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## ABSTRACT

**Background:** 2021 ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma (EC) encourage molecular classification and propose a new prognostic risk stratification based on both pathologic and molecular features. Although deep myometrial invasion (DMI) has been considered as a crucial risk factor in EC, it is unclear if its prognostic value is independent from The Cancer Genome ATLAS (TCGA) groups.

**Aim:** To assess if the prognostic value of DMI is independent from the TCGA groups in EC patients.

**Materials and methods:** A systematic review and meta-analysis was performed by searching through 5 electronic databases, from their inception to March 2021, for all studies that allowed to assess DMI as a prognostic factor independent of the TCGA groups in EC patients.

Pooled hazard ratio (HR) of DMI for overall survival (OS) and disease-free survival (DFS) was calculated at multivariable analyses including TCGA groups as a variable. Superficial myometrial invasion (<50% of myometrial thickness) was considered as a reference. In DFS analyses, locoregional and distant recurrence were separately considered for one study.

**Results:** Five studies with 2,469 patients were included in the systematic review and 3 studies with 1,549 patients in the meta-analysis.

Pooled HR of DMI was 1.082 (CI 95% 0.85-1.377; p=0.524) for OS, 1.709 (CI 95% 1.173-2.491; p=0.005) for DFS, 1.585 (CI 95% 1.154-2.178; p=0.004) for DFS additionally considering locoregional recurrence for one study, and 1.701 (CI 95% 1.235-2.344, p=0.001) for DFS additionally considering distant recurrence for the same study.

**Conclusions:** DMI does not appear as an independent prognostic factor for OS in EC patients; instead, it seems to affect the risk of recurrence independently from the TCGA groups. Further studies are necessary to confirm these findings and to assess the prognostic impact of DMI separately in each TCGA group.



**KEYWORDS:** cancer; tumor; endometrium; prognosis; treatment; risk assessment; PROMISE.

## INTRODUCTION

Endometrial carcinoma (EC) is the most common gynecological cancer in Europe, with 130,051 new cases in 2020 [1-10]. In the last years, incidence has been rising by 80% due to aging and increased obesity of the population. On the other hand, number of deaths has even increased by 300% because of a poorly reproducible histological risk stratification, leading patients to be undertreated or overtreated [2,3,10-15].

After The Cancer Genome Atlas (TCGA) findings [17], the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), has been validated as a novel classifier to make applicable TCGA classification of EC in clinical practice [2,12,13]. In this classifier, immunohistochemical markers have been proposed as surrogate of sequencing as they have lower costs and less technical difficulties to be assessed [18-20]. In fact, ECs may be categorized in four molecular prognostic groups: ultramutated, with the best prognosis and mutations in the exonuclease domain of Polymerase-ε (POLEmt); hypermutated, with intermediate prognosis and mismatch repair proteins deficiency (MMR-d); copy-number high, with the worst prognosis and Tumor Protein 53 (TP53) mutations, accompanied by abnormal p53 expression (p53-abn); copy-number low, with good-to-intermediate prognosis and no specific molecular profile (NSMP) [17]. However, to date, it still appears unclear how to integrate such molecular groups with other prognostic histological factors in the management of EC.

The 2021 joint guidelines of the European Society of Gynaecological Oncology, European Society for Radiotherapy and Oncology and European Society of Pathology (ESGO/ESTRO/ESP) for the management of patients with EC encourage molecular classification, especially in high-grade tumors, and propose a new prognostic risk stratification based on both histological and molecular features [21].

In particular, stage I MMR-d/NSMP patients are classified at low or intermediate/high-intermediate risk based on the presence of deep myometrial invasion (DMI). In the same way, stage I p53-abn and non-endometrioid histotype are classified at intermediate or high risk based on this histological factor. On the other hand, Stage I-II POLE-mt ECs are categorized at low risk regardless of DMI and

other histological factors [21]. Unfortunately, although DMI has been considered as a predictor of both lymph node metastasis and overall prognosis in EC [22], it is unclear whether its prognostic value is independent from the TCGA groups.

The aim of this study was to assess if the prognostic value of DMI is independent from the TCGA groups in EC patients, through a systematic review and meta-analysis.

## MATERIALS AND METHODS

### Study protocol

We *a priori* defined the study protocol defining methods for each review stage. All review stages were independently performed by two authors, with discussion with other authors as disagreements solution. The Preferred Reporting Item for Systematic Reviews and Meta-analyses (PRISMA) statement and checklist [23] were followed for reporting the whole study.

### Search strategy

We performed several searches in 5 electronic databases (i.e. MEDLINE, Web of Sciences, Google Scholar, Scopus, and ClinicalTrial.gov) from their inception to March 2021, by using a combination of the following text words: “ESGO”, “ESTRO”, “ESP”, “ESMO”, “cancer”; “carcinoma”; “tumor”; “tumour”; “malignancy”; “neoplas*”; “endometr*”; “myometr*”; “myometrial invasion”, “prognosis”; “survival”; “ATLAS”; “genome”; “TCGA”; “Proactive Molecular Risk Classifier”; “ProMisE”; “PORTEC”; “TransPORTEC”; “POLE”; “ultramutated”; “copy number”; “mismatch repair”; “MMR”; “MSI”; “microsatellite instability”; “MLH1”; “MSH2”; “MSH6”; “PMS2”; “EPCAM”; “hypermutated”; “TP53”; “p53”; “tumor protein 53”; “immunohistochemistry”; “immunohistochemical”; “marker”. No MeSH terms were used. No geographic or language restrictions were applied.

References list from eligible studies were also screened.

### Study selection

We included all peer-reviewed studies that allowed assessment of DMI as a prognostic factor independent of the TCGA groups in EC patients. In particular, we included all studies that reported multivariate survival analyses with hazard ratios (HR) for DMI and TCGA groups as a variable.

We *a priori* defined reviews and case reports as exclusion criteria.

## Data extraction

P.I.C.O items were followed for data extraction [23]. “Population” of our study was EC patients. “Intervention” (or risk factor) was the presence of DMI (i.e. myometrial invasion >50%). “Comparator” was the absence of DMI (i.e. myometrial invasion <50%). “Outcomes” were overall survival (OS, primary outcome) and disease-free survival (DFS, secondary outcomes). OS (or time to death) was defined as time from surgery until death of any cause. DFS (or time to progression) was defined as time from surgery until there is evidence of recurrent or progressive disease (this was based on either clinical evidence of recurrence or imaging confirmation of recurrence) or if death from the disease occurred prior to the censoring date.

## Risk of bias within studies assessment

The Methodological Index for Non-Randomized Studies (MINORS) was used to assess the risk of bias within studies [24]. We assessed each included study for 7 applicable domains related to risk of bias: 1) Aim (i.e. clear aim); 2) Patient selection (i.e. if patients were randomly or consecutively selected for inclusion in the study); 3) Prospective data collection (i.e. data collection following an *a priori* defined study protocol); 4) Appropriate endpoints (i.e. if OS and DFS were considered); 5) Unbiased assessment of the study endpoint (i.e. if study endpoints were assessed without bias); 6) Appropriate follow-up period (i.e. the follow-up time was at least 2 years); 7) Loss to follow-up (i.e. patients lost to follow-up were less than 5% of total study population).

Authors judged each study at “low risk”, “unclear risk” or “high risk” of bias if data about the domain were “reported and adequate”, “not reported” and “reported but inadequate”, respectively.

## Data analysis

Multivariable survival analyses were used whether DMI had a prognostic value independent of the TCGA groups, with regard to OS and DFS in EC patients. Cox proportional hazard models were adopted in each included study.

We reported hazard ratios (HR) of DMI as individual and pooled estimate on forest plots, with 95% confidence interval (CI), for OS and DFS multivariable analyses. Myometrial invasion <50% was considered as reference.

In the case of HR with asymmetric CI, the CI lower limit was adjusted to the upper one in order to obtain symmetry. In the case of a mistake in the CI upper limit, this was adjusted to the lower one based on CI symmetry.

We assessed statistical heterogeneity among studies through the inconsistency index $I^2$, as previously described [25-28]. In particular, we considered heterogeneity as: null for $I^2=0\%$, minimal for $0<I^2<25\%$, low for $25\leq I^2<50\%$, moderate for $50\leq I^2<75\%$ and high for $I^2\geq 75\%$.

We adopted the random effect model of DerSimonian and Laird for all analyses.

Comprehensive Meta-Analysis (Biostat,14 North Dean Street, Englewood, NJ 07631, USA) and Review Manager 5.4 (Copenhagen: The Nordic Cochrane Centre, Cochrane Collaboration, 2014) were used as software.

## RESULTS

### Study selection

We identified 11,512 articles through electronic databases searches. 3,746 articles remained after duplicates removal and 83 after title screening. After abstract screening, 17 articles were assessed for eligibility [3,13-15,28-40]. Of them, 12 were excluded because of the absence of multivariable survival analysis for DMI [13,14,28,30-37] or the lack of DMI as a prognostic factor [3]. Finally, 5 articles with 2,469 patients were included in the qualitative synthesis [15,29,38-40] and 3 articles with 1,549 patients in the quantitative synthesis [15,29,38] (Supplementary Figure 1).

### Characteristics of the included studies

Two studies evaluated a cohort from a randomized controlled trial (RCT) [15,38], two studies a retrospective cohort [39,40], and one study a prospective cohort [29].

Three studies included all ECs regardless of histotype [29,39,40], while 2 studies included only endometrioid histotype [15,38]; one of the latter ones restricted selection to International Federation of Gynaecology and Obstetrics (FIGO) grade 3 ECs [38] (Supplementary Table 1).

### Characteristics of study population

The mean age of patients ranged from 66 to 68 years (range 33-96) and the mean follow-up time ranged from 4.8 to 10.9 years.

Regarding International Federation of Gynecology and Obstetrics (FIGO) stage, 57% of ECs were at Stage I and 43% at Stage II-IV. From studies with extractable pathological data, 87.6% of ECs had endometroid histotype, 56.2% FIGO grade 1-2, 52% FIGO stage IA and 22.1% FIGO stage IB. 53.7% of patients underwent adjuvant treatment (Supplementary Table 2).

ECs were POLE-mt in 8.4% of cases, MMR-d in 27.6%, NSMP in 47% and p53-abn in 17% (Supplementary Table 3).

## Risk of bias within studies

All included studies were judged at low risk of bias for each domain, with the exception of the "Appropriate endpoints" and "Unbiased assessment of the study endpoint" domains.

In the "Appropriate endpoints" domain, 2 studies were considered at unclear risk of bias [15,29]. In particular, the study by Eriksson et al. [29] did not consider OS, while the study by Stelloo et al. [15] considered separately locoregional and distant recurrence for DFS.

In the "Unbiased assessment of the study endpoint" domain, two studies were judged at high risk because they adopted "None" myometrial invasion rather than "Myometrial invasion <50%" as reference [39,40] (Supplementary Figure 2).

## Meta-analysis

Two studies included in the qualitative synthesis were excluded from the meta-analysis due to high risk of bias in the "Unbiased assessment of the study endpoint" domain [39,40]. Two studies were suitable for OS analysis [15,38] and 3 studies were suitable for DFS analysis [15,29,38]. In the study by Stelloo et al. [15], we separately considered locoregional and distant recurrence for DFS analyses. For the study by Eriksson et al. [29], we only considered HR from multivariable analysis containing all preoperative variables associated with recurrence or progression in univariable analysis.

Pooled HR of DMI was 1.082 (CI 95% 0.85-1.377; p=0.524; $I^2$=0%) for OS (Figure 1), 1.709 (CI 95% 1.173-2.491; p=0.005; $I^2$=0%) for DFS excluding the study by Stelloo et al. [15] (Figure 2), 1.585 (CI 95% 1.154-2.178; p=0.004; $I^2$=0%) for DFS considering locoregional recurrence for the study by Stelloo et al. [15] (Figure 3), and 1.701 (CI 95% 1.235-2.344; p=0.001; $I^2$=0%) for DFS considering distant recurrence for the study by Stelloo et al [15] (Figure 4).

## DISCUSSION

This study shows that DMI does not appear as a prognostic factor for OS in EC patients; otherwise, it seems to affect the risk of recurrence independently from the TCGA groups. This study may be the first systematic review and meta-analysis assessing DMI as a prognostic factor independent from the TCGA groups in EC patients.

In 2013, TCGA molecular groups showed the potential to reduce under and overtreatment of EC patients, improving the poor reproducibility of histological risk stratification at the basis of the negative epidemiological trend of EC in the last two decades [2,3,10-15]. However, considering the TCGA groups as the only relevant prognostic factor appears questionable, with several studies hypothesizing a combination of molecular and histological prognostic factors in order to achieve a more tailored risk stratification of EC patients [4-10].

Although data regarding integrated molecular and histological prognostic factor are still lacking, the 2021 ESGO/ESTRO/ESP guidelines for the management of patients with EC have encouraged an integrated risk stratification system [21]. In particular, stage I-II POLEmt ECs are included in the low-risk group, with recommendation of no adjuvant treatment. Stage IA MMR-d/NSMP low grade ECs with negative or focal LVSI are also classified as low risk, following the same management. On the other hand, stage IB MMR-d/NSMP ECs with negative or focal LVSI are included in the intermediate risk group or in the high-intermediate risk group based on the tumor grade [21]. In these cases, since the PORTEC-3 trial suggested no benefit of chemotherapy [41], omission of adjuvant treatment is considered as an option when a close follow-up can be guaranteed [21]. Stage I p53mt endometrial carcinomas are instead included in the intermediate risk group or in the high-risk group based on the myometrial invasion status. For p53mt ECs without myometrial invasion, adjuvant treatment may or may not be recommended, while, for p53mt carcinomas with DMI, adjuvant treatment is recommended [21]. However, as evidence on the value of adjuvant therapy in stage I p53mt carcinomas without DMI is very limited [42], adjuvant treatment is recommended to be discussed on a case-by-case basis until more prospective data are available [21].

Therefore, like other histological factors, myometrial invasion appears as a crucial variable in the risk assessment and in the decision making for adjuvant treatment, especially in stage I MMR-d/NSMP

231 and p53mt ECs. Instead, POLE-mt ECs are considered less prognostically affected by myometrial
232 invasion and other histological factors.

233 However, assigning patients to a specific risk group and therefore to a specific adjuvant treatment
234 based on an integration of TCGA group and myometrial invasion status (as well as for other
235 histological factors) should presuppose prognostic independence between prognostic factors.

236 Several studies have assessed this issue with conflicting results in the literature. In a cohort of 426
237 ECs from Chinese women, Dan He et al. showed that high stage and DMI were significantly
238 associated with an increased risk of tumor recurrence or progression regardless of POLE mutation
239 status [32]. In a recent meta-analysis, McAlpine et al. confirmed that stage appeared significantly
240 associated with recurrence or death from disease within the *POLE*mt group; other prognostic factors,
241 such as age, histotype, grade, and LVSI, did not seem to carry the same relevance [43].

242 Regarding the MMR-d group, while DMI was identified as an independent predictor for death from
243 disease by Loukovaara et al. [44], it appeared as an independent prognostic factor in endometrioid
244 endometrial carcinomas, but not in MMR-deficient ones in Pasanen et al. study [45]. In the latter
245 study, multivariable analysis confirmed only the independent value of LVSI [45].

246 Our study showed that DMI did not bear an independent prognostic impact on OS, since pooled
247 multivariate HR for OS was not statistically significant. On the other hand, pooled HR for DFS was
248 significant and indicated a 1.5-2-fold increased risk of recurrence for EC patients with DMI
249 independent of the TCGA group.

250 These results add further data in order to elaborate an integrated and more tailored evidence-based
251 risk stratification of EC patients. This risk stratification appears crucial in attempting to subvert the
252 negative epidemiological trend of EC in the last two decades. However, the choice to assign EC
253 patients to a specific risk group and therefore to a specific adjuvant treatment based on myometrial
254 invasion status requires to be further investigated. In fact, our data indicating an impact on
255 recurrence and not on death of any cause make the issue even more intriguing. Reasons for such
256 discrepancy are unclear. It might be hypothesized that a longer follow-up is necessary to detect
257 differences in OS, or that DMI preferentially affects the risk of recurrence of less aggressive tumors.

It is evident that further studies are necessary to assess the independent value of myometrial invasion, as only 2 [15,38] and 3 studies [15,29,38] were eligible for OS and DFS analysis, respectively. Moreover, our study highlights the lack of primary studies assessing the prognostic value of DMI in each single TCGA group. Separately assessing TCGA groups is necessary as the prognostic value of each TCGA groups is differently affected from histological factors, with the POLEmt group appearing as the group least affected [4].

Regarding the difference between histotypes, one of the included studies (Stelloo et al.) only included endometrioid ECs [15], while another one (Bosse et al.) only included G3 endometrioid ECs [38] and the remaining one (Eriksson et al.) included any type of EC [29]. The HR values were very similar for OS (1.077 and 1.090) and were consistently higher for DFS (1.315 to 1.780). This might suggest a limited impact of histotype on the prognostic value of DMI, although further research is needed.

As discussed for DMI, the prognostic value of other histopathological factors considered in the guidelines needs to be further assessed, especially because their significance may change across the TCGA groups. For instance, FIGO grade might not be relevant in MMR-d carcinomas [45]. LVSI is the only factor which showed robust results as a prognostic factor independent of the TCGA groups [15]; furthermore, the reproducibility of LVSI was found to be acceptable for routine assessment [46] In addition, there are other histopathological features, not considered in the current guidelines, that were proposed as possible independent prognostic factors, such as microcystic, elongated and fragmented (MELF) pattern of invasion and tumor budding [47-49]. The prognostic significance of these factors, their reproducibility and their possible integration in the current risk stratification system require further investigation.

## CONCLUSION

DMI does not appear as an independent prognostic factor for OS in EC patients; on the other hand, it seems to affect the risk of recurrence independently from the TCGA groups. Further studies are necessary to confirm these findings and to assess DMI prognostic impact separately in each TCGA group.

## CONTRIBUTION

AR and AT independently assessed electronic search, eligibility of the studies, inclusion criteria, risk of bias, data extraction and data analysis. DR, DN, FR, PC and AS contributed to the elaboration of methods for risk of bias assessment, data extraction and analysis. AR, AT, DN, LI, FZ, GFZ, AM and RS conceived the study; DR, FR, PC, AS and LI worked on the design of the study; AR, AT, DN, DR, FR and LC worked on the manuscript preparation; PC, LI, FZ, GFZ, AM and RS supervised the whole study.

## CONFLICT OF INTEREST STATEMENT

Authors report no conflict of interest.

## FUNDING INFORMATION

No financial support was received for this study.

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## LEGENDS FOR TABLES AND FIGURES

**Figure 1**. Forest plot of individual and pooled hazard ratios for death of any cause at multivariable analysis (including TCGA groups as a variable) in endometrial carcinoma patients with deep myometrial invasion. Myometrial invasion <50% were considered as a reference.

**Figure 2**. Forest plot of individual and pooled hazard ratios for recurrence at multivariable analysis (including TCGA groups as a variable) in endometrial carcinoma patients with deep myometrial invasion. Myometrial invasion <50% were considered as a reference. The study by Stelloo et al. [15] was not included.

**Figure 3**. Forest plot of individual and pooled hazard ratios for recurrence at multivariable analysis (including TCGA groups as a variable) in endometrial carcinoma patients with deep myometrial invasion. Myometrial invasion <50% were considered as a reference. Locoregional recurrence was considered for the study by Stelloo et al. [15].

**Figure 4**. Forest plot of individual and pooled hazard ratios for recurrence at multivariable analysis (including TCGA groups as a variable) in endometrial carcinoma patients with deep myometrial invasion. Myometrial invasion <50% were considered as a reference. Distant recurrence was considered for the study by Stelloo et al. [15].

**Supplementary Figure1.** Flow diagram of studies identified in the systematic review (Prisma template [Preferred Reporting Item for Systematic Reviews and Meta-analyses]).

**Supplementary Figure 2. a)** Assessment of risk of bias. Summary of risk of bias for each study; Plus sign: low risk of bias; minus sign: high risk of bias; question mark: unclear risk of bias; **b)** Risk of bias graph about each risk of bias item presented as percentages across all included studies.

**Supplementary Table 1. Characteristics of the included studies.**

**FIGO:** International Federation of Gynaecology and Obstetrics

**RCT:** Randomized controlled trial

**OS:** overall survival

**DFS:** disease-free survival

**DSS:** disease-specific survival

*: DFS was assessed as locoregional recurrence-free survival and distant recurrence-free survival

**Supplementary Table 2. Characteristics of the study population.**

**FIGO:** International Federation of Gynecology and Obstetrics

**Supplementary Table 3.** TCGA groups and myometrial invasion in the study population.

**Supplementary Table 4.** Variables considered at multivariable analyses in the included studies.