

DNA ploidy in curettage specimens identifies high-risk patients and lymph node metastasis in endometrial cancer

Tormund S. Njølstad^{1,2}, Jone Trovik^{1,2}, Tarjei S. Hveem^{3,4,5}, Marna L. Kjæreng^{3,4}, Wanja Kildal^{3,4}, Manohar Pradhan^{3,4}, Janusz Marcickiewicz^{6,7}, Solveig Tingulstad⁸, Anne C. Staff⁹, Hans K. Haugland¹⁰, Runar Eraker^{11,12}, Klaus Oddenes¹³, Jan A. Rokne¹⁴, Jostein Tjugum¹⁵, Margaret S. Lode¹⁶, ENITEC network/ MoMaTEC study group*, Frederic Amant¹⁷, Henrica M. Werner^{1,2}, Helga B. Salvesen^{1,2}, Håvard E. Danielsen^{3,4,5,18,**}.

Author affiliations

¹Department of Clinical Science, University of Bergen, Haukeland University Hospital, 5021 Bergen, Norway; ²Department of Obstetrics and Gynecology, Haukeland University Hospital, 5021 Bergen, Norway; ³Institute for Cancer Genetics and Informatics, Oslo University Hospital, 0424 Oslo, Norway; ⁴Centre for Cancer Biomedicine, University of Oslo, 0424 Oslo, Norway; ⁵Department of Informatics, University of Oslo, 0310 Oslo, Norway; ⁶Department of Gynecology, Sahlgrenska Academy, University of Gothenburg, S-413 45 Gothenburg, Sweden; ⁷Department of Obstetrics and Gynecology, Halland's Hospital Varberg, 432 37 Varberg, Sweden; ⁸Department of Gynecology, St. Olav's Hospital, 7006 Trondheim, Norway; ⁹Department of Gynecology, Oslo University Hospital, Ulleval, 0424 Oslo, Norway; ¹⁰Department of Pathology, The Gade Institute, Haukeland University Hospital, 5021 Bergen, Norway; ¹¹Department of Obstetrics and Gynecology, Akershus University Hospital, 1478 Lørenskog, Norway; ¹²University of Oslo, Oslo, Norway; ¹³Department of Obstetrics and Gynecology, Haugesund Hospital, 5528 Haugesund, Norway; ¹⁴Department of Obstetrics and Gynecology, Hospital of Vestfold, 3116 Tønsberg, Norway; ¹⁵Department of Obstetrics and Gynecology, Førde Hospital, 6800 Førde, Norway; ¹⁶Department of Obstetrics and Gynecology, Ålesund

Hospital, 6017 Ålesund, Norway; ¹⁷Department of Gynecologic Oncology, UZGasthuisberg, KULeuven, 3000 Leuven, Belgium; ¹⁸Nuffield Division of Clinical Laboratory Sciences, University of Oxford, Oxford, United Kingdom.

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** Correspondence: Professor H. E. Danielsen; e-mail: hdaniels@labmed.uio.no.

Abstract

Background: Preoperative risk stratification is essential in tailoring endometrial cancer treatment, and biomarkers predicting lymph node metastasis and aggressive disease are aspired in clinical practice. DNA ploidy assessment in hysterectomy specimens is a well-established prognostic marker. DNA ploidy assessment in preoperative curettage specimens is less studied, and in particular in relation to the occurrence of lymph node metastasis.

Methods: Curettage image cytometry DNA ploidy in relation to established clinicopathological variables and outcome was investigated in 785 endometrial carcinoma patients prospectively included in the MoMaTEC multicentre trial.

Results: Diploid curettage status was found in 72.0%, while 28.0% were non-diploid. Non-diploid status significantly correlated with traditional aggressive postoperative clinicopathological features, and was an independent predictor of lymph node metastasis among FIGO stage I-III patients in multivariate analysis (OR=1.94, $p=0.033$). Non-diploid status was related to shorter disease-specific survival (5-year DSS of 74.4%, vs. 88.8% for diploid curettage, $p<0.001$). When stratifying by FIGO stage and lymph node status, the prognostic effect remained. However, in multivariate regression analysis, preoperative histological risk classification was a stronger predictor of disease-specific survival than DNA ploidy.

Conclusion: Non-diploid curettage is significantly associated with aggressive clinicopathological phenotype, lymph node metastasis and poor survival in endometrial cancer. The prognostic effect was also observed among subgroups with (presumably) less aggressive traits, such as low FIGO stage and negative lymph node status. Our results indicate curettage DNA ploidy as a possible supplement to existing parameters used to tailor surgical treatment.

Keywords

Endometrial cancer, DNA ploidy, curettage, lymph node metastasis, prognosis.

Introduction

Endometrial carcinoma is the most common gynaecological malignancy in the western world with a reported lifetime risk of 2-3% (Jemal *et al*, 2010). In Norway, it constitutes 6% of all female cancers. A steady increase in incidence over the last decades has been observed (Cancer Registry of Norway, 2012), attributed to an ageing population and increasing incidence of obesity (Sorosky, 2012). In the United States, estimates by The American Cancer Society predict 52,630 new cases and 8,590 endometrial cancer deaths in 2014 (American Cancer Society, 2014; Siegel *et al*, 2014).

Abnormal uterine bleeding is the cardinal symptom of endometrial cancer. Essential preoperative diagnostic work-up includes histological verification by endometrial biopsy or curettage, in combination with a selection of imaging techniques to predict metastatic disease. Surgery remains the cornerstone in primary treatment; with curative intent it includes total hysterectomy and bilateral salpingo-oophorectomy, with or without retroperitoneal lymph node dissection and omentectomy, dependent on risk assessment based on tumour characteristics as well as patient operability (Amant *et al*, 2005).

Prognostic factors in endometrial cancer have been thoroughly investigated, the most important being age, the surgical FIGO stage (International Federation of Gynecology and Obstetrics), myometrial invasion, histological subtype and grade, and lymphovascular invasion (Amant *et al*, 2005; Creutzberg *et al*, 2000; Prat, 2004), serving as important determinants of tailoring postoperative treatment. Fortunately, the majority of endometrial cancer patients are diagnosed at a relatively early stage with disease presumably confined to the uterus. This is reflected by a generally favourable prognosis, with a 5-year relative survival of 84.4% reported for the period 2008-2010 in Norway (Cancer Registry of Norway, 2012), and 5-year disease-specific survival

reported to 86.9% (Trovik *et al*, 2012). However, endometrial carcinoma is a heterogeneous disease with great variations in aggressiveness, exemplified by a recurrence rate after primary surgery among patients with presumed localized disease of 15-20% (Abeler & Kjorstad, 1991). Consequently, much effort has been made to individualize treatment by (preferably preoperatively) more accurately identifying patients with higher risk for poor outcome requiring more aggressive treatment, as well as identifying low-risk patients to avoid overtreatment (Salvesen *et al*, 2012). As reviewed by Salvesen *et al*, today's preoperative risk assessment based on endometrial histology and imaging could be further augmented by preoperatively accessible biomarkers, such as curettage specimen hormone receptor status (Trovik *et al*, 2013), stathmin expression (Trovik *et al*, 2011) and DNA ploidy (Fredstorp-Lidebring *et al*, 2001; Mariani *et al*, 2000; Pradhan *et al*, 2012; Steinbakk *et al*, 2011) for tailored surgical treatment.

Assessment of DNA ploidy status has been shown to be of prognostic value in several epithelial cancers (Merkel & McGuire, 1990). For endometrial cancer patients, DNA ploidy postoperatively acquired from hysterectomy specimens has repeatedly been reported to be of prognostic importance, with aneuploidy or non-diploidy as a marker for aggressive disease (Larson *et al*, 1999; Lundgren *et al*, 2004; Pfisterer *et al*, 1995; Pradhan *et al*, 2012; Salvesen & Akslen, 2002; Susini *et al*, 2007; Wik *et al*, 2009; Zaino *et al*, 1998). However, only few studies have assessed the value of DNA ploidy in preoperatively available curettage specimens, both in terms of prognosis (Fredstorp-Lidebring *et al*, 2001; Mariani *et al*, 2000; Pradhan *et al*, 2012; Steinbakk *et al*, 2011) and in relation to lymph node status (Mariani *et al*, 2005). As results from multivariate analysis are conflicting (Mauland *et al*, 2014; Terada, 2012), the clinical value of DNA ploidy assessment needs further validation.

On this background, we have investigated DNA ploidy in curettage specimens in relation to a panel of clinicopathological variables, lymph node status and outcome for women diagnosed and treated for endometrial cancer in the prospective, international multicentre trial MoMaTEC1 (Molecular Markers in Treatment of Endometrial Cancer).

Materials and methods

Study cohort

With Regional Ethics Committee approval, a total of 1046 consenting patients in the MoMaTEC1-trial (Molecular Markers in Treatment of Endometrial Cancer, Clinical Trial identifier NCT00598845) were included in this study. The study has acquired approval of The Norwegian Data Inspectorate (961478-2), Norwegian Social Sciences Data services (15501), and Regional Committees for Medical and Health Research Ethics (REKIII no. 052.01). A total of 402 endometrial cancer patients were prospectively included when treated at the Department of Gynaecology and Obstetrics, Haukeland University Hospital, Bergen, Norway, from May 2001 through March 2011. Nine other centres contributed with 644 patients treated for endometrial carcinoma prospectively included at their institutions. Sampling was performed by pipelle or dilatation and curettage as per routine for each contributing centre. Formalin-fixed paraffin-embedded tumour tissue from curettage specimens was collected from all participating institutions.

Clinicopathological data, including age at diagnosis, FIGO stage according to 2009 criteria, histology (type and grade) from hysterectomy specimens, and treatment modalities were recorded. Preoperative curettage histology reports were routinely categorized as either high-risk (standardly comprising histological type reported as non-endometrioid, or histological grade 3 endometrioid carcinoma) or low-risk (standardly comprising other histological diagnoses including grade 1 or 2 endometrioid carcinoma, hyperplasia, and benign endometrium). All precision samples selected for DNA ploidy analysis as later described were investigated for presence of malignant tissue by one of the co-authors (MP), irrespectively of the preoperative routine histological diagnosis. Complying with the aim to evaluate DNA ploidy impact in a patient group treated routinely in a prospective multicentre setting, routine histological reports for grading

and subtyping were applied from the participating centres. Follow-up data with records of recurrence and survival was collected from patient records and correspondence with physicians responsible for outpatient controls.

DNA ploidy analyses

Haematoxylin and eosin stained slides from individual curettage specimen were evaluated to identify tissue areas with highest tumour grade. From the selected area, one or two 1 mm cylinders were punched out using a custom made precision instrument (Beecher Instruments) depending on the curettage tissue depth. Prepared monolayers were stained with Feulgen-Schiff as previously described by (Pradhan *et al*, 2006). The nuclear DNA content was measured using the Ploidy Work Station (PWS) Grabber version 1.4.12 (Room 4 ltd, UK) and a Zeiss Axioplan microscope equipped with a 546-nm green filter and a black and white high-resolution digital camera (Axiocam MRM, Zeiss). DNA ploidy histograms were created based on Integrated Optical Density of the nuclei using PWS Classifier (vs 3.06.03, Room 4 ltd, UK). DNA ploidy histograms were classified as diploid (one G_0/G_1 peak and G_2 less than 10%), aneuploid (DNA Index (DI) 1.06-1.89, 2.11-3.79 or >4.2), tetraploid (DI 1.90-2.10) or polyploid (DI 3.8-4.2). A detailed description of the procedure, DNA content measurement, and histogram classification criteria is given elsewhere (Pradhan *et al*, 2006). Samples with less than 200 nuclei, and samples with poor technical quality were not classified.

Statistical analysis

For statistical analysis, SPSS, Statistical Package for the Social Sciences, version 20.0 (IBM, Chicago, IL, USA) was applied. Associations between categorical variables were explored using Pearson's chi-square test, and odds ratios (OR) for lymph node

metastasis by binary logistic regression. For evaluation of prognostic impact, disease-specific survival (DSS) was defined as time from surgery until death from endometrial carcinoma, as documented from each patient's responsible clinician. Univariate survival analyses were conducted by the Kaplan-Meier method with log-rank test for statistical significance, and multivariate survival analyses by Cox regression. All tests were two-sided, and results were considered statistically significant with a probability of less than 0.05.

Results

Of 1046 curettage samples, 236 samples were discarded due to lack of sufficient tissue material, being not measurable, or unclassifiable due to poor technical quality. Additional 25 patients had incomplete clinicopathological data upon study completion, leaving 785 patients for the final analysis. Follow-up information was available for 733 patients, with a mean follow-up among survivors of 38.4 months (range 0-96 months).

Of these 785 patients, 97.2% (n=763) were treated with primary hysterectomy and bilateral salpingo-oophorectomy, including lymph-node staging in 74.4% of the cases (n=584), 2.4% (n=19) were treated by curettage only, and 0.4% (n=3) by cytoreductive surgery. Lymph node sampling was predominantly pelvic, but included para-aortic dissection for a minority of patients (n=27). Performed lymph node dissection was significantly associated with younger age (82.6% of patients under 66 years vs. 66.5% of patients over 66 years, $p < 0.001$), but showed no significant association to preoperative histological risk classification or curettage DNA ploidy (Supplementary table 1). Additionally, 33.6% of the patients (n=264) received adjuvant therapy, consisting of various combinations of radiation (n=115), hormonal treatment (n=13) and/or chemotherapy (n=171). The study complied with standardly applied treatment protocols for adjuvant therapy at the including centre, individually adjusted for each patient by the responsible physicians. Hormonal treatment was given to 13 patients due to high age (median 86 years) and/or inoperability. None of the patients had a prior history of endometrial cancer, and none received neoadjuvant radiation or chemotherapy.

Evaluating DNA ploidy from curettage samples, 72.0% (n=565) were classified as diploid, 20.6% (n=162) as aneuploid, 6.6% (n=52) as tetraploid and 0.8% (n=6) as polyploid. Dichotomizing patients according to diploid and non-diploid curettage

specimens, aneuploid, tetraploid and polyploidy tumours were merged, constituting 28.0% of total patients included. Details for clinicopathological and demographic characteristics of the study cohort are presented in Table 1. Distribution of preoperative and postoperatively available clinicopathological findings in relation to DNA ploidy status are presented in Table 2. Non-diploid curettage specimen was significantly associated with high FIGO stage, non-endometrioid histological subtype in hysterectomy specimen, high histological grade in hysterectomy specimen, and high-risk curettage classification (all $p < 0.001$). Associations between lymph node status, clinicopathological variables and DNA ploidy are presented in Table 3. Interestingly, patients with non-diploid curettage had significantly higher occurrence of lymph node metastasis compared to patients with diploid curettage (20.6% vs. 9.1% respectively, $p < 0.001$). High-risk curettage classification was also associated with lymph node metastasis compared to low-risk curettage (24.8% vs. 8.6% respectively, $p < 0.001$), in concordance with previously established research findings and clinical practice for identifying high-risk patients for more extensive lymphadenectomy.

Biopsy reports were routinely categorized as either low- or high-risk for clinical risk stratification, as previously described (Trovik *et al*, 2011). Among patients with FIGO stage I-III, non-diploid curettage specimens significantly predicted the occurrence of lymph node metastasis both in univariate analysis (OR 2.73, 95% CI: 1.60-4.66, $p < 0.001$) and in multivariate analysis when adjusted for reported preoperative curettage risk stratification (OR 1.94, 95% CI: 1.06-3.55, $p = 0.033$)(Table 4). However, the long established high-risk histology classification had a similar association to positive lymph node status (OR 2.23, 95% CI: 1.20-4.13, $p = 0.011$)(Table 4).

In univariate survival analysis, results from surgical staging incorporating final histopathological diagnosis, FIGO stage, depth of myometrial infiltration, and lymph

node status were, as expected, statistically significant predictors of disease-specific survival (DSS) (Table 5). Among preoperatively available information, age, DNA ploidy and histological risk classification showed significant associations. Patients with non-diploid curettage specimens had significantly shorter 5-year DSS compared to patients with diploid curettage (74.4% compared to 89.6% 5-year DSS, $p < 0.001$) (Fig. 1A). A similar pattern was found when analysing FIGO stage I and II patients in isolation, with a 5-year DSS of 95.4% for diploid curettage, versus 86.3% for non-diploid curettage ($p < 0.001$) (Supplementary table 2 and Fig. 1B). Noticeably, when viewing the subgroup of women with high FIGO stage (III and IV), no significant difference in ploidy-dependent survival was observed, with 5-year DSS for diploid and non-diploid curettage of 41.4% and 38.1% respectively ($p = 0.267$).

Subgroup analysis was also performed according to lymph node status (Supplementary table 2). Comparing ploidy status isolated to patients with positive lymph node status ($n = 66$), negative lymph node status ($n = 485$) or unevaluated lymph node status ($n = 182$), a significant reduction in 5-year DSS for non-diploid status was observed among the two latter. Five-year survival proportions for diploid vs. non-diploid curettages were 59.0% vs. 55.2% for patients with positive lymph node status (Fig. 2A, $p = 0.680$), 95.5% vs. 88.3% for patients with negative lymph node status ($p = 0.003$, Fig. 2B), and 80.5% vs. 47.2% for patients with unevaluated lymph node status ($p < 0.001$, Fig. 2C). Furthermore, considering patients with FIGO stage I/II only and concomitant unevaluated lymph node status only, the prognostic effect of non-diploid status remained. Among lymph node unevaluated patients with localized disease, the survival proportions were significantly lower for patients with non-diploid versus diploid curettage, with 5-year DSS of 93.4% and 62.0% respectively (Fig. 2D, $p = 0.001$).

Results from multivariate Cox regression analyses are listed in Supplementary table 3. Unadjusted for other parameters, non-diploid curettage was associated with a significant increase in risk of disease-specific death among FIGO stage I-III patients (unadjusted HR of 3.63, $p < 0.001$). When adjusting for preoperatively available curettage risk classification, non-diploid curettage status did not remain significant, but showed a 62% increased risk of disease-related death (HR 1.62, 95% CI: 0.90-2.92, $p = 0.111$).

Discussion

In endometrial carcinoma, DNA ploidy from hysterectomy specimens has repeatedly been reported to be of prognostic importance, with either aneuploid or non-diploid tumours associating with more aggressive phenotype (Larson *et al*, 1999; Lundgren *et al*, 2004; Pfisterer *et al*, 1995; Pradhan *et al*, 2012; Salvesen & Akslen, 2002; Susini *et al*, 2007; Wik *et al*, 2009; Zaino *et al*, 1998). However, results from multivariate analysis are conflicting (Mauland *et al*, 2014; Terada, 2012), where some studies demonstrate an independent effect on survival (Lundgren *et al*, 2004; Susini *et al*, 2007; Zaino *et al*, 1998), while others do not (Larson *et al*, 1999; Pfisterer *et al*, 1995). One prospective implementation study has shown an independent prognostic effect in a routine diagnostic setting (Wik *et al*, 2009). Furthermore, the vast majority of studies are performed on hysterectomy specimens, and only a few studies have assessed the value of DNA ploidy in curettage samples as a predictor of poor outcome (Fredstorp-Lidebring *et al*, 2001; Mariani *et al*, 2000; Pradhan *et al*, 2012; Steinbakk *et al*, 2011), or the relationship to lymph node status (Mariani *et al*, 2005). Of these, only one shows DNA ploidy in curettage specimens to be an independent prognostic factor, however results should be interpreted with care as DNA ploidy was only bivariately corrected for u-PA (urokinase plasminogen activator) or PAI-1 (plasminogen activator inhibitor type 1) (Fredstorp-Lidebring *et al*, 2001). Furthermore, a study exploring DNA ploidy and lymph node status for 82 endometrial cancer patients found no significant association (Mariani *et al*, 2005). Our study is thus an important supplement to this inconclusive exploration of the significance of curettage DNA ploidy status.

Noticeably, although the prognostic value of DNA ploidy in curettage specimens perhaps may be disregarded as inferior to hysterectomy samples, the strength of being preoperatively accessible before hysterectomy bears an immediate advantage as a

possible supplement to preoperative risk assessment and prediction of lymph node metastasis. In this regard, our perhaps most important findings are that, among our patient cohort without distant metastasis at diagnosis, DNA ploidy is an independent predictor of lymph node metastasis, corrected for routinely performed curettage histological risk stratification (OR 1.94, $p=0.033$). For this analysis we excluded cases with FIGO stage IV metastatic disease, as their distant metastasis can be detected during preoperative work-up, and as they have poor prognosis and are subjected to highly individualized treatment strategies also dependent on comorbidity. FIGO stage II and III cases were included to avoid selection bias due to postoperative upstaging in a population with a high proportion of patients subjected to staging lymphadenectomy. Curettage risk stratification showed a stronger trend towards positive lymph node status (OR 2.23, $p=0.011$), although it may be considered a more subjective parameter. In this regard, contemplating DNA ploidy and histological risk stratification with other curettage biomarkers such as p53 and hormonal receptor status would be of interest, in particular since the latter methods are more applicable in a routine diagnostic setting. In our subgroup analysis portrayed in Supplementary table 2, we illustrate that DNA ploidy could be of use in a subset of patients to prognosticate disease behaviour, such as patients with unevaluated lymph node status. Noticeably, DNA ploidy was not significantly associated with disease-specific survival for patients with aggressive phenotype such as high FIGO stage and confirmed positive lymph node status. However, for patients with a presumably less aggressive disease such as low FIGO stage or negative lymph node status, patients with non-diploid tumours had significantly shorter disease-specific survival. The observation among patients not subjected to lymphadenectomy, where non-diploid curettage status was associated with shorter disease-specific survival, supports a particular relevance for DNA ploidy assessment

among patients with unevaluated lymph node status. Our findings could be confounded by patient operability; however, excluding patients with high FIGO stage did not alter the significance of our results (Fig 2D). Our data suggest that DNA ploidy evaluated in curettage specimens can represent an important clinical tool in risk stratification compared to surgical lymph node staging with documented short and long-term side effects (Amant *et al*, 2005; Salvesen *et al*, 2012).

As noted, evaluation of DNA ploidy in postoperatively acquired hysterectomy samples may be viewed as superior to curettage specimens. This was exemplified in a comprehensive study by Pradhan *et al*. including patients with stage I and II endometrioid adenocarcinoma, concluding that analysis of hysterectomy specimen exceeded curettage specimens in predicting biological behaviour (Pradhan *et al*, 2010). As a possible explanation they postulate that the biology of the deep infiltrating parts of the tumour, of which samples are obtained after hysterectomy, are more important for recurrence development than the superficial part of the tumour, obtained by curettage. As a consequence of intratumoural heterogeneity, DNA ploidy classification may differ between hysterectomy and curettage specimens, as illustrated in a study comparing the two modalities (Pradhan *et al*, 2010). In an extensive study exploring the genomic landscape of primary renal cell carcinoma, Gerlinger *et al* conclude that the intratumoural heterogeneity undetected by single-tumour biopsy samples may present major challenges to personalized-medicine and biomarker development (Gerlinger *et al*, 2012). This also directly applies to endometrial cancer, where any curettage sample evidently is restricted to not only represent a distinct area of the endometrium, but also a limited fraction of a tumour with potentially heterogeneous characteristics. This is an aspect that must be taken into consideration when implementing curettage biomarkers into clinical practice, including the reproducibility challenges faced with the standardly

applied histological typing and grading (Salvesen *et al*, 2012). On the other hand, DNA ploidy may in this regard be characterized as a robust method taking intratumoural heterogeneity into account compared to other molecular markers, as a large amount of cell nuclei are measured with a high sensitivity of detecting subgroup aberrations.

Tumour aneuploidy is a common genetic aberration in cancer reflecting a trait of genetic instability. Whether this aneuploidization is a cause or consequence of malignant transformation is uncertain (Holland & Cleveland, 2009). As opposed to structural chromosome rearrangements such as deletions, amplifications, or translocations, the role of whole-chromosome aneuploidy in cancer has received less attention (Gordon *et al*, 2012; Pellman, 2007). Aneuploidy has long been hypothesized as a promoter of tumorigenesis (Boveri, 1914). However, recent papers have shed light on the potential activation of oncogenes and inactivation of tumour suppressor genes leading to aneuploidy (Solomon *et al*, 2011), suggesting aneuploidy as a passenger of tumorigenesis. Aneuploidy is no obligatory trait of cancer, nor is cancer an obligatory consequence of aneuploidy. In concordance with other genetic instability, the pathways involved and the genetic context is of importance in developing the cancerous phenotype. The challenge remains to identify such genetic fingerprints, and translating results to individualize treatment, optimizing patient outcome.

Our study is not without limitations. As noted, of the 1046 patients selected for DNA ploidy analysis, only 810 were successfully ploidy classified due to aforementioned reasons. This exclusion of 236 patients could cause a potential selection bias affecting our results. However, testing patients excluded from the study in terms of clinicopathological characteristics displayed in Table 1, no significant difference was observed in comparison to our final study cohort (all $p > 0.05$, results not shown). Additionally, the proportion of patients unamenable for classification can limit the value

of DNA ploidy assessment in routine use, calling for a method with higher rate of successful classification. Our approach of obtaining DNA ploidy material by punching out areas with highest tumour grade may pose a selection bias towards enriching for the more aggressive regions of the tumour in case of heterogeneity. However, this method allows small samples to be analysed, applicable to small endometrial biopsies. Our study cohort portrays a proportion of patients subjected to lymphadenectomy of 74.4%, illustrating a challenge complying with a staging procedure including lymphadenectomy in an elderly and often comorbid patient population. The extent of lymph node dissection was decided by the responsible surgeon, balancing patient comorbidity and risk involved in extended surgery. As expected, patients subjected to lymphadenectomy were significantly younger, while no other systematic biases were observed (Supplementary table 1). This suggests a selection bias of patients subjected to lymph node sampling based on age, but not the other investigated biomarkers.

In conclusion, our study demonstrates that non-diploid curettage samples are associated with aggressive phenotype, lymph node metastasis and poor disease-specific survival. With the significant association between DNA ploidy and lymph node status, DNA ploidy can be of interest preoperatively to identify high-risk patients for tailored surgical treatment. Furthermore, subgroups of patients with presumably less aggressive traits, such as low FIGO stage and negative lymph-node status from staging lymphadenectomy, have significantly shorter disease-specific survival in the case of non-diploid curettage sample. This prognostic effect was also observed among patients not subjected to staging lymphadenectomy. Our results indicate DNA ploidy as a possible supplement to existing parameters used to tailor surgical treatment such as curettage histological risk stratification. However, additional studies, and in particular a

prospective randomized clinical trial, would be important to evaluate the effect of implementing DNA ploidy into routine clinical practice.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Titles and legends to figures

Fig. 1: Disease-specific survival (DSS) for endometrial cancer patients related to curettage specimens classified according to DNA ploidy. A) DSS for all patients, grouped according to DNA ploidy (n=733). B) DSS for patients with FIGO stage I/II according to curettage DNA ploidy (n=623). Patients with aneuploid, tetraploid and polyploid tumors were merged due to similar survival, and classified as non-diploid. The number of cases followed by the number of endometrial carcinoma related deaths is given in parenthesis. P-values are by Kaplan-Meier estimation by the log-rank test.

Fig. 2: Disease-specific survival (DSS) for endometrial cancer patients in relation to curettage specimen DNA ploidy and lymph node status. A) DSS for patients with positive lymph-node status (n=66); B) DSS for patients with negative lymph node status (n=485); C) DSS for patients with unevaluated lymph node status (n=182); D) DSS for patients with FIGO stage I/II and unevaluated lymph node status (n=152). Patients with aneuploid, tetraploid and polyploid tumors were merged due to similar survival, and classified as non-diploid. The number of cases followed by the number of endometrial carcinoma related deaths is given in parenthesis. All p-values are by Kaplan-Meier estimation by the log-rank test.

Fig. 1

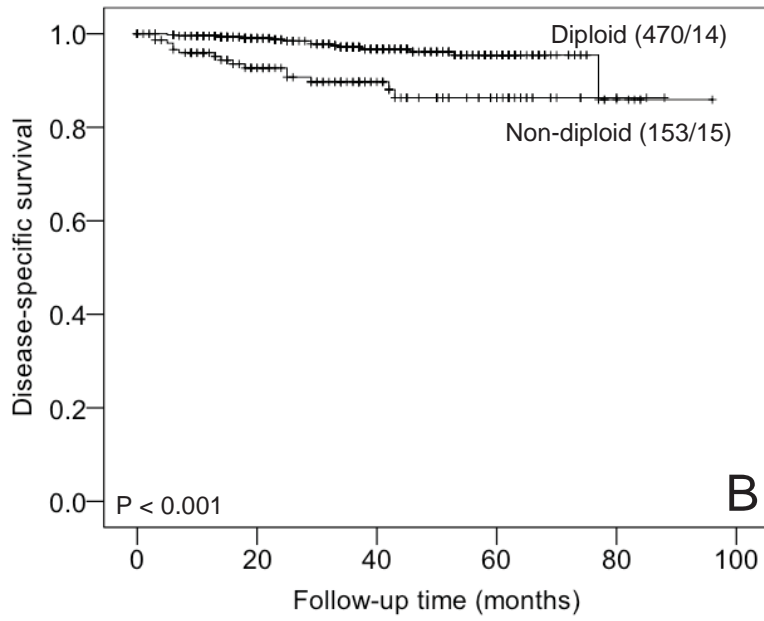
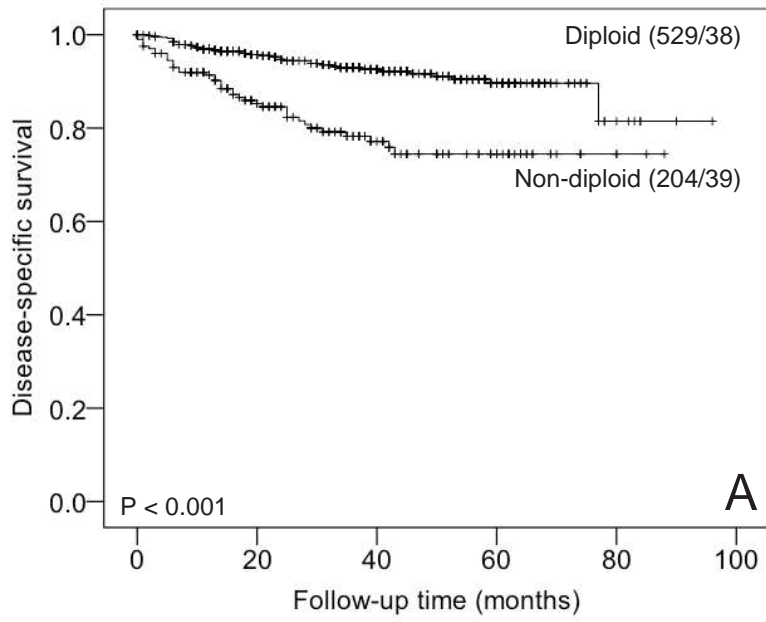
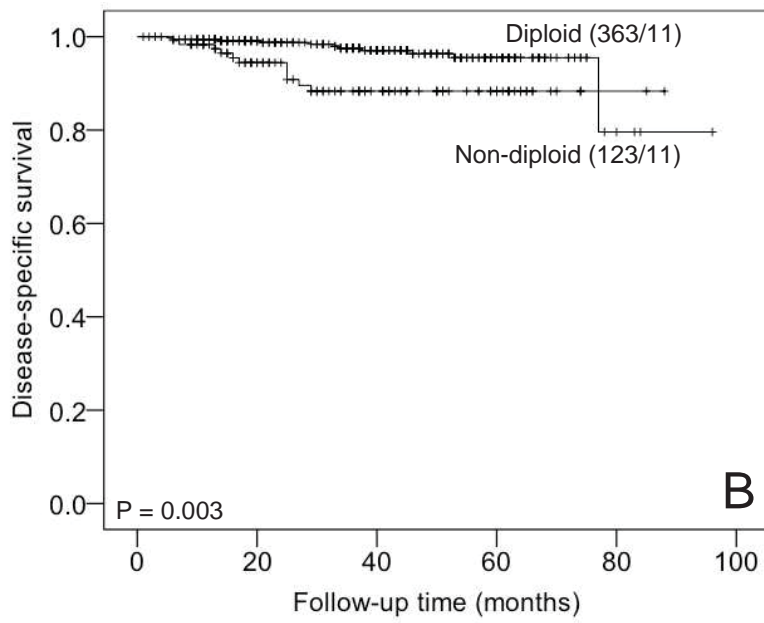
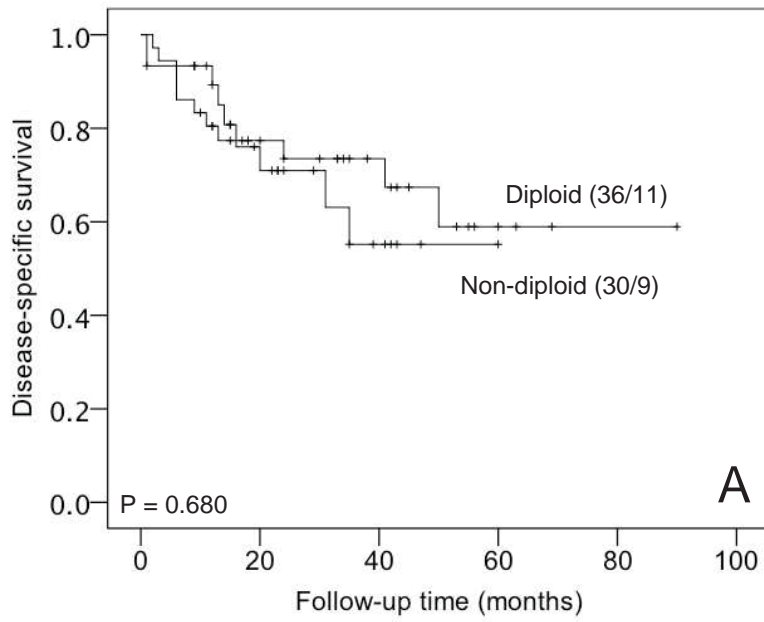


Fig. 2



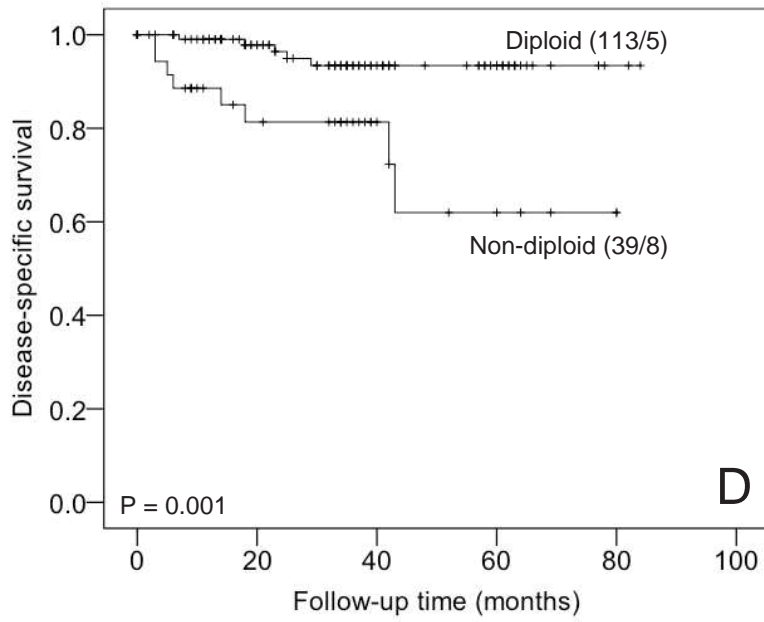
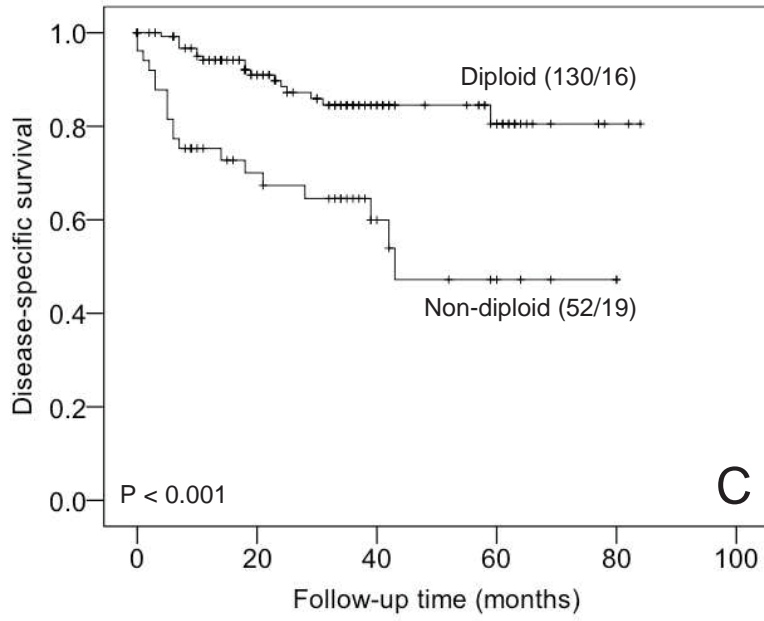


Table 1. Clinicopathological and demographic characteristics for 785 endometrial cancer MoMaTEC* trial patients.

Characteristics	Mean	SD, min-max
Age (years)	66.4	11.1, 28-98
Parity ^a	2.2	1.4, 0-8
Characteristics	Number of cases	Percentage
<i>Information available preoperatively</i>		
Curettage DNA ploidy		
Diploid	565	72.0
Aneuploid	162	20.6
Tetraploid	52	6.6
Polyploid	6	0.8
<i>Information available postoperatively</i>		
Histological subtype ^b		
Endometrioid carcinoma ^c	656	83.8
Serous carcinoma	56	7.2
Clear cell carcinoma	31	4.0
Carcinosarcoma	27	3.4
Undifferentiated carcinoma/other	13	1.7
Histological differentiation ^d		
Grade 1	281	36.0
Grade 2	272	34.9
Grade 3	227	29.1
Myometrial invasion ^e		
<50%	453	64.0
≥50%	255	36.0
FIGO stage (2009 revision)		
I	608	77.5
II	58	7.4
III	91	11.6
IV	28	3.6
Lymph node metastasis ^f		
No	512	87.7
Yes	72	12.3

SD: Standard Deviation. ^aParity information missing for 10 patients. ^bHistological subtype missing for 2 patients. ^cIncluding cases with squamous differentiation. ^dHistological

differentiation missing for 5 patients. ^eData for myometrial infiltration not available for 77 patients. ^fLymph node status evaluated in 584 patients. *Molecular Markers in Treatment of Endometrial Cancer

Table 2. Clinicopathological variables related to DNA ploidy in curettage specimens from 785 patients with endometrial cancer, classified as either diploid or non-diploid (aneuploid, tetraploid and polyploid). All p-values are by Pearson chi-square test.

Variables	Category	Diploid		Non-diploid		P-value
Age at primary treatment	< 66 years	309	80.3%	76	19.7%	<0.001
	≥ 66 years	256	64.0%	144	36.0%	
<i>Information available preoperatively</i>						
Curettage histology classification ^a	Low risk	499	83.2%	101	16.8%	<0.001
	High risk	63	35.0%	117	65.0%	
<i>Information available postoperatively</i>						
FIGO stage (2009)	I / II	503	75.5%	163	24.5%	<0.001
	III / IV	62	52.1%	57	47.9%	
Histological subtype ^b (Hysterectomy specimen)	Endometrioid	530	80.8%	126	19.2%	<0.001
	Non-endometrioid	35	27.6%	92	72.4%	
Histological grade ^c (Hysterectomy specimen)	Grades 1 and 2	462	83.5%	91	16.5%	<0.001
	Grade 3	101	44.5%	126	55.5%	
Myometrial infiltration ^d (Hysterectomy specimen)	< 50%	349	77.0%	104	23.0%	0.008
	≥ 50%	173	67.8%	82	32.2%	
Lymph node metastasis ^e	No	381	74.4%	131	25.6%	<0.001
	Yes	38	52.8%	34	47.2%	
Recurrence ^f	No	443	76.5%	136	23.5%	<0.001
	Yes	58	57.4%	43	42.6%	

^aCurettage histological risk classification as either low risk (benign, hyperplasia or endometrioid grade 1-2) or high risk (comprising non-endometrioid or endometrioid grade 3 histology). Curettage histology risk classification missing for 5 patients. ^bHistological subtype missing for

2 patients. ^cHistological grade missing for 5 patients. ^dMyometrial infiltration not available for 77 patients. ^eLymph node status evaluated in 584 patients. ^fRecurrence only evaluated in patients considered tumour-free after operation (n=680).

Table 3. Lymph node status in 584 endometrial cancer patients subjected to lymphadenectomy in relation to clinicopathological variables and expression of biomarkers. All p-values by Pearson's chi-square test.

Variable	Category	Lymph node negative		Lymph node positive		P-value
Age	< 66 years	279	87.7%	39	12.3%	0.959
	≥ 66 years	233	87.6%	33	12.4%	
<i>Information available preoperatively</i>						
Curettage histology classification ^a	Low risk	406	91.4%	38	8.6%	<0.001
	High risk	103	75.2%	34	24.8%	
Curettage DNA ploidy ^b	Diploid	381	90.9%	38	9.1%	<0.001
	Non-diploid	131	79.4%	34	20.6%	
<i>Information available postoperatively</i>						
Histological subtype ^c (Hysterectomy specimen)	Endometrioid	446	91.8%	40	8.2%	<0.001
	Non-endometrioid	64	66.7%	32	33.3%	
Histological grade ^c (Hysterectomy specimen)	Grades 1 and 2	384	93.0%	29	7.0%	<0.001
	Grade 3	127	75.1%	42	24.9%	
Myometrial infiltration ^d (Hysterectomy specimen)	< 50%	334	97.7%	8	2.3%	<0.001
	≥ 50%	157	79.7%	40	20.3%	

^aCurettage histological risk classification as either Low risk (benign, hyperplasia or endometrioid grade 1-2) or High risk (comprising non-endometrioid or endometrioid grade 3 histology). ^bNon-diploid comprising aneuploidy, tetraploid and polyploidy curettage. ^cHistological subtype and grade missing for 2 patients. ^dMyometrial infiltration available for 539 patients.

Table 4. Prediction of lymph node metastasis based on curettage histology and curettage specimen DNA ploidy in 568 lymph node sampled patients with endometrial cancer FIGO stage I, II or III in the MoMaTEC* multicentre trial. Univariate and multivariate odds ratios (OR) by logistic regression.

Variable	n	Univariate OR	95% CI	P	Multivariate OR	95% CI	P
Curettage histology classification ^a							
Low-risk	441	1			1		
High-risk	127	3.04	1.76-5.24	<0.001	2.23	1.20-4.13	0.011
Curettage DNA ploidy ^b							
Diploid	412	1			1		
Non-diploid	156	2.73	1.60-4.66	<0.001	1.94	1.06-3.55	0.033

^aCurettage histology classified as low-risk (benign, hyperplasia or endometrioid grade 1-2) and high risk (non-endometrioid or endometrioid grade 3). ^bNon-diploid classification comprising aneuploid, tetraploid and polyploid curettage. *Molecular Markers in Treatment of Endometrial Cancer

Table 5. Disease specific survival for 733 endometrial cancer patients related to clinicopathological variables and curettage specimen DNA ploidy. All p-values by Kaplan-Meier survival analysis with log-rank significance test.

Variable	n	Number of deaths	5-year survival	P
Age				
<66	360	21	93.0%	
≥66	373	56	77.0%	<0.001
<i>Information available preoperatively</i>				
Curettage histology ^a				
Low-risk	569	34	91.3%	
High-risk	161	43	62.4%	<0.001
Curettage DNA ploidy ^b				
Diploid	529	38	89.6%	
Non-Diploid	204	39	74.4%	<0.001
<i>Information available postoperatively</i>				
FIGO Stage				
I-II	623	29	93.3%	
III-IV	110	48	38.0%	<0.001
Myometrial infiltration ^c				
<50%	429	17	94.4%	
≥50%	227	37	76.8%	<0.001
Histological type				
Endometrioid	618	37	91.3%	
Non-endometrioid	115	40	52.7%	<0.001
Histological grade ^d				
Grades 1-2	517	25	93.0%	
Grad 3	214	52	67.4%	<0.001
Lymph node metastasis ^e				
Negative	485	22	93.7%	
Positive	66	20	55.7%	<0.001

^aCurettage histological risk classification as either low risk (benign, hyperplasia or endometrioid grade 1-2) or high risk (comprising non-endometrioid or endometrioid grade 3 histology). Information is missing for 3 patients. ^bNon-diploid curettage status includes aneuploid, tetraploid

and polyploidy samples. ^cMyometrial infiltration missing for 77 patients. ^dHistological grade missing for 2 patients. ^eLymph node metastasis evaluated in 551 patients with follow-up information.