The Prognostic Value of DNA Ploidy Determination in Endometrial Cancer

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Abstract
Aneuploidy, defined as an abnormal quantity of DNA in cells nuclei, is the most frequently observed genetic abnormality in cancer cells. Alterations in cell cycle control and chromosomal missegregation very often result in the accumulation of excess genetic material. These genetic reassortments simultaneously imbalance lots of structural and regulatory proteins. This chromosomal instability may be associated with mutations in tumor suppressor genes or loss of function of mismatch repair genes. These are common pathways of cancerogenesis in endometrial cancer. Endometrial cancer represents the most common female genital tract malignancy and is generally associated with favourable outcomes for affected patients. Despite this, recurrence rates and disease-related deaths are consistently reported in published series, also in so-defined low-risk groups of patients. Hence, is reasonable to believe that traditional prognostic factors–surgical stage, histologic type, tumor grading, myometrial deep of invasion, involvement of vascular spaces–do not definitely respond to the clinical needs of a comprehensive management. The determination of DNA ploidy in endometrial cancer has been widely investigated in the last decades and clear evidence is available of its direct correlation with prognosis. Despite this, poor acceptance and skepticism are common believes in the scientific community. Aim of the paper has been the attempt to underline, upon scientific strength, the common believes in the scientific community. A 5-year overall survival rate of 80-85%. Seventy-five percent of women with EC are classified stage I at the time of diagnosis, with an 89% 5-year survival rate reported for these patients; of note, out of the 15-20% of women who die of endometrial cancer, almost half of the cases were early stages of the disease. Thus, although accounting for the great minority of cases, EC-related deaths are significantly reported in this apparently low-risk population. This probably not only reflects the limitations of current prognostic assessment but also the inherent variability in the tumor biology. Surgery, including total abdominal hysterectomy with bilateral salpingo-oophorectomy is considered as the gold standard treatment, although the extent of surgery may vary [1,2]. Adjuvant therapy is reserved to high-risk patients. However, according to this management, survival rates did not substantially improve over the last decades [3]. Several factors such as depth of myometrial invasion, tumor grading, histologic subtype and vascular space invasion have been identified as predictors of relapse. In particular, histological heterogeneity plays a relevant role, determining significant differences in prognosis: the most common histotype, the endometrioid subtype (Type I), accounting for almost 80% of all endometrial cancers, is correlated with a better long-term prognosis and survival than serous and/or clear cell histological type (Type II). However, only few women with disease confined to the uterine corpus will show these traditional high-risk features, and a small proportion of them will ultimately relapse. Therefore it is necessary to define adjunctive prognostic and predictive factors to stratify low and high-risk groups of patients, especially for early stages EC of the endometrioid subtype. In recent years several studies have tried to define new prognostic predictors for identifying those patients at higher risk for extrauterine disease spread and recurrence after primary treatment. More accurate stratification of patients would allow clinicians to improve patients’ selection for radical surgery and adjuvant therapy, avoiding overtreatments and morbidities for those patients with good prognosis.

DNA Ploidy in Endometrial Cancer
Normal human somatic cells with 46 chromosomes (23 pairs) are defined as diploid, whereas cells with fewer or more than 46 chromosomes as considered as aneuploid. Aneuploidy implies an abnormal quantity of genomic material in the cell, being this presumably a consequence of inherited and somatic alterations in proto-oncogenes and tumor-suppressor genes, which play a key role in regulating cell growth, differentiation, angiogenesis, invasion and metastasis.

Flow cytometry and image cytometry
DNA analysis with flow cytometry (FCM) or image cytometry (ICM) quantifies nuclear DNA in cancer cells and classifies the tumor on the basis of its DNA ploidy. Each technique presents both advantages and disadvantages. In most studies DNA analysis is performed by FCM on either frozen or formalin-fixed, paraffin embedded tissues, essentially following the procedures originally described by Hedley [5] with modification according to Schutte [6]. Flow cytometry allows 20,000-50,000 cells to be analyzed; however, tumor cells cannot be separated from normal cells and this may bias the distribution of abnormal cells in DNA histogram. In contrast, ICM visually selects each cancer cell, thus excluding surrounding cells from the analysis. However, because of the small number of cells
analyzed, rare non-diploid cells may be missed. Anyway, there is a good correlation (>80%) between DNA ploidy results obtained from both FCM and ICM [7,8], even if FCM has been more frequently performed.

**Prognostic role of DNA ploidy in EC**

In several studies flow cytometric analysis of DNA ploidy has been shown to provide stronger and independent prognostic information in comparison to standard pathologic parameters both in univariate (UA) and multivariate analysis (MA) [9-35]. Britton [14] analyzed DNA ploidy patterns of 256 EC patients; non-diploid patterns were detected only in 22% of cases, but accounted for 52% of the relapses. The 4-year overall survival rate for non-diploid EC patients was 57% compared to 88% of those with diploid DNA pattern (p=0.001). At multivariate survival analysis, the two most cogent independent prognostic factors were histologic type and DNA ploidy.

Ikeda [19] demonstrated an association between aneuploidy and advanced stage, tumor grade, myometrial invasion, nodal metastasis and positive peritoneal cytology in a study of 76 surgically staged EC patients. Moreover, the authors reported a fivefold (33 versus 6.9%) higher prevalence of lymph nodes metastases in patients with aneuploid tumors compared to patients with diploid tumors. Iversen [11] studied both the DNA ploidy and steroid receptor status as outcome predictors in EC patients. UA showed significant prognostic value for ploidy, surgical stage, grade and depth of myometrial invasion; however, in the stepwise analysis only ploidy and surgical stage were finally significant. Larson [36] reported in a study of 208 EC patients that aneuploid tumors had a significantly higher prevalence of metastases to the cervix, adnexa and omentum, malignant pelvic cytology and advanced surgical stages. Moreover, they found a fivefold higher risk of pelvic lymph nodes metastases and a six fold higher risk of para-aortic lymph nodes metastases in patients with aneuploid tumors compared to those with diploid tumors.

Lundgren [27], in a study of 376 stage I-IV patients with EC, reported that beside stage and histological subtype, DNA ploidy was the strongest outcome predictor and was reliable in predicting the risk of relapse. Mangili [28], in a multivariate analysis study of 203 consecutive cases of EC, reported that among all prognostic factors (including age at diagnosis, grade, peritoneal cytology, node involvement, vascular invasion, myometrial invasion and ploidy) only DNA ploidy performed as an independent prognostic variable.

More interestingly, some investigators studied the prognostic role of ploidy in early stage EC patients. Friberg [33] evaluated patients with stage I-II EC, demonstrating a 5-year survival rate of 92% in patients with diploid lesions and 36% in aneuploid tumors. Britton [34] determined ploidy in 203 patients with surgical stage I EC; non-diploid specimens were observed only in 16% of patients but accounted for 50% of all recurrences. Moreover the Authors reported that ploidy effectively stratified the endometrioid tumors according to their risk of recurrence: diploid and non-diploid endometrioid tumors had a 5-year survival rate of 93% and 74% respectively (p<0.01). Von Minckwitz [22] reported that, in patients with stage I disease, DNA ploidy and S phase fraction were found to be independent and the most significant outcome predictive factors at MA, also including grade, histological type and estrogen receptor status. Mangili [35], in a study of 222 stage I endometrioid EC patients reported that only DNA ploidy and age at diagnosis resulted independent prognostic factors (Cox, p=0.01 and p<0.0001 respectively); furthermore DNA ploidy was the only prognostic factor related to recurrence risk (log rank, p=0.05). The effective role of ploidy as prognostic factor in early stages of EC is still matter of debate [36-43]. Several authors reported a prognostic value of ploidy in terms of survival rates in UA but not in MA. Örbo [39], in a study involving 123 women with EC stage I-IV found that FIGO stage, histologic grade, myometrial infiltration depth, ploidy, vessel invasion and progesterone receptor status were significantly related to survival in the UA. However in the multivariate regression analysis only FIGO stage, vessel infiltration and nuclear perimeter were independently related to survival. Similarly, Lundgren [40], in a small cohort of patients with EC (n=80) found that DNA ploidy loses its significant prognostic value when submitted to MA; the authors speculated that DNA ploidy was unexpectedly not significant at MA probably due to the small number of patients. Terada [41] reviewed ploidy in 100 patients with stage IB and IC EC; results of this study indicate that aneuploidy affects the overall survival rate but does not increase the risk of recurrence if patients are adequately staged at the time of initial surgery. However, given the small number of relapses, the Authors suggested a prognostic role of ploidy in patients who were not appropriately surgically staged. Mariani [38], in a case-cohort study on histopathologic and molecular predictors of distant failure in EC, reported that aneuploidy significantly predicted distant relapses but lost any significance when a logistic regression was performed. Osmanoğlu [42] similarly found that ploidy was an independent prognostic indicator in MA and was correlated with tumor recurrence in UA, however it did not result significant for tumor recurrence in MA.

Conversely, Pfisterer [43] reported that ploidy status did not correlate either with recurrence-free survival or with overall survival in UA. In particular, they found an increased risk of reduced overall survival at UA; however, given the small number of events (n=26), no statistically significant differences were observed. Moreover, Konski [37] reported in a study on 171 stage I EC patients, that patients with diploid tumors had a slightly increased (but not significant) survival rate compared to patients with non-diploid tumors (p=0.12). Recently, a Korean study further confirmed that DNA aneuploidy is an independent prognostic factors and it is useful to identify “high-risk” patients among those usually considered at “low risk”, as stage I endometrial cancer [44]. In large series of consecutive patients with early stage endometrial cancer, DNA ploidy was studied in order to evaluate its reliability as a prognostic factor. Data confirmed that patients with non-diploid tumors have a significant higher risk of relapse and disease-related death, but the superiority of ploidy over the traditional prognostic factors was not demonstrated [45-47]. Pradhan [48] considered DNA Index (DI) of the aneuploid tumor in DNA ploidy results in about 900 cases of endometrioid adenocarcinoma; patients with aneuploid tumor with DI >1.20 had higher recurrence rates, higher distant failures rate and poorer disease-free and overall survival, while patients with aneuploid tumors with DI ≤1.20 mainly relapsed locally and had higher recurrence rates than patients with diploid tumor. These results may suggest that patients with aneuploid tumor with DI ≤1.20 can be clinically followed up or be considered for adjuvant vaginal brachytherapy. It must be however underlined that, in this experience, DNA ploidy was not found to be an independent prognostic factor when traditional ones where taken into account.
our experience [35], patients with aneuploid tumor and DI ≤1.20 were included in the same group of those with diploid tumor in order to decide whether to give adjuvant therapy. In contrast to the aneuploid tumors with DI ≤1.20, the aneuploid tumors with DI >1.20 are more clinically aggressive and relapse mainly outside the pelvis in the same fashion as serous and clear-cell adenocarcinomas, indicating that these patients can be potential candidates for adjuvant chemotherapy. The same authors [49] performed a DNA ploidy analysis in cases of stage I and II serous adenocarcinoma of the endometrium and demonstrated a prognostic significance of these parameters: patients with diploid (n=19), aneuploid (n=29), and tetraploid (n=8) tumor had 5-year recurrence rates of 10, 38, and 53%, respectively (p=0.09). A DNA ploidy parameter, 5c exceeding rate, was found to be a prognostic marker for recurrence (p=0.03), progression-free survival (p<0.01), and overall survival (p=0.02). These results seem to be particularly interesting, as it has been reported that DNA ploidy closely correlates with histopathologic subtypes of endometrial cancer [50].

Ploidy and postoperative management of EC

There is still no consensus on the evidence-based optimal treatment of patients with early stage EC. Surveys by Maggino have shown that the clinical management of the same tumor may vary according to different oncological referral centres in Europe [1] and in the United States [2]. Naumann [44] analyzed the current standards of care for early stage EC adopted by the members of the Society of Gynecologic Oncologists and reported that for stage IB grade 3 and all stage IC most gynaecologic oncologist would recommend some type of adjuvant radiation therapy. Almost all the studies reported in the literature clearly identify DNA ploidy as a strong prognostic factor in EC patients; however, a very recently published editorial by Terada [51] is not consistent with this and did not support the use of DNA ploidy as a replacement of traditional prognostic factors for treatment decision; interestingly the author stated that “...obviously, such a strategy will include some "low-risk" patients who ultimately recur and some "high-risk" patients who will survive without adjunctive therapy”. Unexpectedly, DNA content in EC cases is not routinely screened in clinical practice worldwide. Generally, aneuploid endometrial neoplasms are usually either G3 endometrioid adenocarcinomas, clear-cell carcinomas or uterine papillary-serous adenocarcinomas. Non-endometrioid and high-grade (G3) endometrioid histotypes are consistently accepted to be high-risk features. In clinical practice, the real challenge is to identify high-risk cases among the heterogeneous group of endometrioid EC, accounting for 85% of all endometrial neoplasms. DNA ploidy may play a key role in the subset of patients for whom adjuvant therapy after hysterectomy is still a controversial issue, i.e. moderately differentiated (G2) endometrioid carcinomas stage IB-IC. Susini [31] reported the 10-year results of a prospective study on the prognostic role of ploidy in EC; by multivariate analysis DNA aneuploid pattern was demonstrated to be the strongest independent predictor of poor outcome, followed by age and surgical stage; aneuploid tumors had a significantly higher risk ratio for recurrence (5.03) and death due to the disease (6.50) compared to diploid tumors. These results suggest that even in the subgroup of well-differentiated (G1) tumors it is possible to distinguish patients with excellent prognosis bearing a DNA diploid tumor and patients with less favourable outcome correlated to a DNA aneuploid tumor. More interestingly, in patients with G2 endometrioid tumors, representing a group for which prognosis and need for adjuvant therapy are still unclear, DNA ploidy allows the identification of a subset of more aggressive tumors that definitely requires adjuvant therapy. Other authors recently proposed multiple factor analysis, including DNA content evaluation, to overcome the limits of traditional pathological risk assessment. Lim [45] prospectively assigned 406 consecutive stage I “low-risk” patients (endometrioid EC limited to ≤50% myometrial infiltration and no vascular space invasion or grade 3) to treatment groups based on tumor ploidy. Patients with aneuploid tumors were submitted to vaginal vault radiotherapy (RT), while those with diploid tumors were followed and treated only at relapse. The authors reported that patients with aneuploid tumors treated with RT have the same risk of relapse as untreated patients with diploid tumors, suggesting a useful role of this ploidy-based treatment policy in clinical practice. In addition, Hogberg [46] proposed treating patients according to new risk criteria, including DNA ploidy. In this large study on 335 stage I-II endometrioid EC patients, high-risk cases were defined by the sum of those with grade 3 and/or deep myometrial infiltration and the cases with aneuploid tumors: highly significant differences were reported between the “low risk” and the “high risk” groups regarding overall survival, disease-specific survival and failure rates, despite the administration of adjuvant vaginal brachytherapy to the high risk group. Mangili [35] reported preliminary results of postoperative risk assessment by means of DNA ploidy in stage I endometrioid EC patients. The authors reported that, since ploidy was introduced as a “decision-making” factor, 69.4% of patients in stage IC were spared adjuvant therapy. Neither increase in tumor related mortality in this group nor any statistically significant increase in recurrence rate was observed between patients submitted to adjuvant therapy according to traditional prognostic factors stratification and those managed according to ploidy evaluation. Inconsistently, Lindahl [52] reported that a treatment programme in endometrioid EC based upon DNA ploidy alone, for which adjuvant vaginal brachytherapy was performed in non-diploid tumors, was not the optimal treatment option, suggesting more research towards cytostatics.

Summarized results of the most significant studies regarding the prognostic role of DNA ploidy in endometrial cancer are reported in Table 1.

Conclusions

Considering the large number of studies reported in literature, DNA ploidy appears to be with no doubt very relevant in the prognostic assessment of early stages endometrioid EC patients, allowing the identification of cases at increased risk for disease recurrence and/or cancer-related death; this would signify the recognition of patients who would benefit from adjuvant strategies despite the apparent low-risk. However, it is actually neglected or poorly considered by the large majority of gynaecological oncologists in clinical practice. It must be underlined that, and we strongly support this concept, DNA ploidy determination should not replace the use of traditional and evidence-based validated prognostic indicators, rather, they should complement each other to optimize the clinical workout. Since DNA ploidy has not been positively considered in clinical practice for more than 20 years, the hypothesis of gaining a real clinical value nowadays seems to be quite unrealistic. Chromosomal instability leading to structural and numerical abnormalities of chromosomes has a
Table 1: Summarized results of the principal studies dealing with prognostic relevance of DNA ploidy in endometrial cancer.

<table>
<thead>
<tr>
<th>Author</th>
<th>n° Cases</th>
<th>Stage</th>
<th>Type</th>
<th>Technique</th>
<th>Statistics</th>
<th>Prognostic Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambros [18]</td>
<td>57</td>
<td>I</td>
<td>EN</td>
<td>ICM</td>
<td>UA+MA</td>
<td>( p&lt;0.002^* )</td>
</tr>
<tr>
<td>Britton [14]</td>
<td>256</td>
<td>I-IV</td>
<td>EN+CC+SP</td>
<td>FCM</td>
<td>MA</td>
<td>( p&lt;0.001 )</td>
</tr>
<tr>
<td>Dyas [17]</td>
<td>121</td>
<td>I-II</td>
<td>n.a.</td>
<td>FCM</td>
<td>UA</td>
<td>( p&lt;0.01 )</td>
</tr>
<tr>
<td>Friberg [33]</td>
<td>103</td>
<td>I-II</td>
<td>EN</td>
<td>FCM</td>
<td>UA</td>
<td>( p&lt;0.001 )</td>
</tr>
<tr>
<td>Hogberg [46]</td>
<td>335</td>
<td>I-II</td>
<td>EN</td>
<td>FCM</td>
<td>UA</td>
<td>( p&lt;0.0001 )</td>
</tr>
<tr>
<td>Ikeda [19]</td>
<td>76</td>
<td>I-III</td>
<td>EN</td>
<td>FCM</td>
<td>MA</td>
<td>( p&lt;0.01 )</td>
</tr>
<tr>
<td>Lindahl [9]</td>
<td>166</td>
<td>I-II</td>
<td>EN</td>
<td>FCM</td>
<td>MA</td>
<td>( p=0.015 )</td>
</tr>
<tr>
<td>Lindahl [16]</td>
<td>245</td>
<td>I-II</td>
<td>EN</td>
<td>FCM</td>
<td>UA</td>
<td>( p=0.0001 )</td>
</tr>
<tr>
<td>Lundgren [40]</td>
<td>80</td>
<td>I-IV</td>
<td>EN+CC+SP</td>
<td>ICM</td>
<td>UA</td>
<td>( p&lt;0.001 )</td>
</tr>
<tr>
<td>Mangili [28]</td>
<td>203</td>
<td>I-III</td>
<td>EN</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( p=0.001^* )</td>
</tr>
<tr>
<td>Mangili [35]</td>
<td>222</td>
<td>I</td>
<td>EN</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( p=0.003^* )</td>
</tr>
<tr>
<td>Newbury [15]</td>
<td>233</td>
<td>I-IV</td>
<td>EN+CC+SP</td>
<td>FCM</td>
<td>MA</td>
<td>( p=0.001 )</td>
</tr>
<tr>
<td>Ørbo [39]</td>
<td>123</td>
<td>I-IV</td>
<td>EN+CC+SP</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( P=0.008^* )</td>
</tr>
<tr>
<td>Pfisterer [43]</td>
<td>202</td>
<td>I</td>
<td>EN</td>
<td>FCM</td>
<td>UA+MA</td>
<td>n.s.* **</td>
</tr>
<tr>
<td>Pradhan [48]</td>
<td>937</td>
<td>I-II</td>
<td>EN</td>
<td>ICM</td>
<td>UA+MA</td>
<td>( p=0.02^* )</td>
</tr>
<tr>
<td>Song [47]</td>
<td>217</td>
<td>I</td>
<td>EN+NEN</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( p=0.001^* )</td>
</tr>
<tr>
<td>Sorbe [21]</td>
<td>227</td>
<td>I-IV</td>
<td>EN+NEN</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( P=0.0001^* )</td>
</tr>
<tr>
<td>Suehiro [32]</td>
<td>106</td>
<td>n.a.</td>
<td>EN</td>
<td>ICM</td>
<td>UA+MA</td>
<td>( p=0.012^* )</td>
</tr>
<tr>
<td>Susini [23]</td>
<td>74</td>
<td>I-IV</td>
<td>EN+CC+SP</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( p=0.001^* )</td>
</tr>
<tr>
<td>Susini [26]</td>
<td>50</td>
<td>I-IV</td>
<td>EN+CC+SP</td>
<td>FCM</td>
<td>UA</td>
<td>( p=0.002 )</td>
</tr>
<tr>
<td>Susini [31]</td>
<td>174</td>
<td>I-IV</td>
<td>EN+CC+SP</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( p=0.001^* )</td>
</tr>
<tr>
<td>Terada [41]</td>
<td>100</td>
<td>I</td>
<td>EN</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( P=0.04^* )</td>
</tr>
<tr>
<td>Van der Putten [13]</td>
<td>38</td>
<td>I</td>
<td>EN</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( p&lt;0.01^* )</td>
</tr>
<tr>
<td>Zaino [25]</td>
<td>254</td>
<td>I-II</td>
<td>EN+NEN</td>
<td>FCM</td>
<td>UA+MA</td>
<td>( p=0.001^* )</td>
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</table>


primary role in the malignant alteration of tumors. Chromosomal aneuploidy is found in 20–35% of cases of endometrial cancer and is associated with advanced cancers, high-grade non-endometrioid cancer and deep myometrial invasion in the FIGO staging of carcinoma. We believe that the ever growing need to identify positive prognostic factors in female genital malignancies, together with the increasing importance of fertility-sparing strategies of treatment, will hopefully lead to a greater consideration of this interesting biological aspect of endometrial cancer.

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