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ORIGINAL RESEARCH ARTICLE

Cell proliferation, measured as flow cytometric S-phase fraction, is a strong prognostic indicator in FIGO stage I endometroid endometrial carcinoma: a population-based study

Running headline SPF and prognosis in endometrial cancer

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Conflicts of interests

None of the authors have any conflicts of interests to declare.

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Abstract

Introduction: In early-stage endometrial carcinoma, there is controversy regarding the prognostic value of the flow cytometric variables DNA ploidy (diploid vs. aneuploid) and S-phase fraction. In Sweden, the former is included in national guidelines despite poor scientific support and the latter is not used clinically. This study investigates the prognostic properties of these variables, together with classical histopathological variables, in multivariate analysis in a stringently stratified material.

Material and method: A consecutive, population-based patient material restricted to FIGO 2009 stage I endometroid endometrial carcinoma (n=1140) was retrospectively collected from routinely reported data from medical records. Data on age, FIGO stage, degree of differentiation, S-phase fraction, DNA ploidy status, and adjuvant treatment were included in the study. Cumulative incidence curves with other causes of death as a competing risk were used for univariable analysis for the primary endpoint endometrial cancer death. Cox proportional hazards regression analysis was used for multivariate modeling of all endpoints, and for univariable analysis for the secondary endpoints overall survival and time to progression.

Results: An S-phase fraction value of > 5.5% was associated with worse outcome (for endometrial cancer death: hazard ratio = 2.25; 95% CI 1.38–3.67; p = 0.001, adjusted) and DNA ploidy status was not, for all endpoints tested.

Conclusions: In FIGO stage I endometroid endometrial carcinoma, DNA ploidy status had no prognostic value, while S-phase fraction may be used to identify those with a higher risk of adverse clinical outcome.

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Keywords

aneuploidy; cell proliferation; endometrial neoplasm; prognosis; adjuvant radiotherapy; chemotherapy, adjuvant; flow cytometry; S-phase fraction.

Abbreviations

EC – endometrial carcinoma

FCM – flow cytometry

FIGO – International Federation of Gynecology and Obstetrics

HR – hazard ratio

OS – overall survival

SPF – S-phase fraction

TTP – time to progression

VBT – vaginal brachytherapy

Key message

S-phase fraction gave independent prognostic information in FIGO stage I endometrial carcinoma while DNA aneuploidy showed limited prognostic value regarding clinical outcome. Definition of the optimal cut-off for S-phase fraction is overdue now that the prognostic power has been established.

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Introduction

Being the most common gynecological malignancy in developed countries, even small changes in the management of endometrial carcinoma (EC) can have a substantial effect on morbidity and mortality. In Sweden in 2011, 1,431 new cases were reported and the 5-year relative survival, with all stages included, was 84.0% (1). For early-stage EC, different combinations of prognostic factors are used to select the patients who should receive adjuvant treatment. Despite the fact that there has been a great amount of research, there is still no international consensus regarding the best set of prognostic factors. In Sweden, DNA ploidy is a recognized variable, but acknowledgement of this varies internationally. Flow cytometric S-phase fraction (SPF) is not generally used in standard practice. Both DNA ploidy and SPF have been investigated extensively, but due to variations in inclusion criteria, different risk grouping, and other methodological problems, the results have been conflicting. Some studies have found a strong prognostic value of DNA ploidy (2–7), which has not been found by others (8–11). Likewise, SPF has been found to have (8,12–14) and not found to have prognostic value in different studies(15), and some authors have reported independent prognostic value of both variables in multivariable analysis (16–18).

As previously reported, our laboratory has improved the DNA flow cytometric protocol considerably, leading to more reliable and reproducible measurements of both DNA ploidy and SPF (19).

In this study, we wanted to investigate the prognostic value of DNA ploidy and SPF in International Federation of Gynecology and Obstetrics (FIGO) stage I EC of endometroid histology in a large unselected population-based patient material.

Material and methods

Patients

Data from all reported cases of uterine cancer in the Southern Swedish Healthcare Region between January 2001 and December 2007 (n = 1,547) were collected for this retrospective population-based study on FIGO 2009 stage I endometroid endometrial cancer . Information about diagnosis and date of diagnosis was collected from the Regional Cancer Registry in Southern Sweden, which is part of the Swedish Cancer Registry with a coverage rate of 96.6%

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for cancer in the female genital organs (20). Data including FIGO stage, histological type, degree of differentiation, FIGO grade, and myometrial invasion were obtained from the pathology reports and information about treatment was collected from the patients' medical records at the Department of Oncology, Skåne University Hospital. The latter is a tertiary referral center, and is responsible for the individual treatment plans of all gynecological cancer patients in the Southern Swedish Healthcare Region.

Patients were re-staged according to the FIGO 2009 criteria. Some tumours had not been given a FIGO grade by the surgical pathologist, but the degree of differentiation instead. Thus, we constructed a "well-differentiated" group in which FIGO grade-1 to -2, well-differentiated and moderately differentiated tumours were included and a "poorly differentiated" group, which contained FIGO grade-3 tumours and poorly differentiated tumours. Cases that had a non-endometroid phenotype or lacked information on histology (n = 161) or cases that were of FIGO stage II–IV (n = 246 of the endometroid cases) were excluded, thus leaving 1,140 patients in the present study.

Two consecutive versions of treatment guidelines, summarized in Table 1, were used during the study period. The standard practice during the entire study period was surgery with hysterectomy and bilateral salpingoophorectomy, complemented with lymphadenectomy if the patient qualified for extended surgery due to preoperative risk factors—or at the surgeon's discretion. However, during the study period pelvic lymphadenectomy was not well implemented and paraaortic lymphadenectomy was not performed at all. The postoperative risk factors that qualified patients for adjuvant therapy varied slightly between the two sets of guidelines. In total, 68 out of 1,140 patients had received adjuvant therapy. A single patient could have received more than one treatment modality.

The study was approved by the Regional Ethical Review Board in Lund (journal number 2012/386).

Flow cytometry

DNA flow cytometry (FCM) was performed at one laboratory in Skåne University Hospital as part of routine preoperative risk evaluation. Information on SPF and DNA ploidy status was collected from the patient's original referral.

The procedure for flow cytometric DNA ploidy and SPF analyses in Sweden is summarized in

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national guidelines (21), originally described by Baldetorp *et al.* (22) and Schutte *et al.* (23). A FACSCalibur flow cytometer (Becton Dickinson, San José, CA, USA) connected to a computer running CellQuest (Becton Dickinson) data collection software was used. Histograms were evaluated using ModFitLT software, version 3.1 (Verity Software House, Topsham, ME, USA). Up to 20,000 events were collected for each sample. A DNA aneuploid control sample (with known DNA index and SPF) was run in parallel in order to control for the conditions of the FCM instrument, the preparation of cell nuclei, and the stability and quality of DNA staining. Samples with one G0/G1 peak and a corresponding G2 peak were considered diploid, and those with two or more G0/G1 peaks were considered aneuploid. Tetraploid tumours, defined as having a DNA index of 1.9–2.1, were included in the aneuploid group. In cases of aneuploidy, SPF was reported for the aneuploid population. Since there is no established cut-off value for SPF, the mean SPF value was used in the construction of a high and a low SPF group.

Follow-up

Vital status was checked using the Total Population Register and the Swedish Cause of Death Register. End of follow-up was December 2012 for cause-of-death data, August 2012 for follow-up on progression from medical journals, and January 2014 for death from any cause.

Statistical analysis

The primary endpoint of the study was death from endometrial cancer (i.e. where death with underlying or contributing cause C54 or C55 in International Statistical Classification of Diseases and Related Health Problems – Tenth Revision was considered an event). Secondary endpoints were (1) overall survival (OS) and (2) time to progression (TTP), i.e. time from diagnosis to first local, regional, or distant recurrence.

For death from endometrial cancer, cumulative incidence curves, separated according to SPF and DNA ploidy status, were calculated treating death from other causes as a competing event. SPF was dichotomized using the mean as the cut-point before any prognostic analyses were run. To obtain *p*-values, hazard ratios, and for multivariable modelling, Cox proportional hazard regression was used. For Cox regression for endometrial cancer death, death from other causes was considered to be a censoring event, i.e. cause-specific Cox regression was performed. Similarly, in the analysis of TTP, death from any cause was considered a censoring event.

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Adjusted hazard ratios were obtained by including age (with levels < 65, 65–75, and > 75), FIGO stage (IA or IB), degree of differentiation ("well-differentiated" or "poorly differentiated"), SPF ("low" or "high"), DNA ploidy status (diploid or aneuploid), and adjuvant treatment ("yes" or "no") in the Cox model. Most *p*-values were calculated using Wald tests, except for "age"—for which a likelihood-ratio test was used.

Fisher's exact test was used to compare the distribution of categorical variables between aneuploid and diploid tumours and between tumours of high and low SPF. Student's t-test was used to check for significance of differences in means of continuous variables. Statistical analysis was done using Stata 13.1/SE (StataCorp, College Station, TX, USA).

Power calculation

When planning the study, a power calculation was performed using group and effect sizes from a pilot study of the effects of SPF and DNA ploidy status on 5-year OS. For SPF, we anticipated groups of equal sizes, and that the 5-year OS would be 90% in women with tumours with low SPF. Thus, for a hazard ratio (HR) of 2, five hundred patients would be needed in order to achieve 80% power to detect the effect. For DNA ploidy status, we anticipated that 80% of the women would have diploid tumours, and that the 5-year OS in these women would be 80%. For an HR of 2, three hundred patients would be needed to achieve 80% power. Using seven consecutive years, we expected 1,160 patients in the Southern Swedish Healthcare Region, thus giving comfortable margins for detection of effects, even after accounting for fewer deaths from endometrial cancer than from all causes, and for using multivariable analyses.

Results

Patient characteristics

Median age at diagnosis was 68 years (mean 68, range 32–92). There were 1,137 patients graded for differentiation, 1,030 in the "well-differentiated" group (91%) and 107 in the "poorly differentiated" group (9%). 890 patients (78%) had been graded according to FIGO criteria, and 247 (21.7%) by subjective degree of differentiation. Only 3 patients (0.3%) had no histological grading. There were 865 patients in stage IA (76%) and 275 (24%) in stage IB. Only 68 patients (6%) had received adjuvant treatment (Table 2 and Fig. 1).

Flow cytometry

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DNA ploidy status could be evaluated for 1,069 of the patients (94%), 889 (83%) of whom had diploid tumours and 180 (17%) of whom had aneuploid tumours. For SPF, 234 patients (21%) had no evaluation, generally because of low resolution of the histograms through background debris. For the remaining patients, the mean SPF was 5.5%—giving 553 patients (61%) below the threshold and 353 patients (39%) above the threshold. All cases that could be evaluated regarding SPF could also be evaluated regarding DNA ploidy status (Table 2 and Fig. 1). The mean coefficient of variation of the diploid G0/G1 peak in all DNA histograms was 3.5%.

Death from endometrial cancer

Median follow-up time for death from EC was 8.9 years for patients who were still alive at the end of follow-up. In total, 105 patients died of endometrial cancer during the follow-up period, 85 of them within the first 5 years after diagnosis. Figure 2 shows the cumulative incidence curves of death from endometrial cancer, according to SPF and DNA ploidy status. In univariable analysis, a high SPF value was associated with worse outcome (HR = 2.95; 95% CI 1.9–4.7; $p < 0.001$) than a low SPF value, and similarly for aneuploid *vs.* diploid tumours (HR = 2.0; 95% CI 1.3–3.1; $p = 0.002$). In multivariable analysis, SPF retained its prognostic value (HR = 2.3; 95% CI 1.4–3.7; $p = 0.001$) whereas DNA ploidy status showed no independent prognostic value (HR = 1.3; 95% CI 0.72–2.2; $p = 0.4$). All variables included were associated with a worse outcome, except for adjuvant treatment. The same relationships were true with a follow-up period of 5 years (Tables 3 and 4). Removal of adjuvant treatment status as a confounding factor had no substantial effect on *p*-values or confidence intervals for the variables included (data not shown). When we used the multivariable model on only those who had not received adjuvant treatment, we obtained very similar results for the hazard ratios regarding SPF and DNA ploidy status (SPF: HR = 2.6; 95% CI 1.5–4.4; $p < 0.001$; and DNA ploidy status: HR = 1.3; 95% CI 0.71–2.4; $p = 0.4$).

Overall survival

Median follow-up time for OS was 9.2 years for patients who were alive at the end of follow-up. Altogether, 315 patients died, 170 of whom had died within 5 years. A high SPF value was associated with worse outcome, both in univariable analysis (HR = 1.65; 95% CI 1.28–2.13; $p < 0.001$) and in multivariable analysis (HR = 1.50; 95% CI 1.15–1.95; $p = 0.003$), whereas DNA ploidy status did not retain its prognostic value after adjustment for measured confounders.

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These relationships were the same for both points in time (Table 4). An extended list of univariable and multivariable results regarding OS and TTP can be found in Supporting Information Table S1.

Time to progression

Median follow-up time for TTP was 7.8 years for patients who were alive without recurrence at the end of follow-up. Altogether, 125 patients (11%) had a recurrence during the follow-up period, 114 of whom had a recurrence within 5 years. There were 73 vaginal recurrences (59%), 12 pelvic recurrences (10%), 39 distant recurrences (31%), and 1 (1%) with missing location. A high SPF value was highly associated with worse outcome when adjusted for included factors (HR = 2.24; 95% CI 1.5–3.4; $p < 0.001$, full follow-up period). This applied to both time frames. DNA ploidy status was not associated with worse outcome (Table 4).

Discussion

Our results show that SPF has independent prognostic value, whereas DNA ploidy does not, when included in multivariable analysis of endometrial cancer death, OS and TTP.

Some of the patients with aneuploid tumours, with other concomitant risk factors, had undergone adjuvant vaginal brachytherapy (VBT) and/or external pelvic beam radiotherapy (EBRT) as part of routine care. This could possibly explain some of the loss of prognostic information for DNA ploidy status. To assess the direct effect of ploidy on prognosis, not mediated by treatment effect, we adjusted for treatment in our main analysis. In a stability analysis, we investigated the influence of SPF and DNA ploidy status in untreated patients only. Selection of untreated patients may introduce bias due to the other risk factors (FIGO stage and degree of differentiation) that, together with DNA ploidy status, affect the use of adjuvant therapy. By adjusting for these risk factors, however, the problem is reduced. Altogether, only 52 of 905 patients included in the multivariable analysis (6%) received adjuvant treatment, 34 of whom had aneuploid tumours and 18 of whom had diploid. The increased hazard of aneuploidy was very similar, adjusted for the other risk factors, irrespective of whether treated patients were included or excluded. In all, the loss of prognostic information for DNA ploidy status could not be explained by increased use of adjuvant therapy in the aneuploid group.

In this study, death from EC was defined as death with EC as the underlying or contributory

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cause. Physicians in Sweden are required by law to report all deaths to the Swedish Cause of Death Register, but the actual cause of death can sometimes be difficult to ascertain. The variability between doctors can thus be great, and probably not all of the patients reported to have died from EC in the population actually died from their cancer—but rather *with* their cancer, or even cured from their cancer. This could explain why the number of women reported to have died from EC was high (105) compared to what would be expected from the number of recurrences (125 in total, 73 of which were vaginal). However, inclusion of patients who did not actually die from EC in a survival analysis on EC-related death will make the endpoint tend towards OS, lessening the effect of the variables included (by producing a bias towards the null hypothesis). Thus, it is plausible that the prognostic importance of SPF and DNA ploidy status is actually somewhat greater than has been shown in our analysis.

Generally, little difference in recurrence rate and survival has been shown in studies on early stage endometriod EC incorporating DNA ploidy status in risk grouping for adjuvant treatment with VBT. Lim *et al*. reported no difference between treated aneuploid cases compared to untreated diploid cases (24). Terada *et al*. reported similar results, but in a completely untreated—although smaller—population (25). Högberg *et al*. did report a significant difference in both recurrence rate and OS when they treated high-risk cases with VBT and left low-risk cases untreated but concluded that VBT likely was not effective in preventing recurrences since a majority of recurrences were distant (26). In a randomized controlled trial by Sorbe *et al*. on low-risk cases, no effect was seen in the VBT-treated arm (27). Thus, it appears that aneuploidy does not add an increased risk of recurrence and that VBT does not alter the recurrence rate significantly on histopathologically low-risk cases.

Variations in prognostic value for SPF and DNA ploidy can often be traced back to differences in inclusion criteria, with different studies using various mixes of stages and histological types. The studies that have found DNA ploidy to be significant as a prognostic factor have often included all stages, e.g. Lundgren *et al*. (2), Susini *et al*. (4), and Zaino *et al*. (28), which would possibly explain the discrepancies. In an attempt to test this hypothesis, we included all FIGO stages in our database and obtained prognostic significance for DNA ploidy (data not shown).

Gudmundsson *et al*. found that DNA ploidy and SPF have different prognostic value depending on the histological grade. In their low-risk group, SPF was found to be a very strong independent predictor of survival whereas DNA ploidy had no prognostic value. This contrasted with their high-risk group in which DNA ploidy was an independent prognostic factor while SPF lost significance in the multivariate analysis (13). By copying their study design, which included all

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FIGO stages, we too found DNA ploidy to be significant in the high-risk group but not in the low-risk group (data not shown).

The discrepancies between the results in our study and those cited above can thus be explained by differences in inclusion criteria and methodology, which might explain some of the controversy in this field. It highlights the importance of taking such differences into account when comparing studies.

In accordance with our study, Wagenius *et al*. showed independent prognostic value for SPF, but not for DNA ploidy, in a prospective study stratified for FIGO stage I–II; they also showed that there was a correlation between higher SPF and earlier recurrence (29).

Image cytometry has been shown to be superior to FCM in identifying aneuploid cases (30), but it cannot give a reliable estimation of SPF due to the low cell numbers in the histograms. FCM is also superior to Ki-67 immunohistochemistry for quantification of cell proliferation in EC (14). In our material, 234 cases (21%) could not be evaluated regarding SPF. However, since the DNA FCM protocol in the national guidelines was updated in 2008, there have been dramatic improvements in DNA histogram quality, i.e. less contribution from debris and a shift in the coefficient of variation from 4.5–5.5% to 2.0–2.5% for the diploid G0/G1 peak. Thus, nowadays SPF is reported in ~95% of the EC samples analyzed (unpublished data).

It would be valuable for future studies to look into optimizing the cut-off value for SPF when FCM is used on hysterectomy and curettage samples. Studies concentrating on survival analysis and recurrence patterns should carefully stratify patients according to international FIGO criteria for stage and grade, in order to avoid erroneous conclusions based on arbitrary group constructions.

In conclusion, in the largest population so far reported, we have found that SPF is an independent prognostic marker for FIGO stage I EC.

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References

1. Socialstyrelsen. Cancer i siffror 2013 [National Board. Cancer of the figures in 2013]. Socialstyrelsen; 2013.

2. Lundgren C, Auer G, Frankendal B, Moberger B, Nilsson B, Nordström B. Nuclear DNA content, proliferative activity, and p53 expression related to clinical and histopathologic features in endometrial carcinoma. Int J Gynecol Cancer. 2002;12(1):110–8.

3. Baak JP a, Snijders W, van Diermen B, van Diest PJ, Diepenhorst FW, Benraadt J. Prospective multicenter validation confirms the prognostic superiority of the endometrial carcinoma prognostic index in international Federation of gynecology and obstetrics stage 1 and 2 endometrial carcinoma. J Clin Oncol. 2003 Nov 15;21(22):4214–21.

4. Susini T, Amunni G, Molino C, Carriero C, Rapi S, Branconi F, et al. Ten-year results of a prospective study on the prognostic role of ploidy in endometrial carcinoma: DNA aneuploidy identifies high-risk cases among the so-called "low-risk" patients with well and moderately differentiated tumors. Cancer. 2007 Mar 1;109(5):882–90.

5. Mangili G, Montoli S, De Marzi P, Sassi I, Aletti G, Taccagni G. The role of DNA ploidy in postoperative management of stage I endometrial cancer. Ann Oncol. 2008;19:1278–83.

6. Song T, Lee J-W, Kim H-J, Kim MK, Choi CH, Kim T-J, et al. Prognostic significance of DNA ploidy in stage I endometrial cancer. Gynecol Oncol. 2011;122(1):79–82.

7. Lundgren C, Auer G, Frankendal B, Nilsson B, Nordström B. Prognostic factors in surgical stage I endometrial carcinoma. Acta Oncol. 2004;43(1):49–56.

8. Pfisterer J, Kommoss F, Sauerbrei W, Rendl I, Kiechle M, Kleine W, et al. Prognostic value of DNA ploidy and S-phase fraction in stage I endometrial carcinoma. Gynecol Oncol. 1995;58:149–56.

For Peer Review

9. Pisani A, Barbuto D, Chen D, Ramos L, Lagasse L, Karlan B. HER-2/neu, p53, and DNA Analyses as Prognosticators for Survival in Endometrial Carcinoma. Obstet Gynecol.1995;85(5):729–34.

10. Salvesen HB, Iversen OE, Akslen LA. Prognostic Impact of Morphometric Nuclear Grade of Endometrial Carcinoma. Cancer. 1998;83(5):956–64.

11. Steinbakk A, Malpica A, Slewa A, Skaland I, Gudlaugsson E, Janssen E a M, et al. Biomarkers and microsatellite instability analysis of curettings can predict the behavior of FIGO stage I endometrial endometrioid adenocarcinoma. Mod Pathol. 2011;24(9):1262–71.

12. Rosenberg P, Wingren S, Simonsen E, Stàl O, Risberg B, Nordenskjöld B. Flow cytometric measurements of DNA index and S-phase on paraffin-embedded early stage endometrial cancer: an important prognostic indicator. Gynecol Oncol. 1989;35(1):50–4.

13. Gudmundsson TE, Högberg T, Alm P, Anderson H, Baldetorp B, Fernö M, et al. The prognostic information of DNA ploidy and S-phase fraction may vary with histologic grade in endometrial carcinoma. Acta Oncol. 1995;34(6):803–12.

14. Nordström B, Strang P, Bergström R, Nilsson S, Tribukait B. A Comparison of Proliferation Markers and Their Prognostic Value for Women with Endometrial Carcinoma. Cancer. 1996;78(9):1942–51.

15. Pradhan M, Abeler VM, Danielsen HE, Sandstad B, Tropé CG, Kristensen GB, et al. Prognostic importance of DNA ploidy and DNA index in stage I and II endometrioid adenocarcinoma of the endometrium. Ann Oncol. 2012;23(5):1178–84.

16. Friberg L, Norén H, Delle U. Prognostic value of DNA ploidy and S-phase fraction in endometrial cancer stage I and II. A prospective 5-year survival study. Gynecol Oncol. 1994;53:64–9.

17. Kaleli S, Kösebay D, Bese T, Demirkiran F, Öz U, Arvas M, et al. A Strong Prognostic Variable in Endometrial Carcinoma: Flow cytometric S-phase Fraction. Cancer. 1997;79(5):944–51.

For Peer Review

18. Wimberger P, Hillemanns P. Evaluation of prognostic factors following flow-cytometric DNA analysis after cytokeratin labelling: II. Cervical and endometrial cancer. Anal Cell Pathol. 2002;24:147–58.

19. Baldetorp B, Bendahl P-O, Fernö M, Stål O. Improved DNA flow cytometric, DNA ploidy, and S-phase reproducibility between 15 laboratories in analysis of breast cancer using generalized guidelines. Cytometry A. 2003;56(1):1–7.

20. Barlow L, Westergren K, Holmberg L, Talbäck M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009;48(1):27–33.

21. Svensk förening för flödescytometri. Guidelines for DNA FCM analysis of fresh and/or frozen tumor tissue, and the interpretation of DNA histograms measurements [Internet]. SFFF; Available from: www.sfff.se

22. Baldetorp B, Dalberg M, Holst U, Lindgren G. Statistical evaluation of cell kinetic data from DNA flow cytometry (FCM) by the EM algorithm. Cytometry. 1989;10(6):695–705.

23. Schutte B, Reynders MM, Bosman FT, Blijham GH. Flow cytometric determination of DNA ploidy level in nuclei isolated from paraffin-embedded tissue. Cytometry. 1985;6(1):26–30.

24. Lim P, Aquino-Parsons CF, Wong F, Dupuis B, Phillips D, Zhou C, et al. Low-risk endometrial carcinoma: assessment of a treatment policy based on tumor ploidy and identification of additional prognostic indicators. Gynecol Oncol. 1999;73(2):191–5.

25. Terada K, Mattson D, Goo D, Shimizu D. DNA aneuploidy is associated with increased mortality for stage I endometrial cancer. Gynecol Oncol. 2004;95(3):483–7.

26. Hogberg T, Fredstorp-Lidebring M, Alm P, Baldetorp B, Larsson G, Ottosen C, et al. A prospective population-based management program including primary surgery and postoperative risk assessment by means of DNA ploidy and histopathology. Adjuvant radiotherapy is not necessary for the majority of patients with FIGO stage I-II endometrial cancer. Int J Gynecol Cancer. 2004;14(3):437–50.

For Peer Review

27. Sorbe B, Nordström B, Mäenpää J, Kuhelj J, Kuhelj D, Okkan S, et al. Intravaginal brachytherapy in FIGO stage I low-risk endometrial cancer: a controlled randomized study. Int J Gynecol Cancer. 2009;19(5):873–8.

28. Zaino RJ, Davids AT, Ohlsson-Wilhelm BM, Brunetto VL. DNA content is an independent prognostic indicator in endometrial adenocarinoma. Int J Gynecol Pathol. 1998;17:312–9.

29. Wagenius G, Bergström R, Strang P, Gerdes U, Rogo K, Tribukait B, et al. Prognostic significance of flow cytometric and clinical variables in endometrial adenocarcinoma stages I and II. Anticancer Res. 1992;12(3):725–32.

30. Kaern J, Wetteland J, Tropé CG, Farrants GW, Juhng SW, Pettersen EO, et al. Comparison between flow cytometry and image cytometry in ploidy distribution assessments in gynecologic cancer. Cytometry. 1992;13(3):314–21.

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Supporting Information Table S1. Extended list of univariable and multivariable results regarding overall survival and time to progression.

Legends of Figures and Tables

Fig 1. Patient characteristics.

Fig 2. Cumulative incidence of death from endometrial cancer. SPF – S-phase fraction.

Table 1. Standard treatment of FIGO stage I endometrial cancer during the period of the study.

Table 2. Clinical and histopathologic characteristics to DNA ploidy and S-phase fraction status*.

Table 3. Uni- and multivariate analysis of endometrial cancer death.

Table 4. Summary of main findings.

Tabell1

Table 1

Year	Surgery	Postoperative risk factors	Postoperative risk groups
2001-2004			
	TH+BSO+AWC Lgl extirpation if suspicious/palpable nodes	Poor differentiation, MI>50%	*Low risk* ; well or moderatly differentiated tumors with MI<50% *Medium risk* ; poorly differentiated tumors and/or MI>50%
2005-2007			
	TH+BSO+AWC Lgl extirpation if aneuploid and/or FIGO grade 3 and/or MI>50% on ultrasonography	Aneuploidy, MI>50%, FIGO grade 3	*Low risk* ; no risk factor *Medium risk* ; maximum one risk facto *High risk* ; two or more risk factors

EC, endometrial carcinoma; TH, total hysterectomy; BSO, bilateral salpingoophorectomy; AWC, abdominal wash cytology; lgl, lymph glands; MI, myometrial invasion; V
ERBT, external beam radiotherapy; HDR, high dose rate

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Adjuvant treatment
None
VBT 20 Gy/5 fractions, HDR
None
None
Inclusion in the NSGO-EC-9501 tria
or ERBT 46 Gy/23 fractions
BT, vaginal brachythe

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Table 2

Characteristics	DNA-ploidy						SPF-status					
	Diploid (*n*=889)		Aneuploid (*n*=180)		*p*-value	missing (*n*=71)	Low (*n*=553)		High (*n*=353)		*p*-value	missing (*n*=234)
	n	%	*n*	%		*n*	*n*	%	*n*	%		*n*
Age, mean (SD)	68.0 (10.4)	-	69.1 (10.2)	-	0.18[s]	71	68.1 (10.7)	-	69.1 (9.9)	-	0.16[s]	234
Age, by group					0.4[a]						0.20[a]	
<65 (443)	343	39	66	37		34	209	38	125	35		109
65-75 (416)	335	38	63	35		18	214	39	126	35		76
≥76 (281)	211	24	51	28		19	130	24	102	29		49
FIGO stage (*n*)					0.007[a]						0.033[a]	
IA (865)	688	77	122	68		55	429	78	251	71		185
IB (275)	201	23	58	32		16	124	22	102	29		49
Differentiation group (*n*)					<0.001[a]						<0.001[a]	
Well differentiated (1030)	828	93	141	78		61	532	96	294	83		204
Poorly differentiated (107)	59	7	39	22		9	20	4	59	17		28
missing (3)	2	0.2	0	0		1	1	0.2	0	0		2
SPF status (*n*)					0.001[a]							
Low (<5.5%) (553)	485	55	68	38		0						
High (>5.5%) (353)	280	32	73	41		0						
missing (234)	124	14	39	22		71						
DNA ploidy status (*n*)											0.001[a]	
Diploid (889)							485	88	280	79		124
Aneuploid (180)							68	12	73	21		39
missing (71)							0	0	0	0		71
Adjuvant treatment (*n*)					<0.001[a]						0.11[a]	
Any (68)	19	2	43	24		6	26	5	26	7		16
None (1072)	870	98	137	76		65	527	95	327	93		218
Chemo (*n*)					<0.001[a]						0.25[a]	
Yes (12)	3	0.3	7	4		2	3	0.5	5	1		4
No (1128)	886	99.6	173	96		69	550	99.5	348	99		230
VBT (*n*)					<0.001[a]						0.4[a]	
Yes (30)	9	1	18	10		3	11	2	11	3		8
No (1110)	880	99	162	90		68	542	98	342	97		226
EBRT (*n*)					<0.001[a]						0.17[a]	
Yes (48)	14	2	29	16		5	18	3	18	5		12
No (1092)	875	98	151	84		66	535	97	335	95		222

SD, standard deviation; VBT, vaginal brachyterapy; EBRT, external beam radiotherapy; SPF, S-phase fraction
*For ratios and *p*-value calculation 'missing' is omitted
[s]Student's t-test
[a]Fisher's exact test

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Table 3

Factor	5-year endometrial cancer death						full time endometrial cancer death, median follow-up 8.9 years					
	Unadjusted HR[θ]	95% CI	*p*-value	Adjusted HR[ϑ]	95% CI	*p*-value	Unadjusted HR[θ]	95% CI	*p*-value	Adjusted HR[ϑ]	95% CI	*p*-value
Age, by group			**<0.001**			**0.001**[L]			**<0.001**			**<0.001**[L]
<65	Ref.			Ref.			Ref.			Ref.		
65-75	2.6	1.3-5.1		2.1	0.96-4.6		3.0	1.6-5.6		2.5	1.2-5.0	
≥76	6.9	3.7-13		4.1	1.9-8.8		7.8	4.3-14		4.3	2.1-8.5	
FIGO stage												
IA	Ref.			Ref.			Ref.			Ref.		
IB	3.5	2.4-5.3	**<0.001**	2.7	1.6-4.4	**<0.001**	3.2	2.2-4.6	**<0.001**	2.5	1.6-4.0	**<0.001**
Differentiation group												
Well differentiated	Ref.			Ref.			Ref.			Ref.		
Poorly differentiated	4.1	2.5-6.6	**<0.001**	2.4	1.3-4.3	**0.006**	3.8	2.4-5.9	**<0.001**	2.3	1.3-4.0	**0.004**
SPF status												
Low (<5.5%)	Ref.			Ref.			Ref.			Ref.		
High (>5.5%)	3.5	2.1-6.0	**<0.001**	2.6	1.5-4.5	**0.001**	2.9	1.9-4.7	**<0.001**	2.3	1.4-3.7	**0.001**
DNA ploidy status												
Diploid	Ref.			Ref.			Ref.			Ref.		
Aneuploid	2.0	1.2-3.3	**0.005**	1.4	0.8-2.6	0.3	2.0	1.3-3.1	**0.002**	1.3	0.7-2.2	0.4
Adjuvant treatment												
None	Ref.			Ref.			Ref.			Ref.		
Any	0.97	0.4-2.4	0.9	0.5	0.2-1.4	0.2	1.5	0.8-3.0	0.2	0.8	0.4-1.9	0.6

p-values<0.05 in bold face
[L] *p*-value by lr-test
[θ] by univariable cause specific Cox proportional hazard regression
[ϑ] by multivariable cause specific Cox proportional hazard regression

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Table 4

FCM variable	Endpoint	Follow-up time											
		5 years						full time					
		Unadjusted			Adjusted			Unadjusted			Adjusted		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
SPF status													
	endometrial cancer deat	3.5	2.1-6.0	**<0.001**	2.6	1.5-4.5	**0.001**	2.9	1.9-4.7	**<0.001**	2.3	1.4-3.7	**0.001**
	OS	1.9	1.3-2.7	**<0.001**	1.5	1.1-2.2	**0.02**	1.7	1.3-2.1	**<0.001**	1.5	1.2-2.0	**0.003**
	TTP	2.7	1.8-4.1	**<0.001**	2.4	1.5-3.6	**<0.001**	2.5	1.7-3.8	**<0.001**	2.2	1.5-3.4	**<0.001**
DNA ploidy status													
	endometrial cancer deat	2.0	1.2-3.3	**0.005**	1.4	0.8-2.6	0.3	2.0	1.3-3.1	**0.002**	1.3	0.7-2.2	0.4
	OS	1.4	0.9-2.0	0.1	0.95	0.6-1.5	0.8	1.2	0.9-1.6	0.2	0.8	0.6-1.2	0.3
	TTP	1.5	0.9-2.3	0.09	0.8	0.4-1.4	0.4	1.5	0.9-2.2	0.09	0.7	0.40-1.3	0.2

FCM, flow cytometry; SPF, S-phase fracion; OS, overall survival; TTP, time to progression

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Supporting Information Table S1.

Factor	5-year overall survival (OS5)						all time overall survival (OS), median follow-up 9.2 year						5-year time to progression (TTP5)						all time time to progression (TTP), median follow-up 7.8 year					
	Unadjusted HR[a]	95% CI	*p*-value	Adjusted HR[b]	95% CI	*p*-value	Unadjusted HR[c]	95% CI	*p*-value	Adjusted HR[d]	95% CI	*p*-value	Unadjusted HR[e]	95% CI	*p*-value	Adjusted HR[f]	95% CI	*p*-value	Unadjusted HR[g]	95% CI	*p*-value	Adjusted HR[h]	95% CI	*p*-value
Age, by group			**<0.001**			**<0.001**			**<0.001**			**<0.001**			**0.001**			**0.002**			**<0.001**			**0.002**
<65	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
65-75	2.6	1.6-4.3		2.3	1.3-4.0		2.9	2.1-4.2		2.8	1.8-4.2		2.2	1.4-3.6		2.4	1.3-4.1		2.1	1.3-3.3		2.3	1.4-3.9	
>75	7.6	4.8-11.9		6.2	3.6-10.6		9.3	6.6-13.0		8.4	5.7-12.6		3.3	2.0-5.4		2.5	1.4-4.5		2.9	1.8-4.6		2.3	1.3-4.0	
FIGO stage																								
Ia	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Ib	2.3	1.7-3.2	**<0.001**	1.5	1.0-2.1	**0.04**	2.0	1.6-2.5	**<0.001**	1.2	0.9-1.6	0.1	2.7	1.8-3.9	**<0.001**	2.4	1.6-3.6	**<0.001**	2.6	1.8-3.7	**<0.001**	2.3	1.6-3.5	**<0.001**
Differentiation group																								
Well differentiated	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Poorly differentiated	3.0	2.1-4.4	**<0.001**	2.2	1.4-3.5	**0.001**	2.3	1.7-3.1	**<0.001**	1.7	1.1-2.4	**0.009**	2.8	1.8-4.4	**<0.001**	2.1	1.2-3.6	**0.007**	2.8	1.8-4.4	**<0.001**	2.1	1.2-3.5	**0.007**
SPF status																								
Low (<5.5%)	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
High (≥5.5%)	1.9	1.3-2.7	**<0.001**	1.5	1.1-2.2	**0.02**	1.7	1.3-2.1	**<0.001**	1.5	1.2-2.0	**0.003**	2.7	1.8-4.1	**<0.001**	2.4	1.5-3.6	**<0.001**	2.5	1.7-3.8	**<0.001**	2.2	1.5-3.4	**<0.001**
DNA ploidy status																								
Diploid	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Aneuploid	1.4	0.9-2.0	0.1	0.95	0.6-1.5	0.8	1.2	0.9-1.6	0.2	0.8	0.6-1.2	0.3	1.5	0.9-2.3	0.09	0.8	0.4-1.4	0.4	1.5	0.9-2.2	0.09	0.7	0.4-1.3	0.2
Adjuvant treatment																								
None	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Any	0.9	0.4-1.7	0.7	0.6	0.3-1.4	0.2	0.99	0.6-1.6	1.0	0.8	0.4-1.5	0.4	0.9	0.4-1.8	0.7	0.7	0.3-1.7	0.4	0.6	0.3-1.0	**0.04**	0.97	0.4-2.1	0.9

p-values<0.05 in bold face

[a]*p*-value by lr-test

[b]by univariable Cox proportional hazard regression

[c]by multivariable Cox proportional hazard regression

[d]by univariable cause specific Cox proportional hazard regression

[e]by multivariable cause specific Cox proportional hazard regression

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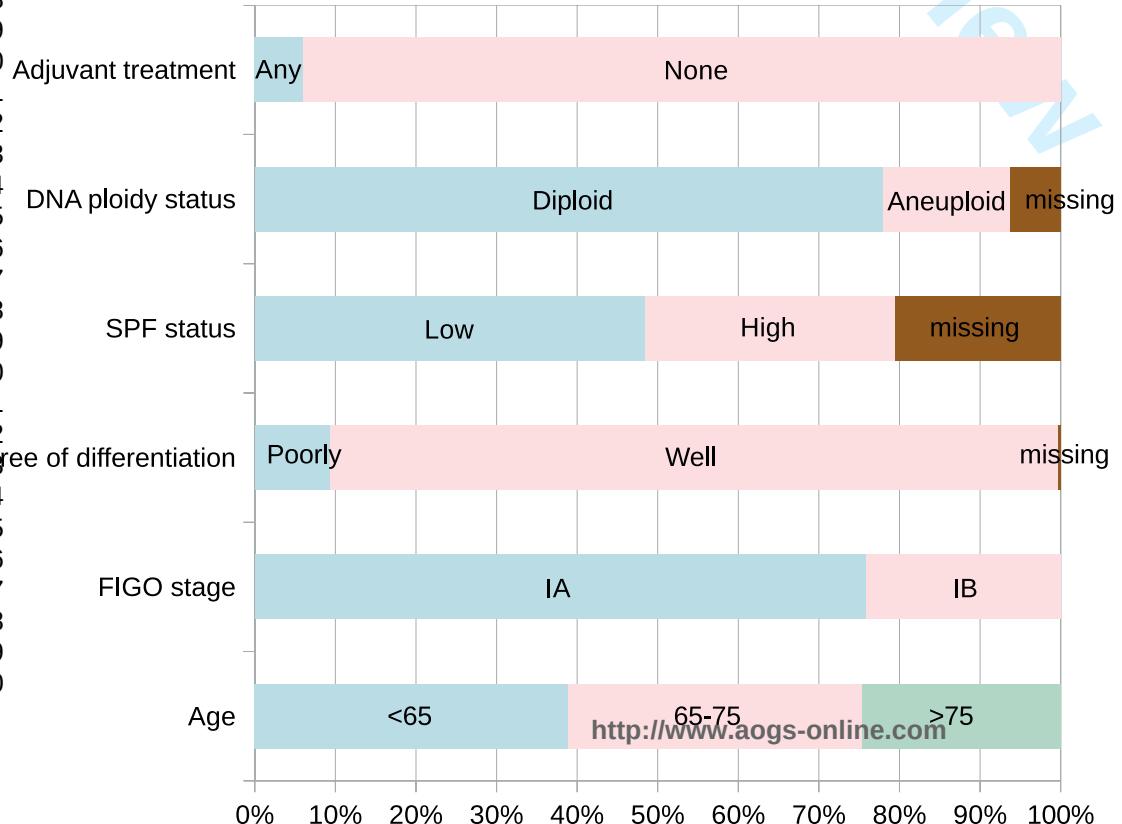

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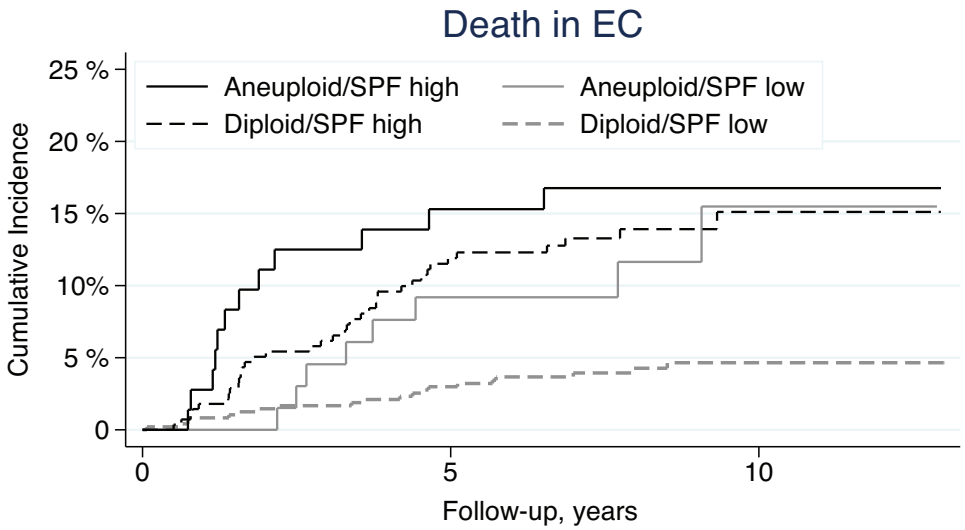

At risk

(Ploidy / SPF)			
A / high	73	60	23
D / high	280	224	46
A / low	68	58	11
D / low	485	434	143

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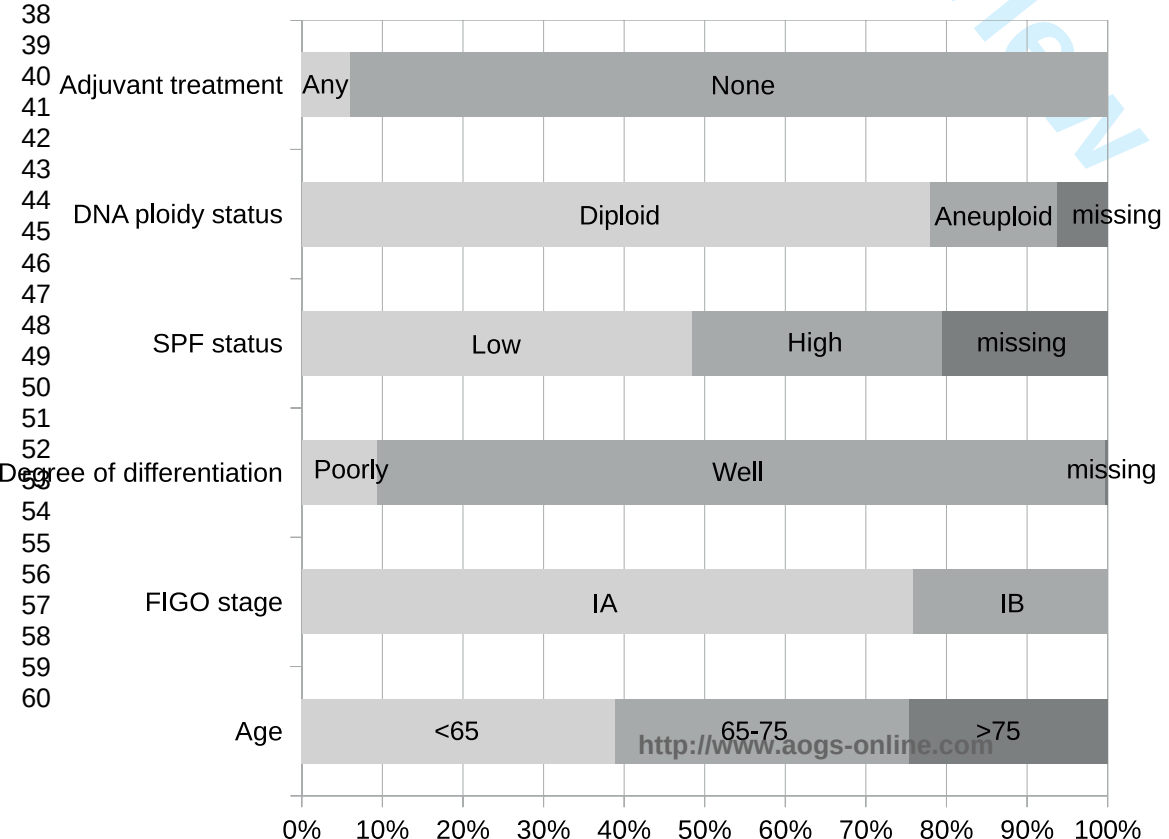

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