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1 **Prognostic value of myometrial invasion and TCGA groups of endometrial carcinoma**

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28 **ABSTRACT**

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

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

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

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

42 **Results:** Five studies with 2,469 patients were included in the systematic review and 3 studies with
43 1,549 patients in the meta-analysis.

44 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;
45 p=0.005) for DFS, 1.585 (CI 95% 1.154-2.178; p=0.004) for DFS additionally considering
46 locoregional recurrence for one study, and 1.701 (CI 95% 1.235-2.344, p=0.001) for DFS additionally
47 considering distant recurrence for the same study.

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

52

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

54 INTRODUCTION

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

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

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

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

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

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

86

87 **MATERIALS AND METHODS**

88 **Study protocol**

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

93

94 **Search strategy**

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

104 References list from eligible studies were also screened.

105

106 **Study selection**

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

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

111

112

113 **Data extraction**

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

122

123 **Risk of bias within studies assessment**

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

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

134

135 **Data analysis**

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

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

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

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

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

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

152

153 **RESULTS**

154 **Study selection**

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

161

162 **Characteristics of the included studies**

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

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

168

169 **Characteristics of study population**

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

172 Regarding International Federation of Gynecology and Obstetrics (FIGO) stage, 57% of ECs were
173 at Stage I and 43% at Stage II-IV. From studies with extractable pathological data, 87.6% of ECs
174 had endometrioid histotype, 56.2% FIGO grade 1-2, 52% FIGO stage IA and 22.1% FIGO stage IB.
175 53.7% of patients underwent adjuvant treatment (Supplementary Table 2).

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

178

179 **Risk of bias within studies**

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

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

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

188

189 **Meta-analysis**

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

197 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%
198 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
199 95% 1.154-2.178; $p=0.004$; $I^2=0\%$) for DFS considering locoregional recurrence for the study by
200 Stelloo et al. [15] (Figure 3), and 1.701 (CI 95% 1.235-2.344; $p=0.001$; $I^2=0\%$) for DFS considering
201 distant recurrence for the study by Stelloo et al [15] (Figure 4).

202

203 **DISCUSSION**

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

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

214 Although data regarding integrated molecular and histological prognostic factor are still lacking, the
215 2021 ESGO/ESTRO/ESP guidelines for the management of patients with EC have encouraged an
216 integrated risk stratification system [21]. In particular, stage I-II POLEmt ECs are included in the low-
217 risk group, with recommendation of no adjuvant treatment. Stage IA MMR-d/NSMP low grade ECs
218 with negative or focal LVSI are also classified as low risk, following the same management. On the
219 other hand, stage IB MMR-d/NSMP ECs with negative or focal LVSI are included in the intermediate
220 risk group or in the high-intermediate risk group based on the tumor grade [21]. In these cases, since
221 the PORTEC-3 trial suggested no benefit of chemotherapy [41], omission of adjuvant treatment is
222 considered as an option when a close follow-up can be guaranteed [21]. Stage I p53mt endometrial
223 carcinomas are instead included in the intermediate risk group or in the high-risk group based on the
224 myometrial invasion status. For p53mt ECs without myometrial invasion, adjuvant treatment may or
225 may not be recommended, while, for p53mt carcinomas with DMI, adjuvant treatment is
226 recommended [21]. However, as evidence on the value of adjuvant therapy in stage I p53mt
227 carcinomas without DMI is very limited [42], adjuvant treatment is recommended to be discussed on
228 a case-by-case basis until more prospective data are available [21].

229 Therefore, like other histological factors, myometrial invasion appears as a crucial variable in the risk
230 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.

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

264 Regarding the difference between histotypes, one of the included studies (Stelloo et al.) only
265 included endometrioid ECs [15], while another one (Bosse et al.) only included G3 endometrioid ECs
266 [38] and the remaining one (Eriksson et al.) included any type of EC [29]. The HR values were very
267 similar for OS (1.077 and 1.090) and were consistently higher for DFS (1.315 to 1.780). This might
268 suggest a limited impact of histotype on the prognostic value of DMI, although further research is
269 needed.

270 As discussed for DMI, the prognostic value of other histopathological factors considered in the
271 guidelines needs to be further assessed, especially because their significance may change across
272 the TCGA groups. For instance, FIGO grade might not be relevant in MMR-d carcinomas [45]. LVSI
273 is the only factor which showed robust results as a prognostic factor independent of the TCGA groups
274 [15]; furthermore, the reproducibility of LVSI was found to be acceptable for routine assessment [46]

275 In addition, there are other histopathological features, not considered in the current guidelines, that
276 were proposed as possible independent prognostic factors, such as microcystic, elongated and
277 fragmented (MELF) pattern of invasion and tumor budding [47-49]. The prognostic significance of
278 these factors, their reproducibility and their possible integration in the current risk stratification
279 system require further investigation.

280 **CONCLUSION**

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

285

286 **CONTRIBUTION**

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

293

294 **CONFLICT OF INTEREST STATEMENT**

295 Authors report no conflict of interest.

296

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298 No financial support was received for this study.

299

300 **REFERENCES LIST**

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440

441 **LEGENDS FOR TABLES AND FIGURES**

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

445

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

450

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

455

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

460

461 **Supplementary Figure 1.** Flow diagram of studies identified in the systematic review (Prisma
462 template [Preferred Reporting Item for Systematic Reviews and Meta-analyses]).

463

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

467

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

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

470 **RCT:** Randomized controlled trial

471 **OS:** overall survival

472 **DFS:** disease-free survival

473 **DSS:** disease-specific survival

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

475

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

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

478

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

480

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