The prognostic value of DNA ploidy in a total population of uterine sarcomas

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Background: The diagnosis of uterine sarcoma is associated with poor outcome for the patient and there is a need for reliable prognostic markers. Most previous studies on the prognostic value of DNA ploidy include few uterine sarcomas and report conflicting results.

Materials and methods: We examined the prognostic value of DNA ploidy and its association with clinicopathological parameters and crude survival in a total population of 354 sarcomas.

Results: In univariate analyses, we observed significantly better crude survival for endometrial stromal sarcomas (ESS) and adenosarcoma (AS) patients with diploid as compared with nondiploid tumors, but not for patients with leiomyosarcomas (LMS). In Cox multivariate analyses, DNA ploidy was the only significant predictor of survival for patients with AS. In LMS, mitotic index (MI), tumor size, tumor extent and tumor margins, whereas for ESS, MI, tumor extent and tumor necrosis obtained independent significance of survival. DNA ploidy was a significant predictor of survival for LMS patients in Cox regression analyses when excluding MI.

Conclusion: DNA ploidy might be useful as a prognostic marker in patients with LMS and AS.

Key words: endometrial stromal sarcoma, leiomyosarcoma, DNA ploidy, prognostic factors, uterine sarcomas

Introduction

The three main histological types of uterine sarcomas are leiomyosarcomas (LMS), endometrial stromal sarcomas (ESS) and adenosarcomas (AS) [1]. ESS was formerly divided into low-grade and high-grade ESS. The latter is now considered as undifferentiated uterine sarcomas (UUS). In addition, some other rare types exist. LMS are associated with prominent nuclear atypia and abundant mitotic activity. Even though LMS are often confined to the uterus at the time of diagnosis, the frequency of recurrence is high [2, 3]. The 5-year survival is in the range 50%–60% in stage I, but only 15% in more advanced stages [4]. In contrast, ESS are associated with minimal nuclear atypia and low mitotic activity. The 5-year survival ranges from 67% to nearly 100% [5–7]. The UUS have marked atypia and abundant mitotic activity. UUS are highly aggressive tumors, and most patients die within 3 years after surgery [8]. The rare sarcomas are all very aggressive.

Multiple studies have been conducted to find reliable prognostic factors for patients with uterine sarcomas. Stage of disease, free resection margins at surgery, grade, histological subtype, tumor size, age, DNA ploidy, TP53 expression and mitotic index (MI) have all been reported as possible prognostic markers [9]. Most of these studies include a limited number of cases and the results are contradictory, but most studies conclude that stage is a predictor of recurrence and survival for patients with LMS [10, 11] and ESS [5, 8]. For patients with uterine sarcomas confined to the uterus, tumor size is also found to be of prognostic value [12, 13].

Previous reports on the prognostic value of DNA ploidy in uterine sarcomas show contradictory results [14]. Some reports have found prognostic impact of DNA ploidy [10, 13, 15–19], while others have not [20–22].

The aim of the present study was to evaluate the prognostic value of large-scale genomic instability as determined by DNA ploidy, in a population-based study of 419 uterine sarcomas.

Materials and methods

All the 587 uterine sarcomas registered from 1970 to 2000 at the Norwegian Cancer Registry, which gathers information on all cancer events in Norway, were initially included in our study. The tumors were reclassified by an experienced gynecological pathologist (VMA) according to the recommendations by the World Health Organization histological classification of tumors of the uterus corpus [23]. The diagnosis of uterine sarcoma was confirmed in 419 of the 587 patients [24]. Of the 419 patients, 29 were not admitted to surgery and were not included in the analyses. Further, tissue blocks with tumor material could not be obtained in 15...
cases, and in 21 cases, we could not obtain a DNA ploidy classification because of poor quality of the tumor material. Survival data for all patients were obtained from the Norwegian Cancer Registry in October 2007. The study was approved by the Regional Ethics Committee.

DNA ploidy analysis was carried out as described previously [25]. In brief, paraffin-embedded formalin fixed tissue selected by the pathologist was used for preparation of nuclei suspensions. Monolayers were prepared and the nuclei were stained by Feulgen–Schiff. The Fairfield DNA Ploidy System (Fairfield Imaging LTD, Kent, UK) was used for image processing and analysis. The DNA ploidy histograms were classified using the following criteria: a sample was considered to be diploid if there was only one peak, located at the 2c position, and the number of nuclei at the 4c position did not exceed 10%. If the 4c peak was >10% or there was a peak present in the 8c position, the sample was considered tetraploid. A tumor was defined as polyploid if there was a peak in the 2c position, and the number of nuclei at the 4c position did not exceed 10% or there was a G2 peak in the 16c position. The sample was considered aneuploid when a peak appeared outside 2c, 4c or 8c ranges. On average, 1288 (326–2437) tumor nuclei were included from each case. The mean coefficient of variation (CV) of the diploid tumor peak was 4.75.

### statistical analysis

SPSS software (SPSS 15, SPSS, Chicago, IL) was used for calculation of statistics. Comparison between parameters and groups was carried out by Pearson χ² analyses. Crude survival was calculated from date of diagnosis to death or end of follow-up, using the Kaplan–Meier method. The median follow-up was 93.86 months (ranging from 0 to 430). The log-rank test was used for univariate analyses and for multivariate analyses a Cox proportional hazards regression model, including the variables that were significant in univariate analyses, was used. The variables were grouped as follows: DNA ploidy as diploid versus nondiploid; histological subtype as LMS, ESS, AS, UUS or other sarcomas; MI as below or above 10 per high-power field (HPF); tumor extent as confined to uterus or not; tumor size as below or above 10 cm; tumor margins as pushing or infiltrating; histological subtype is presented in Table 2. There were significant correlations between DNA ploidy and histological subtype, as the majority of the LMS, UUS and sarcoma NOS were aneuploid, whereas the majority of the ESS and AS were diploid (Table 1). In addition, there were significantly more aneuploidy cases with >10 mitoses per HPF (P < 0.001), severe cellular atypia (P < 0.001), the presence of tumor necrosis (P < 0.001), the presence of hyaline necrosis (P = 0.002), >10 cm tumor size (P = 0.010), the absence of vascular invasion (P = 0.011) and pushing tumor margins (P = 0.014). There was no significant correlation between DNA ploidy and tumor extent.

### outcome

For LMS, the 5-year crude survival was 64.3%, 51%, 62.1% and 32.8% for patients with diploid, tetraploid, polyploid and aneuploid tumors, respectively (P = 0.033). For ESS, the 5-year crude survival was 82.5%, 38% and 50% for patients with diploid, tetraploid and aneuploid tumors, respectively (P < 0.001).

When comparing crude survival for patients with diploid and nondiploid tumors (Figure 1), the difference did not reach statistical significance for LMS (P = 0.051). The difference was of statistical significance for ESS (P < 0.001) with a 5-year survival of 82.5% for patients with diploid tumors and 40.0% for nondiploid tumors, and AS (P = 0.033) with a 5-year survival of 76.9% for diploid and 62.5% for nondiploid cases. No difference was seen for UUS or other sarcoma types.

An analysis on the 267 cases with disease confined to the uterus showed statistical significant differences in survival as related to DNA ploidy for the 52 cases of ESS (P = 0.047), whereas the differences in 175 LMS (P = 0.065) and 20 AS (P = 0.064) were not.

### prognostic factor analyses

In Cox regression analyses, the independent prognostic factors for patients with LMS were MI [P < 0.001, hazard ratio (HR) 2.1, 95% confidence interval (CI) 1.5–2.97] and tumor size (P < 0.001, HR 2.1, 95% CI 1.46–3.14) followed by tumor extent (P = 0.011, HR 1.5, 95% CI 1.09–1.95) and tumor

### results

### characteristics of patients

Among the 354 cases with DNA ploidy classification, there were 222 LMS, 78 ESS, 21 AS, 16 UUS and 17 ‘other’ [10 sarcomas not otherwise specified (NOS), four rhabdomyosarcomas, two giant cell tumors with or without LMS and one perivascular epithelioid cell tumor (PEComa)] as shown in Table 1. The patients diagnosed with LMS had a mean age of 57 years at time of diagnosis, while it was 53 years for patients with ESS and 63 years for the other histological subgroups. The distribution of DNA ploidy and histological subtypes is shown in Table 1. The description of clinicopathological factors related to histological subtype is presented in Table 2. There were significant correlations between DNA ploidy and histological subtype, as the majority of the LMS, UUS and sarcoma NOS were aneuploid, whereas the majority of the ESS and AS were diploid (Table 1). In addition, there were significantly more aneuploidy cases with >10 mitoses per HPF (P < 0.001), severe cellular atypia (P < 0.001), the presence of tumor necrosis (P < 0.001), the presence of hyaline necrosis (P = 0.002), >10 cm tumor size (P = 0.010), the absence of vascular invasion (P = 0.011) and pushing tumor margins (P = 0.014). There was no significant correlation between DNA ploidy and tumor extent.

### Table 1. DNA ploidy distribution in the different histological subtypes of 354 cases of uterine sarcomas

<table>
<thead>
<tr>
<th>Histological subtype</th>
<th>DNA ploidy classification, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diploid</td>
<td>Tetraploid</td>
</tr>
<tr>
<td>LMS</td>
<td>28 (12.6)</td>
<td>49 (22.1)</td>
</tr>
<tr>
<td>ESS</td>
<td>63 (80.8)</td>
<td>8 (10.3)</td>
</tr>
<tr>
<td>AS</td>
<td>13 (61.9)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>UUS</td>
<td>3 (18.8)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Rare tumors a</td>
<td>3 (17.6)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>Total</td>
<td>110 (31.1)</td>
<td>63 (17.8)</td>
</tr>
</tbody>
</table>

aRare tumors includes sarcoma not otherwise specified (10), rhabdomyosarcomas (four), giant cell tumor with or without LMS (two) and PEComa (one).

LMS, leiomyosarcoma; ESS, endometrial stromal sarcoma; AS, adenosarcoma; UUS, undifferentiated uterine sarcoma.
The histological diagnosis of uterine sarcomas is challenging [24, 26], and due to their rarity few pathologists have large experience in diagnosing these tumors. The best prognostic marker for both LMS and ESS in this material was MI, which in other studies has been shown to have a low reproducibility [27, 28]. Hence, there is a need for additional prognostic markers in uterine sarcomas.

Most previous studies on DNA ploidy in uterine sarcomas have used flow cytometry for measurement. However, image cytometry has been shown to be superior to flow cytometry in detecting small aneuploid peaks and nuclei with high DNA content [14, 29]. Further, flow cytometry should be carried out on fresh material, while image cytometry is carried out on formalin-fixed material, which is easily accessible. We have used a novel automated system for DNA ploidy analysis by image cytometry, which can acquire a large number of nuclei that are morphologically controlled to ensure that only single, well-preserved tumor nuclei are analyzed. The mean CV for the diploid tumor peak was 4.75, thus, by using our automated system, we have obtained reliable DNA measurements from tissue blocks up to 36 years old. In addition, the most representative tumor area of the sections was carefully selected by the reviewing pathologist. Furthermore, heterogeneity in DNA ploidy classification has been observed in some types of tumors [30, 31], but has to our knowledge not been reported for uterine sarcomas.

Previous reports on DNA ploidy analysis in uterine sarcomas have shown contrasting results. Some groups have found...
prognostic impact of DNA ploidy \[10, 13, 15–19\], while others have not \[20–22\]. However, most of these studies included a small number of cases, which might in part explain the lack of consensus. In addition, some of the above-mentioned studies \[16, 19, 22\] are also weakened by the lack of Cox regression analyses. To our knowledge, the largest number of LMS and ESS previously studied are 70 cases \[13\] and 48 cases \[21\], respectively, and these cases are included in our series. Hence, this study of 354 cases represents the largest study conducted on DNA ploidy in uterine sarcomas and includes Cox multivariate analyses.

Most earlier reports on patients with LMS have found DNA ploidy to be a prognostic factor in both univariate \[10, 13, 16\] and multivariate analyses \[10\]. However, in our study, we did not observe any significant difference in crude survival between the diploid and nondiploid LMS in multivariate analysis. This is in agreement with the study by Nordal et al. \[13\] on 70 cases of LMS. The lack of significance of DNA ploidy in our multivariate analysis might to a large extent be explained by the fact that MI have a strong discrimination power in this study. Since the MI has been shown to be of restricted value in other studies due to low reproducibility \[27, 28\], we also carried out Cox multivariate analyses without this factor. In these analyses, DNA ploidy was a significant predictor of outcome.

Considering the low reproducibility of MI, the argument of using DNA ploidy as an objective additional marker in LMS is enforced.

For ESS, the two largest reports on DNA ploidy show contrasting results. Nola et al. \[15\] reported that DNA ploidy was a predictor of outcome in 26 patients, whereas Nordal et al. \[21\] did not find significant differences in survival in 48 cases of ESS. In contrast, even though most of their cases are included in the present study, we found significant difference in survival between patients with diploid versus nondiploid ESS in univariate analyses of 78 patients. The previous reports have included both high- and low-grade ESS, while we have separated the cases into ESS and UUS according to the recent criteria for classification \[23\]. This might explain the observed discrepancy since DNA ploidy was not a predictor of outcome in UUS. In addition, the previous studies were carried out by flow cytometry. In multivariate analysis, DNA ploidy was not a significant prognostic marker in ESS in the present study. When MI was omitted from the Cox analysis on ESS, DNA ploidy was of marginal significance with a HR of 2.3. Due to the relative small number of patients with ESS, we cannot conclude on the prognostic importance of DNA ploidy in this group.

DNA ploidy was the only significant predictor of outcome in 21 patients with AS, which is not in agreement with an earlier study showing no relationship between DNA ploidy and survival in 11 patients with uterine AS \[32\]. However, the results for AS must be considered with caution due to the limited number of cases included in both of these studies.

In conclusion, DNA ploidy might give valuable information on the outcome for patients with LMS and AS.

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references


