ORIGINAL ARTICLE

Comparison of clinical staging of benign and malignant ovarian tissues with DNA flow cytometry

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Abstract

The prevention, diagnosis and treatment of ovarian cancer are major issues. The outcome of patients with advanced ovarian cancer is poor despite aggressive therapy including surgery, combination drug chemotherapy and radiation treatments. From the literature, the direct correlation between DNA ploidy and survival is greatly enhanced using high resolution DNA measurements, which results in a coefficient of variation (CV) range between 1%-2% (1.42 ± 0.19, n=66) for trout red blood cells (TRBC) and 2%-3% (2.18 ± 0.46 SD, n=22) for tonsil nuclei derived from formalin-fixed, paraffin-embedded tissues (deparaffinated). DNA nuclear determinations from 50 ovarian cancer and 21 benign patients is presented. This was accomplished by measuring the DNA content of nuclei simultaneously isolated from deparaffinated tissues, stained with the fluorescent DNA specific dye, 4', 6-diamidino-1-phenylindole (DAPI) and analyzed on a high resolution flow cytometer. A high percentage of aneuploidy (92.0%) was determined from the ovarian cancer patients, especially of the aneuploid DNA histogram types, such as hypodiploid, multiploid and hypertetraploid (64.0%), which have shown poor prognosis in a variety of cancers including ovarian. Furthermore, aneuploidy was detected in 23.8% of the benign patients. DNA flow cytometry may complement pathological assessment of ovarian cancer to better determine malignancy, thus justifying a closer follow-up with more specialized approaches to treatment in clinical trials that involve new therapies including the use of chemically defined, natural products, which may help offset the overall poor prognosis seen in ovarian cancer.

Key words

DNA histogram, Flow cytometry, Ovarian cancer

1 Introduction

The five-year survival rates for ovarian cancer are among the lowest of the 21 most common cancers in England. Epithelial ovarian cancer is one of the major causes of cancer death among women and is the first among gynecologic malignancies [1]. These relatively low survival rates can be attributed in part to the fact that 29% of cases of the ovarian cancer are emergency presentations [2]. The ovarian cancer five year, stage specific relative survival rates (ages 15-99)

clearly show the survival rates with little significant improvement except for a slight increase in Stage 2 patients since 2000. The outcome of patients with advanced ovarian cancer is poor despite aggressive therapy including surgery and combination chemotherapy [3]. Patients with no macroscopic residual disease after a primary cytoreductive operation are considered to have the most favorable prognosis among subjects with advanced ovarian carcinoma. Nevertheless, over half of these patients eventually die of recurrent disease [4]. Clearly, the prevention, diagnosis and treatment of ovarian cancer are major issues. Ovarian cancer has been an object of extensive medical research [5, 6]. Most patients with epithelial ovarian cancer present at the time of initial diagnosis with advanced disease and are subjected to multimodality, aggressive chemotherapy and radiation treatments after surgery.

Research studies have investigated the possibility of in vitro chemosensitivity testing to determine the efficacy of chemotherapy. Unfortunately, although the accuracy of in vitro systems in predicting drug resistance is very high, the accuracy in predicting sensitivity is very low [7]. The application of in vitro results to individual patients has therefore been limited to avoid the administration of drugs that are ineffective in vitro.

Studies of the predictive accuracy of biomarkers in determining prognosis and treatment response in epithelial ovarian cancer have shown DNA ploidy as one of the most intensively investigated biologic features [8-17]. Also, proliferative rate [9, 12, 15, 17-24], growth factors and their receptors [25], lamina receptors [26] and functional and structural alterations of oncogenes and oncosuppressor genes [21, 22, 25-29] have been studied. Biomarkers directly involved in drug resistance, such as proteins associated with multidrug resistance detoxification [30-36], have also been determined as indicators of clinical outcome. However, due to the marked biologic, pathologic, and clinical tumor heterogeneity, outcome and interpretation of translational studies are often not equivocal. Clinical outcome is the result of biological aggressiveness and treatment efficacy, which are balanced to varying degrees in the different ploidy tumor types [37, 38]. A confounding interference could occur in interpreting results in terms of prognosis or treatment efficacy, because biomarkers related to such events often work in opposite directions. This situation is commonplace in ovarian carcinoma, a disease that generally receives first-line systemic therapy [38].

Coulson, Thornthwaite, et al. [39] investigated the predictive role of DNA ploidy as it relates to histological diagnosis, recurrence and survival of breast cancer. Lee et al. [40] and Silvestrini et al. [38] later studied the relationship of DNA ploidy and ovarian cancer with similar results. Brescia et al. [41] examined the DNA content of 99 ovarian carcinomas by flow cytometric analysis of nuclei obtained from paraffin-embedded tissue. They showed the determination of DNA ploidy in ovarian carcinomas may be used as an adjunct in predicting tumor behavior, response to chemotherapy, and late recurrence of disease.

The clinical importance of ploidy in ovarian neoplasms is dependent on the techniques, reagents and instruments used to obtain DNA histograms, especially with paraffin embedded tissues. The data should be interpreted with caution before the data can be used for clinical assessment [42].

We have been pioneers in developing better ways to measure DNA in tissues [39, 40, 43-46]. The purpose of the research presented here is to show the best procedures for measuring DNA in nuclei derived from paraffinated tissues. We are able to obtain lowest coefficients of variation (CV) for the DNA flow cytometry. We use the term “high resolution” DNA flow cytometry to describe standard measurements in the CV= 1%-2% range. A comparison will be presented between DNA ploidy types and benign vs. malignant data. For example, hypodiploid is a poor prognostic group, and we find hypodiploid is common in ovarian cancer unlike our breast cancer studies [39], thus predicting the aggressive outcomes of this disease.

In this paper, we will address developing a DNA flow cytometric protocol to assist the pathologist in the determination of aggressiveness as measured by DNA histogram type determination of primary ovarian cancers and more clearly define the distinction between benign and malignant.

2 Materials and methods

2.1 Sample preparation

Paraffinated human ovarian tissues under human subject's approval were obtained from St. Mary's Hospital and King's College in London. The formalin-fixed paraffin embedded tissue blocks were cut into 30μm thick strips using a microtome. Each tissue strip was subjected to deparaffination and enzymatic dissociation (PARA Kit TN Scientific). After incubation of one section per 1ml of PARA reagent in a 37ºC water bath for 30 minutes, the dissociated cells were filtered through a 37μm nylon mesh into a 15mL conical centrifuge tube. A 9ml portion of Dulbecco's phosphate buffered saline (PBS) without calcium and magnesium was added to each conical tube. The 10mL portions were centrifuged at 200xg at room temperature. The pellets were suspended in 1mL of Nuclear Isolation Medium II- 4', 6-diamidino-1-phenylindole (NIMII-DAPI TN Scientific), filtered through a 37μm nylon mesh, and left on ice for at least two minutes before DNA flow cytometric analysis.

2.2 Standard preparation

Trout erythrocytes were used as the DNA internal standard (TN Scientific). The DNA standard was mixed with DAPI-NIM, and filtered through a 37μm filter.

The DNA standard served three purposes. Firstly, it was use to establish the flow cytometer was operating within the precise boundaries of CV= 1%-2%. Secondly, during a sample run, the DNA standard peak at channel 50 was maintained by utilizing the optimized maximum fluorescence signal on the sample-sheath stream of nuclei using the X-Y-Z (focus) stepping motors to maintain the DNA standard in channel 50 (equivalent to 5.0 pg/nucleus for the TRBC). Finally, the position in channel 50 was used to assure the PARA reagents were functioning by using human tonsil to determine the optimum CV= 2%-3% and the relative DNA value for the G0 human tonsil nuclei of 7.4 pg/nucleus.

2.3 Flow cytometry

DNA per nuclei values were determined by utilizing a fluorescence-electronic cell volume flow cytometer (NPE Systems). DNA flow cytometry measurements were obtained using the DAPI excitation-emission dichroic mirror-filter sets, which isolated the 365-nm mercury emission line from a stabilized 100 watt mercury lamp to excite the DAPI fluorochrome, and a 400-nm dichroic mirror with a 450-nm interference filter to optimize measurement of the DAPI fluorescence emission at 450 ± 20 nm.

2.4 DNA data analysis

A data acquisition and analysis system as described by Thornthwaite et al. [46] was used in these studies to acquire the 10 bit analog to digital resolution DNA histograms. The percentage coefficient of variation (CV) was determined by an assembler/ basic interface computer analysis program using the following formula:

$$CV = \frac{\text{Width at 1/2 ht}}{\text{Peak channel no.} \times 2.3} \times 100 \quad (1)$$

The numbers of G0/1 cells were calculated by integrating the minimum cell number values immediately to the left and to the right of the G0/1 curve, respectively. A similar integration was performed to obtain the cell number in the G2 + M curve. The remaining cells comprised the S-phase population. This integration procedure, using a high-resolution flow system with CVs in the range of 1 to 3%, is sufficient for obtaining precise data [46].

2.5 DNA index (DI)

The DI was determined by dividing the G0/1 peak channel number of the aneuploidy tumor cells by the peak G0/1 channel number of normal diploid ovarian cells run in the same experiment. The DI of the aneuploid nuclei represents the change

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in DNA content of the aneuploidy tumor when compared to normal ovarian cell nuclei. Normal tonsil nuclei derived from paraffinated tissues and subjected to the NIM-DAPI procedure were also used to determine the diploid ovarian population. A relative DNA Index of the tonsil nuclei was determined by dividing the peak tonsil G0 population by the peak DNA TRBC standard DNA population.

2.6 Percentage of Aneuploid Nuclei

Data from graphs were converted into the number of cells in diploid G0/1, S, and G2 + M, and estimates of the number of aneuploidy nuclei in G0/1, S, and G2 + M were made after subtracting an estimate of overlap with the diploid population. In most cases the population overlaps made an accurate measurement of the S-phase problematic. Except where noted, the estimated percent S-phase of the aneuploidy was not attempted. This percentage of aneuploidy nuclei value is an estimate of the aneuploidy tumor load [39, 46]. The overlap of aneuploidy cells in many tumor samples was quite large, which did not allow for an accurate estimate of percentage of aneuploidy.

The most important measurement was the determination of the DNA histogram type, which described a DNA population as diploid [DIP] (DI= 1.0); hyperdiploid [HDIP] (DI= 1.05-1.99); hypodiploid [HYPO] (DI<0.9-0.5) or mutiploid [MULTI] with multiple aneuploid G0/1 populations.

3 Results

Figure 1 presents typical DNA histograms (CV=1.42 ± 0.19 SD, n=66) of the DNA Standard composed of stabilized trout red blood cells that were converted to DAPI stained nuclei with NIM-DAPI. The DNA Standard was utilized to act as an internal instrument standard to maintain the NIM-DAPI stained TRBC nuclei in channel 50 utilizing three stepping motors to optimize the X-Y-Z position of the sample stream while focusing on the stream with a 100X objective. The DNA Standard was also mixed with the tonsil and ovarian samples and used as an internal standard on which to locate the diploid populations in the tonsil and ovarian samples.

In Figure 2, paraffinated tonsil was used as a human diploid standard to ensure the PARA reagents were functioning properly and to determine, along with the DNA standard, the diploid populations in the benign and cancer samples. The data for 22 diploid, normal tonsil samples resulted in an average G0 coefficient of variation of 2.18 ± 0.46 SD, an average DNA index (peak channel of normal tonsil divided by the peak of DNA standard) of 1.42 ± .033 SD, and an average S-phase fraction of 4.37 ± 1.37 SD. The DNA content of TRBC is 5.0 pg/nucleus [40]. The DNA content of the diploid populations was about 7.4 pg/nucleus, which more closely resembled non-paraffinized tissue G0 populations of 7.9 pg/nucleus [39, 40, 45, 46]. The fixation and paraffination affected the ability of DAPI to bind to the same extent to A-T regions as in fresh tissues. In any event, the diploid populations in the tumor samples were very consistent in their DI with the DNA standard as shown in Figures 2-5.

Figure 3a, c, and d shows normal, diploid DNA histograms from pathologically diagnosed benign tumors. The flow cytometry revealed that these samples also displayed a diploid characteristic, which is consistent with what the pathology should show. However, 5/21 (23.8%) of the pathologically benign cancers were aneuploid as shown in the five aneuploidy benign tumors shown in Figures 3b and 4a,b,c,d.

Figure 4 again shows pathologically diagnosed benign tumors. However, these samples displayed aneuploidy. These data revealed that although a mass may appear to display normal pathology, their DNA is atypical. This finding is well supported in the literature [39, 40, 50, 52, 64]. The presence of this atypical DNA suggests a prognosis that is poorer than a sample with normal DNA [6, 8, 11, 13, 14, 16, 24, 39, 41, 43, 52, 59, 60, 66, 70]. The aneuploid characterization of these benign tumors should at least require a closer, independent pathological examination. In Figure 4, the yellow regions show the aneuploidy, while the red regions are diploid. Without high-resolution DNA flow cytometry, the hypodiploid portions (a poor prognosis) are difficult to see as shown in Figure 4c. Usually, the hypodiploid DNA histograms also show

hyperdiploid populations, which would identify them as multiploid (still a poor prognosis) (Figure 4d). Clinical follow-up on these patients was not done because of the low sampling size.

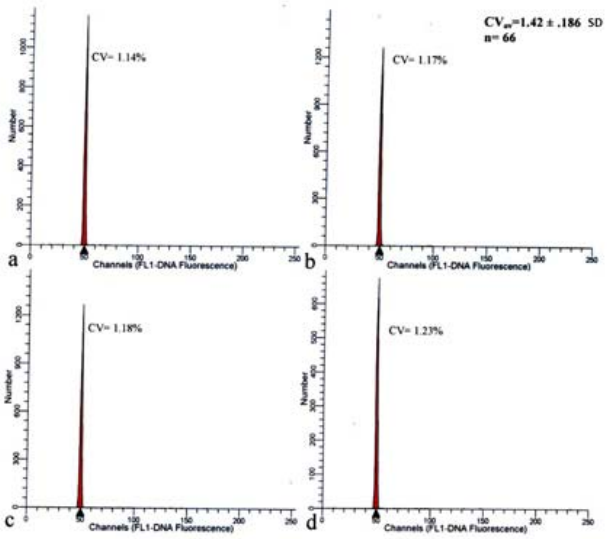

Figure 1. Typical DNA Standard Trout Red Blood Cell (TRBC) Values. CV = 1.42 ± 0.19 SD, n=66. SD = Standard Deviation

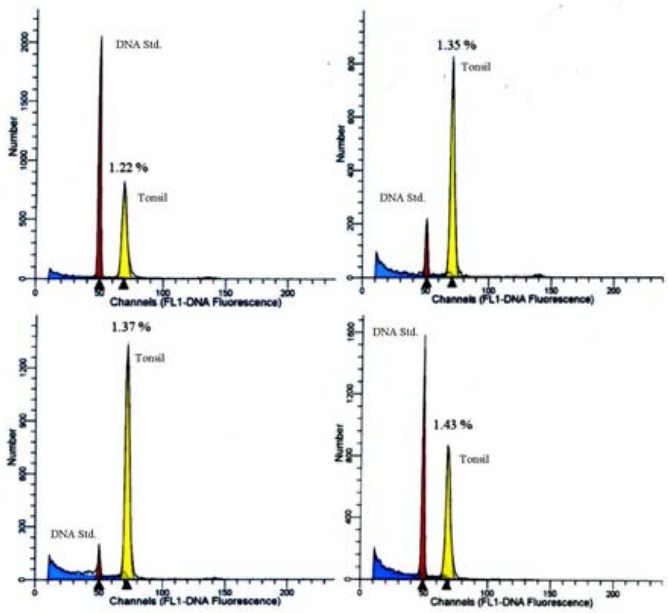

Figure 2. Tonsil with TRBC Standard. Examples. The data for 22 samples resulted in an average coefficient of variation of 2.18 ± 0.46 SD, an average DNA index of 1.42 ± 0.03 SD, and an average S-phase fraction of 4.37 ± 1.37 SD

Figure 5 graphically displays representative DNA histogram types of various ovarian cancers. The debris population (blue) to the left of the diploid population (red) in Figure 5 may be used to show the degree of necrosis of the tumor. This necrotic population showing broken cells may be the result of ovarian cancers being quite large before surgery resulting in the possibility of necrotic regions. No pathological evidence of this necrosis was performed. The DNA standard may be "buried", as shown in Figures 4 and 5, in the DNA debris population (blue). However, an accurate DNA index can be determined from the relative peak DNA concentrations before determining the peak aneuploid/diploid populations. In Figure 5a, the DNA histogram is from a patient with a borderline diagnosis, which correlates with the diploid tumor (7.4 pg DNA/nucleus). Figure 5b is a DNA histogram from a late stage III- IV cancer DNA histogram displaying hypodiploidy

(6.5 pg DNA/nucleus), thus indicating a poor prognosis. Figure 5c shows a slightly hypodiploid population (DI= 0.90), which is from an early stage I/II cancer. Figure 5d reveals a DNA histogram from a patient with a late stage III/IV cancer. Multiple G0/1 cancer populations can be seen. These multiploid cancers (shown in yellow) may be very refractory to treatment due to the each ploidy tumor type having a different response if any to treatment.

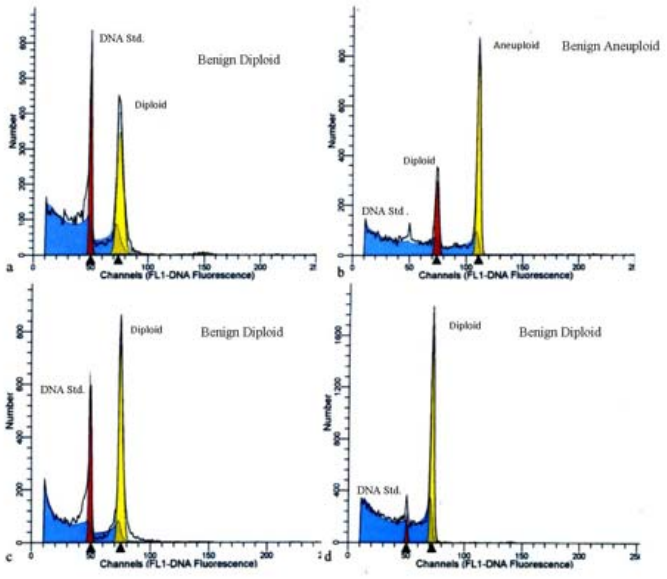

Figure 3. DNA histogram types of pathologically diagnosed benign tumors. (a, c, and d), diploid (typical), (b) hyperdiploid (atypical). Aneuploidy was seen in 23.8% (5/21) of the benign cases

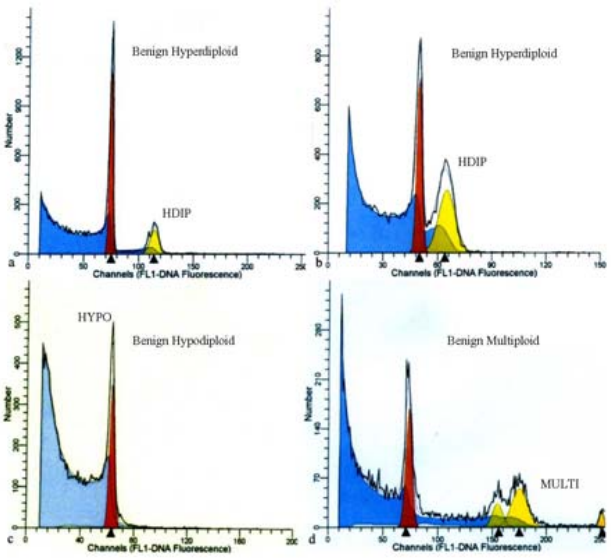

Figure 4. Pathologically defined benign tumors showing non-diploid populations. Aneuploidy was seen in 23.8% (5/21) of the benign cases

The distributions of benign ovarian tumors by DNA histogram type are seen in Figure 6. Most often, those tumors diagnosed as benign were diploid as shown by the examples in Figures 3a,c,d. However, 5 out of 21 of the benign tissues (Figures 3b; 4a, b, c, and d) examined showed aneuploidy. This is an indication that the morphology did not match the normal DNA content of the tumor. In these cases, a more thorough pathological review of the tissue sections with possible treatment may be necessary to decrease the likelihood of local recurrence and metastatic disease.

Figure 7 shows the distribution of DNA histogram types in cancers of all stages. Although most of the samples (46/50) showed an aneuploidy population, four display diploid characteristics typical of what is shown in Figure 6a. These results

correlate with the degree of aggressiveness of ovarian cancer, where there were a relative high percentage of the most aggressive DNA types (hypo, multi, and hypertetraploid).

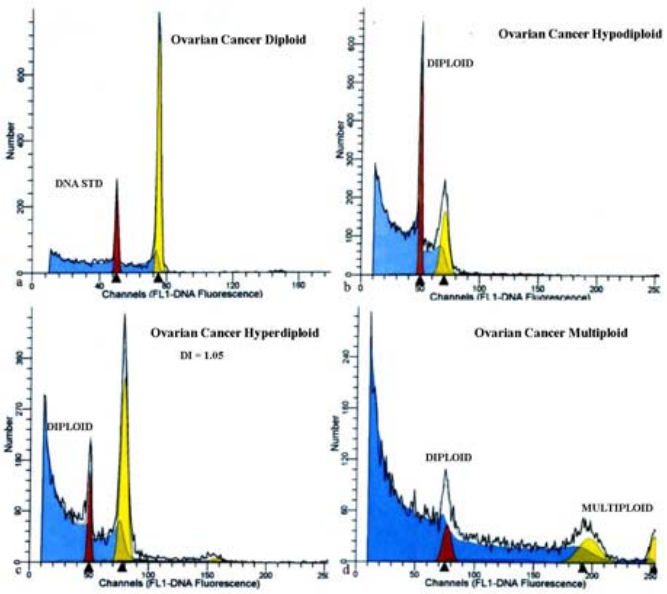

Figure 5. Ovarian Cancer Histogram Types. (a) stage borderline, diploid CV = 1.31. (b) stage III-IV, hypodiploid DI = 0.93 DNA standard run in higher channels to show the hypodiploid population. (c) stage I-II, hyperdiploid DI = 1.05. (d) stage III-IV, multiploid DI = 2.57, 3.29

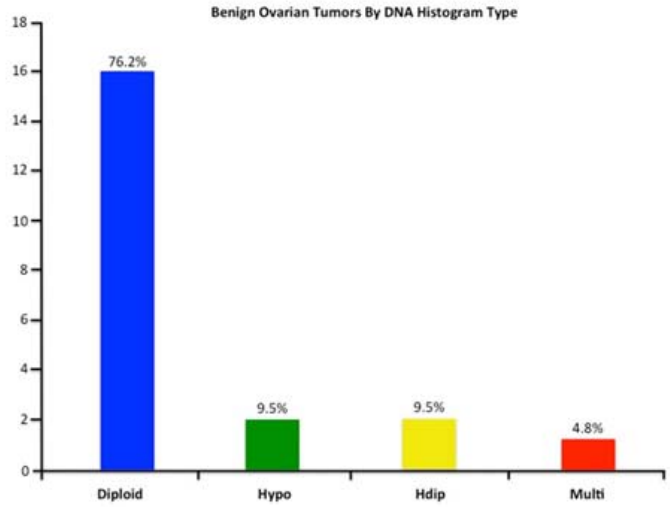

Figure 6. Benign Ovarian Tumors By DNA Histogram Type. Diploid-76.2%. Aneuploid-23.8%

4 Discussion

The direct correlation between DNA ploidy and survival is greatly enhanced using high resolution DNA measurements, that can result in CV = 1%-2% range for TRBCs and 2%-3% for deparaffinized tonsil nuclei. Papers that do not publish their coefficients of variation and do not show representative DNA histograms should be reviewed with caution [39, 40, 43-46]. Furthermore, preparation techniques using formalin-fixed, paraffinated tissues that use a low pH of 2.0 and non-DNA specific staining methods, such as propidium iodide, are less than ideal in performing the proper sample preparation [47].

The sample preparation in this study utilized near physiological pH and the DAPI DNA specific stain. The lack of proper resolution of the DNA measurement will result in near hyperdiploid and hypodiploid cancers appearing as diploid, a generally acceptable good prognostic cancer [39]. Some studies show very high percentages of diploid ovarian cancer patients such as 86% (60), compared to only 8% diploidy (Figure 7) seen in this study. The aggressiveness of ovarian cancer more closely correlates to the high percentage of 64% of the aggressive DNA ploidy types and low percentage of diploidy (8%) for the 50 ovarian cancer patients in this study. Therefore, DNA ploidy using internal standards and objective, machine-based determinations, is complementary to the predicative value of pathology in judging one the most important diagnoses in medicine.

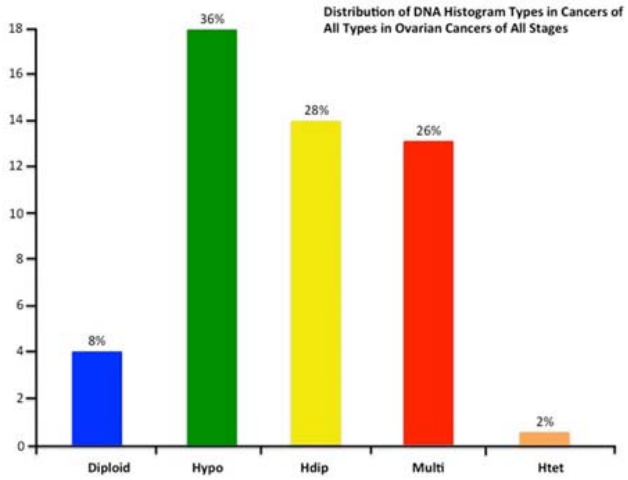

Figure 7. Distribution of DNA Histogram Types in Cancers of All Types in Ovarian Cancers of All Stages. The most aggressive aneuploidy DNA histogram types, hypodiploid (HYPO), multiploid (MULTI), hyper tetraploid (HTET) comprising the 92% of the aneuploidy

Borderline epithelial ovarian tumors pose a significant problem to surgical pathologists as they may morphologically show very similar features to non-invasive malignant epithelial tumors. However it is important to separate these from their invasive counterparts because of their superior prognosis. Lodhi et al. [52] focused on the prognostic value of flow cytometric analysis of DNA ploidy in borderline epithelial ovarian tumors. This data study suggest that aneuploidy if ever demonstrated in histologically confirmed borderline tumors should prompt more sampling of the tumor and a close follow up. Similar results have been seen in by other studies [53-56]. Lai et al. [55] found that reproducible DNA aneuploidy of high DI may be predicting a poor outcome in 50 borderline ovarian cases. Only stage, DNA ploidy, progesterone, and CA 125 were found to be of significant value to predict relapse. Multivariate analysis identified DNA ploidy as an independent prognostic variable for both relapse and survival [56]. Figure 5a shows the borderline pathology assessment correlated with a diploid DNA histogram type.

Vergote et al. [57] studied 290 patients with stage I epithelial invasive ovarian carcinoma. Multivariate analysis identified degree of differentiation as the most powerful prognostic indicator of disease-free survival followed by deoxyribonucleic acid ploidy and the International Federation of Gynecology and Obstetrics (1986) stage. DNA flow cytometry was described as an important independent prognostic factor in stage I ovarian carcinoma [57].

Khoo et al. [58] conducted a study that supported previous findings that tumor ploidy is an important prognostic indicator in ovarian cancer, showing aneuploidy to be associated with a poorer clinical outcome in stage II disease, regardless of the amount of residual tumor after primary surgery and the degree of cellular differentiation. Valverde et al. [59] in a study of 77 cases of stage I-II ovarian cancer after comprehensive surgical staging showed that flow cytometric DNA quantification was the main independent prognostic factor of relapse and survival in these women. Pfisterer et al. [60] measured DNA content and S-phase fraction, with flow cytometry, of tumor specimens from 162 women with no pretreated surgically

staged FIGO stage I endometrial cancer using clearly defined inclusion criteria. A total of 139(86%) cases were found to be diploid, whereas 23 (14%) were aneuploid. The results suggested that abnormalities of the nuclear DNA content and S-phase fraction in this homogenous group of patients are associated with clinical and morphological prognosticators.

Lage et al. [61] using multivariate analysis has shown DNA aneuploidy remains significantly associated with cancer stage and grade, both known predictors of survival in ovarian cancer. In their study of 37 patients with stage III and IV ovarian carcinomas, patients with aneuploidy tumors had significantly shorter survival rates than did those with diploid tumors. Patients whose tumors showed a high percentage of aneuploidy or a high proliferation pool (S-phase and G2/M cell proportion) seemed to die earlier [62]. Friedlander et al. [63] showed in 91 patients with advanced ovarian cancer 69% of the tumors were aneuploid and 31% were diploid. Numerous other studies have shown the importance of DNA ploidy in the prognosis of epithelial ovarian carcinoma [3, 54, 64]. Osanagaoglu et al. [65] reported that DNA ploidy is a significant factor in univariate analysis, correlates with tumor recurrence and could be a useful factor in prognosis.

DNA-ploidy has been shown to be as powerful measurement for predicting clinical outcome in advanced ovarian cancer. Kimmig et al. [3] recommended that DNA-ploidy should be introduced to currently recurring phase III studies for therapy of ovarian cancer for better definition of prognostic subgroups. Skirnisdottir et al. [66] showed aneuploidy of ovarian tumors were strongly associated with tumor grade. There is also a strong association between p53 expression of the tumors and DNA aneuploidy (DNA index >1.10 and S-phase fraction >11.5%). However, they concluded tumor grade remains the most important prognostic factor with regard to the risk of tumor recurrence and the cancer-specific survival rate in early stage ovarian carcinoma [66]. In a study of 26 epithelial ovarian cancer patients, Ozalp et al. [67] concluded DNA ploidy and DNA indices are important prognosticators for malignant epithelial ovarian tumors. Kimball et al. [68] examined a group of thirty-five women with stage III or IV epithelial ovarian cancer. They found diploidy of the primary tumor is a positive predictor of long-term survival. Interestingly, they also showed that that a high proportion of tumor deposits found in metastatic lymph nodes are diploid with a low S-phase fraction. Therapeutic pelvic and aortic lymph node dissection removes disease that, on the basis of flow cytometric characteristics, may be predicted to be resistant to chemotherapy and radiation therapy [68]. Roush et al. [69] examined 18 granulose cell tumors of the ovary, and followed the patients for an average of 10 years. They found that aneuploidy in granulose cell tumors is associated with other adverse histopathology parameters and shows an apparent trend toward aggressive behavior. Gajewski et al. [13] examined 87 patients, and found with Cox proportional/hazards analysis that DNA content is an independent prognostic factor for survival in epithelial ovarian cancer. Yoon et al. [70] performed a prospective analysis on 46 ovarian tumor patients. Their data suggest that the assessment of the DNA ploidy, S-phase fraction and the expression of cyclin A may provide important information for predicting the prognosis of epithelial ovarian cancer. Khoo et al. [16] surveyed 133 patients with ovarian adenocarcinoma. They showed that although stage and cellular differentiation were found to be significant associations with survival and relapse, multivariate analyses identified only residual disease and ploidy status (and the related DNA index and percentage of aneuploidy cells) as independent prognostic variables. The magnitude of the effect of ploidy depended on the amount of residual disease. Among patients with less disease (< 2 cm), the mortality rate was nearly fourfold higher for those with aneuploidy tumors than for those with diploid tumors. They concluded ploidy determination in ovarian cancer was an important prognostic indicator, especially for a subgroup of patients with minimal residual disease.

There are a number of studies which support the role of proliferation rate in prognosis of epithelial ovarian cancer [9, 12, 15, 17-19, 21-24, 37, 38, 72]. The determination of S-phase in an aneuploidy population is difficult because of diploid population overlap. This is illustrated in comparing the diploid S-phase of 5a compared to the aneuploidy population in figure 5c. These cases illustrate the importance of performing high resolution DNA flow cytometry with the TRBC internal marker. Figure 5c could easily be interpreted as diploid with a slightly elevated S-phase, when almost the entire population is aneuploid.

In conclusion, we have shown from 50 ovarian cancers and 21 benign patients that formalin-fixed, paraffin-embedded samples can accurately assess the DNA content after deparaffination, enzymatic release of the cells/nuclei with subsequent

nuclear isolation and simultaneous staining with the DNA specific dye, DAPI, and high resolution flow cytometric analysis. These data suggest that DNA flow cytometry could be used as a companion to pathologic analysis of benign and malignant ovarian tissues to determine the degree of aneuploidy. There is a very high percentage of aneuploidy (92.0%), especially for the DNA histogram types, such as hypodiploid, multiploid and hypertetraploid (64.0%), which have shown poor prognosis for a variety of cancers. The existence of a high percentage of the poor prognostic DNA histogram types suggests that the ovarian cancers may have been allowed to mutate over a long time. The "silent killer" designation for ovarian cancer comes from the fact that minimal pain centers exist allowing a relatively large unencumbered volume to grow and the reluctance in some areas of the medical community to provide ultrasound as part of a woman's routine physical exam.

Usually, after aggressive surgical, radiation and chemotherapy for late stage cancers, there is little that can be done except to wait for recurrence. We believe there is a place for DNA flow cytometry in ovarian tumor analysis. Furthermore, we advocate an aggressive approach should be taken for patients that will most surely have recurrence, as is true in the majority of cases. Clinical trials should be performed to study the preventative effects of using chemically defined, natural products, hopefully to offset the poor prognosis seen in ovarian cancer [73, 74].

Acknowledgements

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