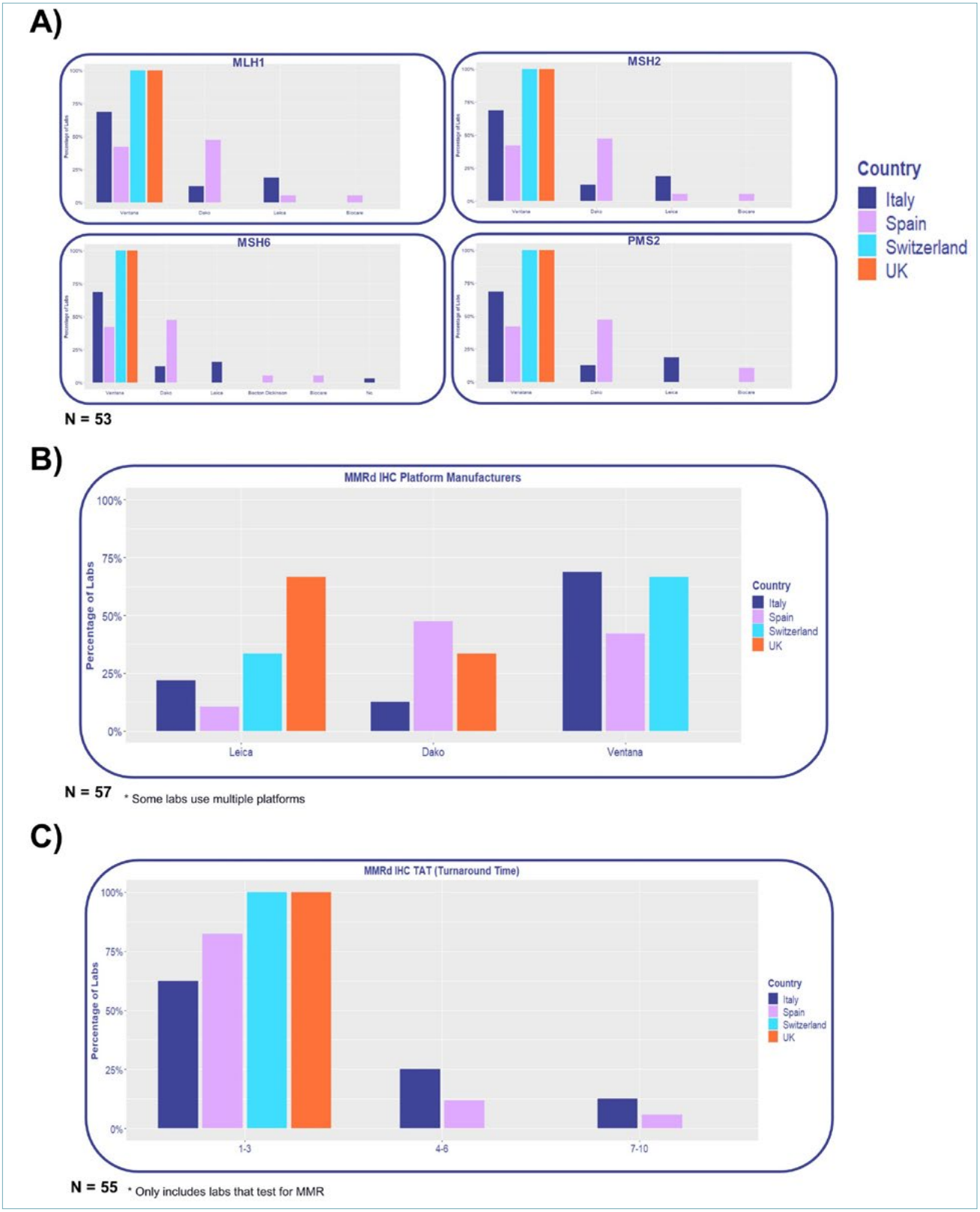

Figure 2. Kits, platforms, and turnaround time(TAT) for MMRd testing in IHC. (A) Laboratories kit adoption (based on manufacturers) for MLH1, MSH2, MSH6, PMS2 by countries for the assessment of MMRd. N: total number of laboratories from the different countries.(B) Platforms used by the different laboratories in Italy, Spain, Switzerland, UK for the assessment of MMRd by IHC. N: total number of laboratories from the different countries.(C) TAT for MMd testing by country: average of time in days from when the sample is received by the laboratories to the final report of the result. N: total number of laboratories from the different countries.

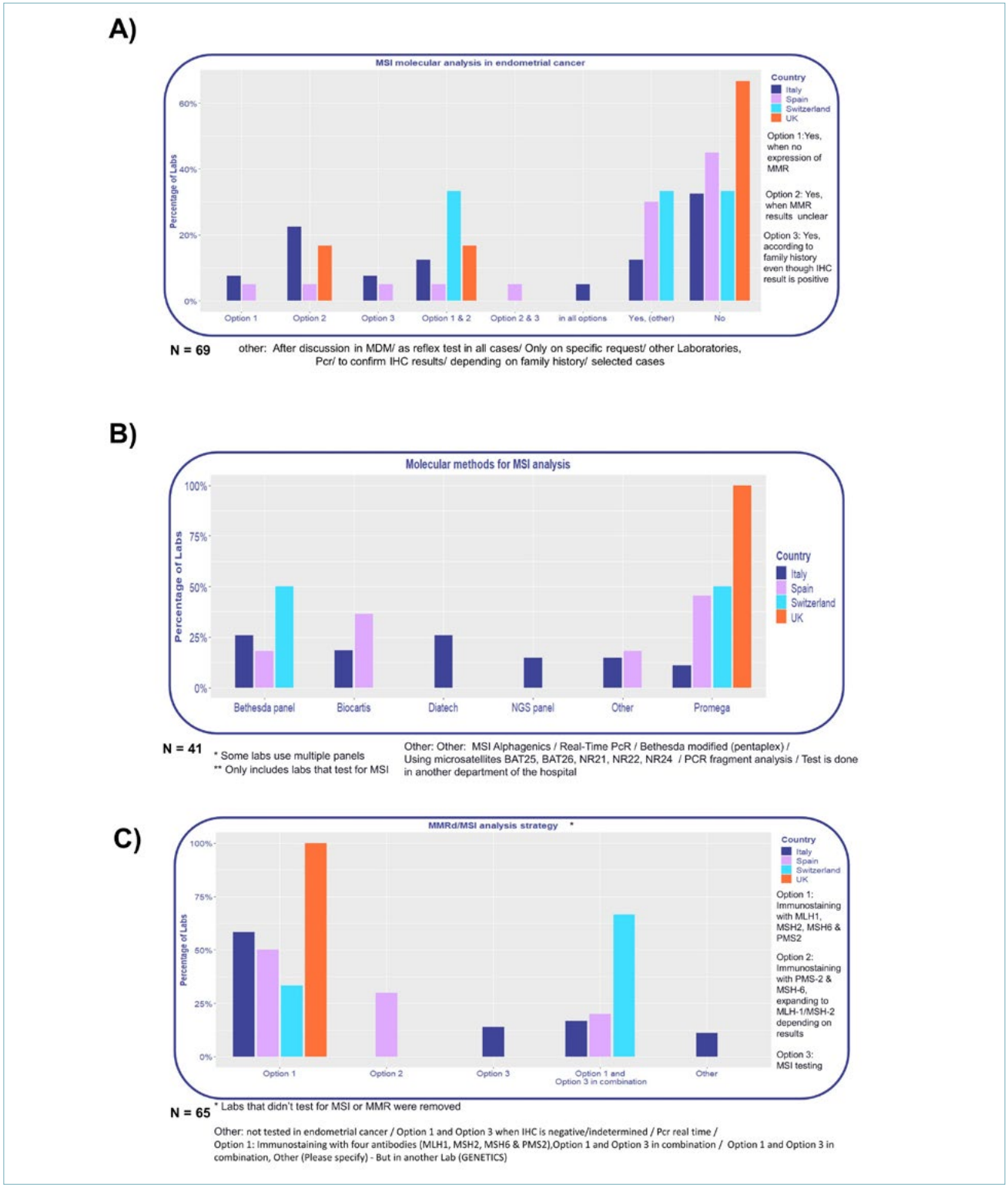

Figure 3. Molecular analysis of microsatellite instability (MSI) in endometrial cancer when and how? (A) Criteria used by the Italian, Spanish, Switzerland, UK laboratories for MSI assessment. N: total number of laboratories. (B) Molecular methods used by the different laboratories for MSI testing. N: total number of laboratories.(C) MMRd/MSI analysis strategy in endometrial cancer. Strategy used by the different laboratories of the different countries for MMRd and/or MSI assessment. N: total number of laboratories excluding those that did not perform MMR or MSI testing.

Molecular analysis: technologies and Kit Employed for MSI assessment

What kind of method do you prefer for molecular analysis?
Answers to the above-mentioned question were provided by 41 responders.
Some labs referred the use of multiple panels (Fig. 3B).
Spanish laboratories: the most frequently used molecular methods for MSI analysis are the Promega panel (MSI Analysis System Version 1.2) (45%) and RT-PCR based Biocartis Idylla MSI test (36%). Less frequently, laboratories use the Bethesda Panel and other tools (MSI Alphagenics / Real-Time PCR / Bethesda modified pentaplex / microsatellites BAT25, BAT26, NR21, NR22, NR24 / PCR fragment analysis / Test done in another department of the hospital - molecular biology).
Italian laboratories: the most frequently used molecular methods for MSI analysis are the Bethesda panel (26%) and Diatech test (26%).
Swiss laboratories: the most frequently used molecular methods for MSI analysis are the Promega panel (MSI Analysis System Version 1.2) (50%) and the Bethesda panel (50%)
UK laboratories: all the responders use the Promega panel (MSI Analysis System Version 1.2) (100%)

MMRd/MSI analysis

MMR/MSI analysis strategy

What kind of method do you prefer to biologically characterize EC, regarding microsatellite/MMR protein status?
Answers to the above-mentioned question have been provided by 65 responders.
Responders have provided different type of answers:

- option 1: Immunostaining with MLH1, PMS2, MSH2, MSH6;
- option 2: Immunostaining with PMS2 and MSH6, expanding to MLH1 and MSH2 depending on results;
- option 3: MSI testing;
- option 1 and 3 in combination;
- other, including not tested in endometrial cancer / Option 1 and Option 3 when IHC is negative-undetermined / RT-PCR / Option 1, Option 1 and Option 3 in combination or Other (Please specify) - but in another Lab (GENETICS).

For Italian, Spanish and UK labs, the most common approach MMR analysis in EC is IHC uses the four proteins (PMS2, MSH6, MSH2, MLH1) (58% - 50% - 100% of labs, respectively) or using 2 MMR proteins (PMS-2, MSH-6), expanded to a 3rd one (MSH-2, MLH-1) for the 30% of Spanish labs.
For Swiss labs the preferred approach is represented by the combination of IHC and MSI molecular analysis (Fig. 3C).

MMR/MSI assessment in EC in 2020: selection of study cases

During the 2020 survey, in the different European countries EC cases were selected to establish the modality of MMR/MSI characterization: 2911 cases were tested in Italy, 1250 in Spain, 960 in UK and 130 in Switzerland. In all countries we can confirm that MMR analysis is mainly carried out using IHC (Fig. 4A).
Spanish laboratories: 75% of cases were tested by IHC and among them 21% combining IHC and molecular method. In 25% of cases no integrative analysis was performed.
Italian laboratories: 51% of cases were tested by IHC and among them 12% combining IHC and molecular method. In a minority of cases (0.5%) only MSI analysis was performed.
Swiss laboratories: all cases (100%) were tested by IHC and among them 38% combining IHC and molecular method.
UK laboratories: most cases (80%) were tested by IHC and among them only the 0.5% combining IHC and molecular method.

MMR/MSI assessment in EC in 2021: selection of study cases

During the 2021 survey, in Italy and Switzerland EC cases were selected to establish the modality of MMR/MSI characterization: 1096 cases were tested in Italy, and only 33 in Switzerland. In all the countries we can confirm for 2021 that MMR analysis was mainly carried out by using IHC. No no data on numbers was obtained for Spain and UK for 2021 survey (Fig. 4B).
Italian laboratories: 64% of cases were tested by IHC and among them 10% combining IHC and molecular method. In a minority of cases (0.8%) only MSI analysis was performed.
Swiss laboratories: all cases (100%) were tested by IHC and among them 42% combining IHC and molecular method.

Combination of IHC and molecular analysis

In case of negative MLH1/PMS2 do you perform further analyses?
Answers to the above-mentioned question were provided by 59 responders.
Responders provided different type of answers and some labs use multiple options:

- option 1: analysis of MLH1 promoter hypermethylation;
- option 2: analysis of MLH1 promoter hypermethylation and BRAF V600 mutations;
- others: BRAF V600 / genetic counselling / MSI + BRAF V600E Pyrosequencing / RT-PCR / BRAF V600 mutations analyzed In Genetics / MLH1 pro-

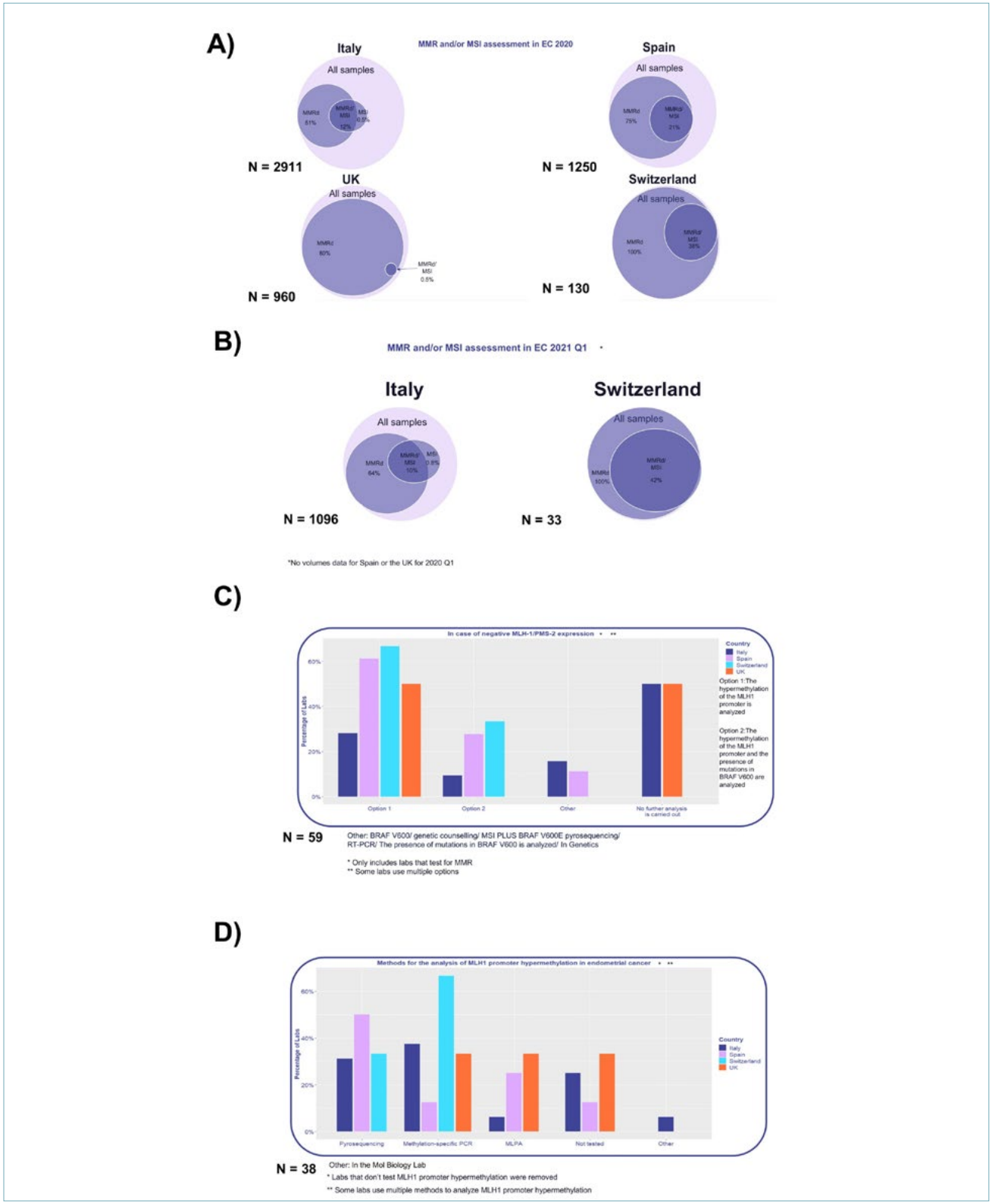

Figure 4. MMR/MSI testing and MLH1 promoter hypermethylation assessment inEC. (A) Percentage of samples tested for MMR and/or MSI in the different European countries in 2020. N: total number of samples by countries.(B) Percentage of samples tested for MMR and/or MSI in Italy and Switzerland during the first quarter of 2021. N: total number of samples by countries. (C) Strategy used in case of negative MLH-1/PMS-2 expression. N: total number of laboratories testing for MMR. (D) Molecular methods used to analyze the hypermethylation status of the MLH-1 promoter. N: total number of labs testing the hypermethylation of the MLH-1 promoter.

moter hypermethylation or Other (Please specify) analyzed in Genetics;
- no further analysis carried out.

In cases showing negative MLH-1/PMS-2 expression, further analyses are carried out, which varied in the different countries (Fig. 4C).

Spanish laboratories: MLH1 promoter hypermethylation is carried out in 61% of labs; in 28% of labs MLH1 promoter hypermethylation and BRAF V600 mutations analysis are both performed.

Italian laboratories: MLH1 promoter hypermethylation is carried out in 28% of labs; 16% of labs select Other options, but 50% of labs do not carry out further analysis.

Swiss laboratories: MLH1 promoter hypermethylation is carried out in 67% of labs; in 33% of labs MLH1 promoter hypermethylation and BRAF V600 mutations analysis are both performed.

UK laboratories: 50% of labs perform MLH1 promoter methylation analysis; no further analysis is carried out in the remaining labs.

MLH1 promoter methylation analysis: methods

How do you perform it?

Answers to the above-mentioned question have been provided by only 38 responders.

Some labs use multiple methods to analyze MLH1 promoter hypermethylation.

Spanish laboratories: The most frequent method for the analysis of MLH1 promoter hypermethylation is pyrosequencing (50%), followed by MLPA (25%).

Italian laboratories: The most frequent method for the analysis of MLH1 promoter hypermethylation is Methylation specific PCR (38%), followed by pyrosequencing (31%)

Swiss laboratories: The most frequent method for the analysis of MLH1 promoter hypermethylation is Methylation specific PCR (67%), followed by pyrosequencing (33%)

UK laboratories: they equally perform analysis of MLH1 promoter hypermethylation by methylation specific PCR (33%) and MLPA (33%) (Fig. 4D)

Other biomarkers

Do you perform the IHC evaluation of additional molecular makers?

Answers to the above-mentioned question were provided by 69 responders.

There are other viable biomarkers that can be used for EC characterization, and these include:
- p53, analyzed in most laboratories in all countries (85% in Italy, 90% in Spain, 67% in Switzerland and 83% in UK);
- POLE, analyzed mostly in Spain (50%) and Switzerland (67%);
- PTEN analyzed mostly in Spain (45%) and Switzerland (67%).

Some labs use multiple biomarkers for EC biomolecular characterization.

Other biomarkers such as Beta-catenin, PR, ER, p16, L1CAM, ARID1A and E-cadherin are studied only in a minority (10%) of Italian labs, for scientific purposes (Fig. 5A).

Algorithm for biomarker analysis in EC

Thus, the most common biomarker analysis strategy in EC includes the analysis of MMR by IHC in all samples, the analysis of MLH1 promoter hypermethylation in case of negative MLH-1/PMS-2 expression, and the analysis of other biomarkers such as p53 and, to a lesser extent, PTEN and POLE.

Guidelines

Which national/international guidelines do you consult to categorize prognostic risk in EC?

Answers to the above-mentioned question were provided by 44 responders.

Swiss labs did not provided answers to this question.

Some labs referred to use multiple guidelines.

ESGO/ESTRO/ESP guidelines are the most used in Italian and Spanish labs (45% and 53% respectively), followed by (College of American Pathologists) CAP guidelines (18% and 41% respectively). 18% and 14% of Italian laboratories also adopt Associazione Italiana di Oncologia Medica - Società Italiana di Anatomia Patologica e Citopatologia (AIOM-SIAPEC) and WHO 2020 guidelines. 100% of UK labs refer to BAGP guidelines.

Spanish Society of Pathology (SEAP) recommendations, Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), European Society for Medical Oncology (ESMO), Armed Forces Institute of Pathology (AFIP) Atlases and National Comprehensive Cancer Network (NCCN) guidelines and/or recommendations are utilized in a minority of cases (Fig. 5B).

Discussion

The present study evaluated current daily practice regarding biomarker analysis in endometrial cancer in 20 Spanish, 40 Italian, 3 Swiss and 6 UK laboratories, focusing on MMR and MSI. Recorded data underline the fact that MMR analysis by IHC in EC samples is a common practice. The advantages of IHC-based analysis are numerous: it is a well-established and readily available method, its cost is relatively low, it has a fast

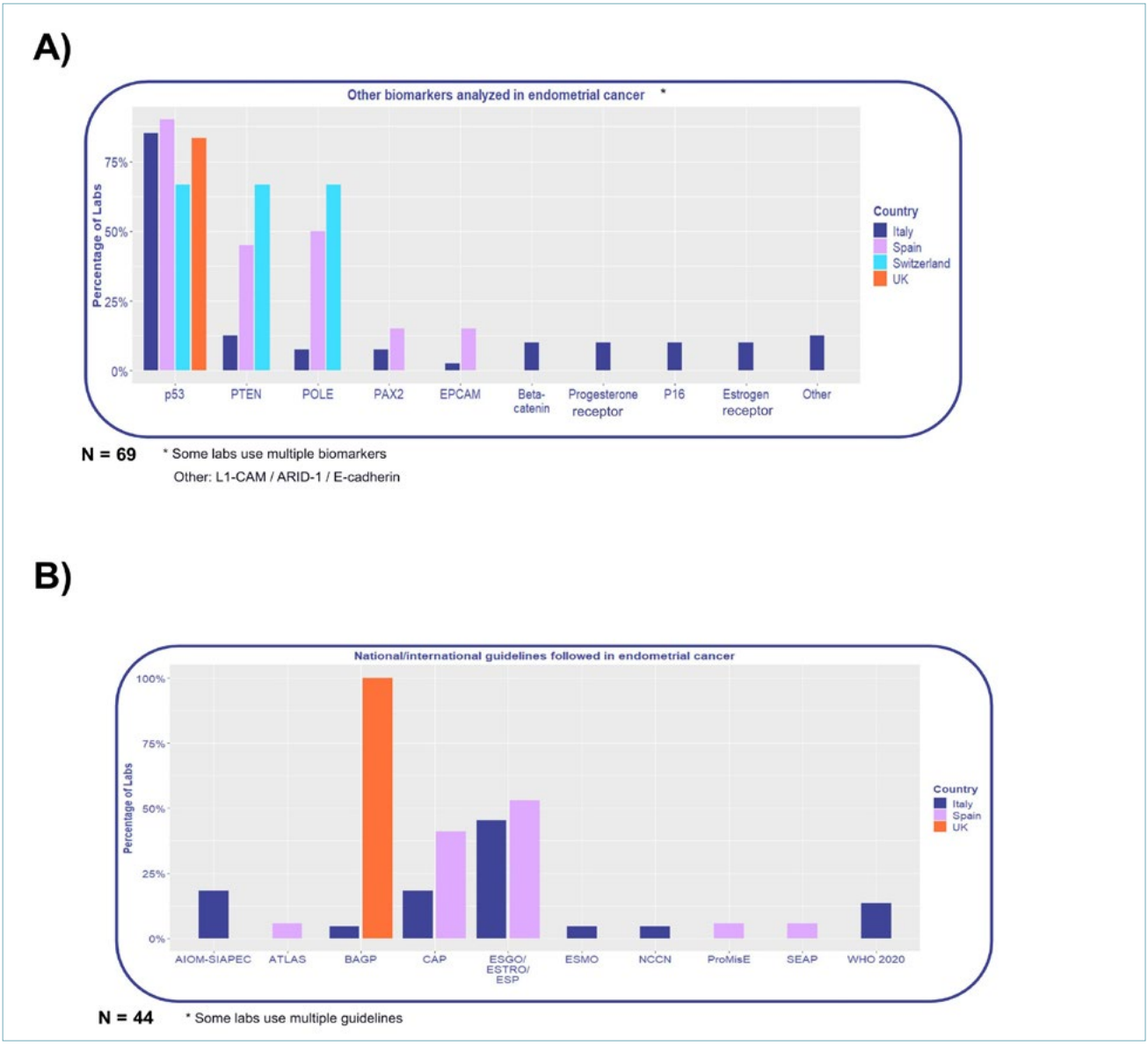

Figure 5. Other biomarkers tested and guidelines followed in Italy, Spain, Switzerland, UK for EC. (A) Biomarker assessment by country in EC. N: number of laboratories. (B) Guidelines followed by the labs of different countries. N: number of laboratories.

turn-around time, correlation with morphology is rapid and easy, it can be applied to any kind of specimen (ranging from small biopsies to surgical resections) and it can be used even in cases with a restricted quantity of tissue.

Most European laboratories (100% in Switzerland and UK; 80% in Spain and 47.5% in Italy) perform MMR by IHC in all EC diagnoses, differently a minority of laboratories (20% in Spain and 32.5% in Italy proceed with this analysis only in selected cases (suspicion of Lynch Syndrome or early-onset cancers).

Even if the association of only two antibodies (MSH6 and PMS2) might decrease the cost without substantially diminishing the diagnostic accuracy, the evaluation of all 4 MMR proteins by IHC is validated as first choice method in 58% of Italian labs, 50% of Spanish labs, in 33% of Swiss labs and in 100% of UK labs.

In contrast, MSI analysis by molecular method is uncommon and restricted to cases showing an ambiguous MMR IHC result, in case of positive Lynch

Syndrome (LS) family history or on clinician request. In this regard, Italian labs mostly provided Option 2 (ambiguous IHC) (22.5%), Spanish Labs opted for Other (family history with a retained IHC MMR profile) (30%), Swiss Labs selected Options 1+2 (MMR deficient or unclear/ambiguous result) (33.3%) and Other (33.3%), UK labs choose Option 2 (16.7%) and Option 1+2 (16.7%).

The two methods (IHC and MSI) have similar sensitivity and show nearly 96% concordance, meaning that IHC analysis can be regarded as an accurate surrogate of MSI molecular testing in EC. One of the reasons for which some pathological guidelines prefer IHC to MSI is because MSI can miss some MSH6 mutations [13,14].

Several centers also carry out MLH1 promoter hypermethylation analysis and BRAF p.V600 mutational analysis in case of MLH1/PMS2 negative tumors to investigate the presence of somatic mutations. In particular, MLH1 promoter hypermethylation is carried out in in 67% of Swiss labs, 61% of Spanish labs, in 50% of UK labs and in 28% of Italian labs.

For what concerns the use of other biomarkers, it appears that p53 is the most studied (85% in Italy, 90% in Spain, 67% in Switzerland and 83% in UK) followed by POLE and PTEN.

Pathogenic somatic mutations in the exonuclease domain of the replicative DNA polymerase epsilon (POLE) distinguish a subcategory of ultramutated tumors (POLE-ultramutated) within the category of ECs. Patients with these mutations tend to have superior immune response and improved clinical outcome. ESGO/ESTRO/ESP guidelines suggest that POLEmut carcinomas up to FIGO stage II, independently of FIGO grade, histotype, and presence or absence of LVSI, are categorized as low-risk group and thus, a watch-and-wait strategy can be adopted, with no need for adjuvant therapy [5,6]. The most common pathogenetic POLE mutations are P286R, V411L, S297F, A456P, and S459F; however, the management of patients bearing less frequent POLE mutations remains uncertain [5,6]. Furthermore, there are cases showing simultaneously two or three molecular signatures. These account for 3% of ECs and are called "multiple classifiers", in detail: MMRd/p53abn; POLEmut/p53abn; MMRd/POLEmut/p53abn; MMRd/POLEmut. In these subgroups, the prognosis depends on the driver molecular subtype. Specifically, the POLEmut signature prevails over the other signatures, giving a good prognosis and the MMRd signature prevails over the p53abn signature [15].

From our survey data, POLE testing is performed in 50% of Spain, 67% of Swiss and only in 10% of Italian laboratories, but this trend is still increasing. In particular, in Italy the POLE analysis is adopted only by a minority of labs, because this test is not still reimbursed by the National Health Service. Thus, generally physicians restrict POLE sequencing to low-risk ECs showing abnormal or sub-clonal p53 staining and intermediate/high intermediate risk patients but omitted in advanced (stage III-IV) ECs since adjuvant therapy is always adopted regardless of molecular classification. In UK, NGS testing is accessible via the national genomic test directory (https://www.england.nhs.uk/publication/national-genomic-test-directories/) which included POLE testing in endometrial carcinoma in 2022. This occurred after the survey reported here. Since 2022, BAGP guidelines (https://www.bgcs.org.uk/wp-content/uploads/2022/04/BAGP-POLE-testing-in-Endometrial-cancer-v1.1-2022-04-08.pdf) have been followed by most UK laboratories.

Results in the context of published literature

Worldwide, EC places seventh among all female cancers occurring mostly in postmenopausal women. In Europe, it ranks fourth among female neoplasia with an incidence of 12-20:100,000 [16]. However, the number of newly diagnosed cases is expected to rise, and mortality rates have also been increasing by 1.9% per year on average, mainly because of the increasing incidence of obesity and because of aging of the population [17]. The different 5-year overall-survival (OS) rates between patients diagnosed with stage III/IV and those diagnosed with stage I/II (respectively, 57-26% versus 74-94%) [18] still poses the attention on the importance of patient risk stratification in order to proceed with targeted therapies.

Starting from the original The Cancer Genome Atlas (TCGA) proposal of four molecular EC subgroups, the pathological diagnosis and prognostic classification of the disease are constantly evolving. Indeed, molecular classifiers have been included in the new 5th edition of the WHO classification of tumors of the female genital tract. Based on integrated genomic characterization, EC subgroups include ultramutated, hypermutated, and somatic copy-number low and high subtypes. These groups were shown to have significant prognostic differences with ultramutated (POLEmut) ECs having a favorable outcome, hypermutated (MMRd) ECs having an intermediate prognosis and p53abn ECs showing the poorest clinical outcome. These data permit therapeutic stratification in addition to risk stratification: POLEmut ECs can avoid adjuvant therapy while p53abn ECs may benefit from the addition of chemotherapy [19].

According to the National Comprehensive Cancer Network guidelines, ancillary molecular investigations for POLE mutations, MMR/MSI definition, and p53 status

are highly recommended in order to improve and complete the diagnostic pathway of ECs [20]. IHC can be used as first-choice test to establish EC profile; however, POLE mutations cannot be detected by using surrogate immunohistochemical markers and, thus, DNA sequencing is still required [21]. MMR/MSI analysis is recommended in all ECs, independently of age, in order to:

- perform Lynch Syndrome screening;
- correctly define a histo-molecular diagnosis of EC according to the TCGA classification;
- predict MMRd tumor response to targeted treatment with immune checkpoint inhibitors;
- apply a molecular prognostic risk stratification.

In fact, it has been shown that cases with MLH1/PMS2 loss and MLH1-promoter hypermethylation (met-EC) had a lower proportion of grade 1 tumors, a higher proportion of stage III/IV tumors and worse overall and progression-free survival, thus identifying a molecular MSI EC class as the main target for anti-PD-1 antibody treatment. Conversely, patients with Lynch Syndrome associated ECs showed a trend towards better recurrence-free survival, but higher risk for second cancers compared with patients with met-ECs. A significant limitation associated with both MLH1 IHC testing is the inability to differentiate between MLH1 loss caused by a germline mutation or by an uninherited somatic epimutation. For this reason, BRAF V600E mutation together with MLH1 methylation testing has been considered an important addition to the triage process for colo-rectal cancer (CRC) [22,23]. Unlike CRC, the BRAF V600E mutation occurs so infrequently in EC that it has no role in triage for MMR gene mutation testing, being disregarded as a possible surrogate for MLH1 methylation analysis or as a useful molecular marker for the prediction of germline MMR mutations, or to predict any other clinical criteria [24].

Strengths and weaknesses

This study is the first survey analysis centered on the most frequently used immunohistochemical and molecular analyses to classify ECs in European laboratories, aiming to compare the procedures utilized in different countries. Recently, Zannoni et al. also published another survey, focusing on the most commonly adopted immunohistochemical and molecular biomarkers in daily clinical characterization of a diagnosed endometrial carcinoma in Italian labs [25].

Even if complete tumor typing, including molecular analysis, is highly recommended, our survey suggests that in Europe the extensive panel, including p53, POLE and PTEN analysis, is not adopted in the totality of laboratories. However, we considered only laboratories from Spain, Italy, UK and Switzerland and thus the study should be further expanded.

Implications for practice and future research

Given that histological and molecular profiling of ECs appears to be essential for prognosis and therapeutic strategies, it could be helpful to develop a network of certified laboratories focused on this assessment. Indeed, this choice could simplify patient risk stratification, proper use of targeted therapies and thus improve the quality of care.

Conclusions

From our survey we observed that IHC method appears to be the most common choice for ECs biomarker analysis. The advantages are numerous: IHC is widely adopted in daily diagnostic practice, it is easily viable, manageable, and accurate, with a good inter-observer reproducibility especially when based on strict laboratory protocols and established guidelines [26-28].

The most common assessment for ECs consists in the analysis of MMR by IHC in all 4 European countries (Italy due to the cohort of labs and regional organization includes molecular labs performing only MSI assessment), followed by analysis of MLH1 promoter hypermethylation and/or BRAF mutation in case of negative MLH-1/PMS-2 expression in Spain and Switzerland (50% of Italian and UK labs do not perform further analysis). Even if with a lower frequency, there are other parameters that are frequently analyzed and these include mainly p53 in all countries, POLE and PTEN in Spain, Switzerland and more occasionally in Italy. UK labs have started assessing POLE in selected endometrial cancers by NGS testing since April 2022. Indeed, these additional biomarkers allow to identify ECs subgroups (namely, MMR deficient/proficient, p53 mutant/wild-type and multiple classifiers) and move toward personalized and targeted therapies to improve therapeutic and follow-up pathways.

Finally, also the recent new FIGO 2023 staging system encourages the performance of complete molecular classification (POLEmut, MMRd, NSMP, p53abn) in all endometrial cancers [29].

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Acknowledgements

We would like to thank Diaceutics PLC who provided data and analytic support on their DXRX platform for this research.

Conflict of interest statement

The authors declare no conflict of interest.

Funding

This research received no external funding

Ethical consideration

Our study was performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (1975, revised in 2013). Patients' initials or other personal identifiers did not appear in any image. Analysed data were collected as part of routine diagnosis.

Author contributions

Conceptualization: AS and GFZ; methodology: EB, BB, MB, RG, XMG, FC, VG; software: PG, YP, JTL, BB; validation: AS, GFZ, VG, EB, MB; formal analysis: PG, YP, JTL, BB; investigation: AS; resources: Collaborators; writing/original draft preparation: AS, BB; writing/review and editing: AS, GFZ; supervision: RG, XMG, FC, GFZ; project administration: GFZ. All authors have read and agreed to the published version of the manuscript.

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