

Past, present and future of flow cytometry in breast cancer – a systematic review

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ABSTRACT

Breast cancer is the most common malignancy in women worldwide. In this systematic review 28 studies were taken into account, in order to evaluate the role of DNA content and cell cycle phases, measured by flow cytometry in breast cancer. Presence of aneuploidy and S-phase fraction have been extensively studied as a prognostication tool. With the current dawn of the age of intraoperative flow cytometry the present systematic review provide an insight of the current role of flow cytometry in breast cancer and future horizons.

INTRODUCTION

Breast cancer is the most common type of cancer among women according to the World Health Organization (WHO) and affects about 2.1 million women each year [1]. Early detection and screening is of key importance, in order to improve breast cancer outcomes and survival [1,2]. Breast cancer is divided into several subtypes and can either be invasive or non-invasive [Table 1]. In breast cancer diagnosis the next important step is staging for treatment options and prognostic information. In the present study, we performed a systematic review on the value of flow cytometry, presence of aneuploidy and cell cycle fractions, in breast cancer.

FLOW CYTOMETRY

Flow cytometry provides simple, fast and accurate data collection, from a heterogeneous fluid mixture that contains cells or cell particles. Quantification of nuclear DNA content by flow cytometry provides information on ploidy status, DNA Index and % S phase fraction [3,4]. Fresh cells, frozen specimens, ethanol- or formalin-fixed cells, and formalin-fixed,

paraffin-embedded tissues can all be examined for these variables [5-7]. Assessment of S-phase fraction has been proved to be a very useful tool for defying high-risk groups of patients in breast cancer [8]. According to another study, in which they focused on the relationship between Chromosomal Instability (CIN) and DNA ploidy in 46 patients with invasive breast carcinoma, DNA ploidy is likely to be determined during the early stages of carcinogenesis [9]. CIN is among the main reason of aneuploidy, an abnormal chromosome number in cancer cells [6]. Generally, the aneuploid chromosome set differs from wild type by only one or a small number of chromosomes [10]. Aneuploidy has been suggested as a cause more than a century and is characterized as the main driver of cancer progression [6]. Flow cytometry can readily identify DNA ploidy. Aneuploidy has been associated with poor prognosis [11]. Fernö et al. proposed to categorize the ploidy of breast cancer cell populations based on DNA Index (DI) distribution as hypodiploid (DI < 0.95), diploid (DI = 0.95–1.04), near-hyperdiploid (DI = 1.05–1.14), hyperdiploid (DI = 1.15–1.91), tetraploid (DI = 1.92–2.04), hypertetraploid (DI ≥ 2.05), and multiploid [2].

Table 1 Types of breast cancer

Noninvasive	Invasive
Ductal carcinoma in situ (DCIS)	Invasive Ductal carcinoma (IDC)
Lobular carcinoma in situ (LCIS)	Invasive Lobular carcinoma (ILC)
	Medullary carcinoma
	Mucinous carcinoma
	Tubular carcinoma
	Papillary carcinoma
	Inflammatory breast cancer

S-phase fraction (SPF) when combined with mitotic activity, had the same prognostic impact as the lymph node status in breast cancer [12]. This type of cancer is heterogeneous and clinicopathological features which are currently used for prognostication purpose may fail to predicting the behavior of the tumor in each individual case [13,14]. Thus, investigation for novel prognostic markers is of paramount importance. Current prognostic factors are age, tumor size, histological grade, histopathological type, lymph node status, and mitotic index [12]. Also, the status of estrogen (ER) and progesterone (PgR) receptors, epidermal growth factor receptor status, c-erbB-2 oncogene expression, expression of Ki-67 and other predictors of disease progression have been included in the list [15]. The status of axillary lymph nodes is generally recognized as the most powerful prognostic factor in invasive breast carcinoma [16-20]. The presence of internal mammary node metastases also appears to be of great importance in forming the prognosis [16]. In the present study, we set out to investigate the role of flow cytometry in breast cancer.

MATERIAL AND METHODS

The present systematic review has adopted the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [21]. Eligible studies that provided data on Flow cytometry and breast cancer were identified by searching MEDLINE. The following combination of search strings was used in the database search: "breast cancer", "flow cytometry" and "DNA content". No language or other restrictions were imposed. Last literature search was conducted on 1/3/19. Reference lists of all articles that met the inclusion criteria and of relevant review articles were examined to identify studies that may have been missed by the initial database search. All retrieved studies and

reference lists were scanned independently by two reviewers (GA and MM).

RESULTS

MEDLINE database search yielded 837 studies. After excluding 2 duplicate studies, the remaining studies were screened for eligibility criteria. After retrieving the full-text version of 50 potentially eligible studies, 22 studies were excluded for not providing data on either diagnostic value or tumor grading. Twenty-eight studies were included [see Table 2 at the end of articles].

The prognostic role of DNA aneuploidy in breast cancer

In favor

In a multivariate analysis, DNA ploidy was significantly associated with long term survival, with 58% of 393 patients having aneuploid tumors. Interestingly in a subgroup of patients with grade 2 tumors (n=195), aneuploidy (n=111) as compared to diploidy (n=84), was an indicator of worse prognosis for Disease-Free Survival (DFS, p=0.011) and Disease-Specific Survival (DSS, p=0.045) [22]. In a study of 584 patients, patients with hypoploid tumors (5.5%), had a 23±8% survival rate at 5 years and no patients were alive at 10 years, compared to the group of patients with diploid and near-diploid tumors with 98±1% survival rate at 5 years and 98±1% after 10 years of follow up. This suggests that DNA hypoploidy (DI<0.95) was a strong, independent prognostic factor of worse survival in short-term clinical outcome in multivariate analysis [8]. Another study, compared a Population Screening (PS) group of 70 patients with Invasive Ductal Carcinoma (IDC) with a 33.3% rate of aneuploidy, to a Hospital group of 225 patients diagnosed at the same period. DNA ploidy was found significant for prognosis in both groups (p=0.016, p=0.015 respectively). The DNA index added prognostic value to Mitotic Activity

Index (MAI) for small tumors and tumors with small nuclei, because a diploid pattern in these cases was correlated with a 95% 10-year survival rate [23]. The prognostic significance of FC DNA analysis in node-negative breast cancer patients was the main interest in another study. Among 155 patients, 41% had aneuploid tumors, and had significantly shorter relapse-free ($p=0.0001$), as 36% of those relapsed, and shorter overall survival (OS) ($p=0.0001$), than those with diploid tumors. Crude survival was also significantly lower for the group of patients with aneuploid tumors ($p<0.03$). Of those with IDC (74%), 41% had aneuploid tumors. These patients with aneuploid tumors and IDC had significantly shorter OS than patients with IDC but diploid tumors, ($p<0.002$). The multivariate analysis showed ploidy status to be the only significant variable in predicting relapse-free survival ($p=0.02$), and also the most significant factor in predicting overall survival, ($p=0.02$) [18].

As for survival, it was found in another study with 565 primary breast cancers from patients treated in the period 1975-1984, that OS was lower for the group of patients with aneuploid tumors ($p=0.4$). When tumors with a low portion of aneuploidy ($DI<1.4$) and diploid tumors were combined into one group, the difference in OS and Distant Relapse Free Survival (DRFS) between them and the remaining aneuploid group was increased ($p=0.006$, $p=0.003$). In multivariate analysis aneuploidy correlated significantly only with OS ($p=0.02$) [19]. At the end of a study 42% of patients with diploid tumors had distant metastasis compared to 72% of the aneuploid ones, with a high statistical significance ($p<0.001$). Also, one-third of the patients with diploid cancers died of the disease, compared to two-thirds of the patients with aneuploid cancers ($p<0.001$).

With a follow up of 11.5 years, the DNA aneuploidy of the tumor showed a significant association with decreased survival, as 65% of

patients with aneuploid tumors had died from breast cancer during the follow-up, in comparison with 33% of those with diploid tumors, ($p<0.00$) [24]. FC provides additional indirect information on aggressiveness associated with DNA ploidy. Aneuploid tumors in this study had a rate of 47%. This study suggests that tumors with a Ki-67 labeling index of 50% or above are highly proliferative or aneuploid, which means they carry a bad prognosis. Those with lower values require investigation, since aneuploid tumors with a low SPF may also have low Ki-67 indexes. This suggests that the Ki-67 labeling index just reflects the proportion of cells in S-phase, whereas DNA aneuploidy reflects something else in addition, closely associated with a bad prognosis [7].

Aneuploidy was significantly associated with tumors of lacking hormone receptor activity (estrogen receptors and progesterone receptors) [22]. In a study of 807 patients by Stahl et al., 73% of the non-diploid tumors (60%) were ER-positive compared to the 86% of the diploid tumors ($p<0.001$). DNA ploidy was also significantly correlated with tumor size in this particular study. While more than half of the tumors with a diameter <11 mm were DNA diploid, more than 70% of those larger than 20 mm were non-diploid ($p<0.001$) [25].

A higher rate of aneuploid tumors was also found in the ER- group, compared with that in the ER+ group, and this difference appeared to be pronounced in patients with negative lymph nodes (N0) than in those with positive lymph nodes (N+), ($p<0.05$). ER status had a significant effect on OS, but not on Crude Survival (CS) [19]. In another study, focused on the relationship of DNA ploidy level to histology and ER receptor among 155 patients, it was shown that tumors with lower DNA ploidy, tended to be of low grade and ER+ and exhibited a better prognosis ($p=0.01$). Those with higher DNA ploidy were more likely to be of higher grade, more

anaplastic and ER- [26]. No significant correlation between ploidy and receptor content was found in a study, although there was a slight tendency for diploid tumors to be receptor-positive, (69% vs. 58%) [27]. In node negative patients, aneuploidy was significantly correlated with an unfavorable prognosis for DFS [22].

Patients with a high degree of axillary node involvement (10 or more positive nodes), showed a higher incidence of aneuploidy than patients with lower or zero nodes involved ($p < 0.05$). For the group of patients with 3 or more positive nodes (N+), both DRFS and OS were significantly better for patients with diploid tumors [19]. A significant association was also observed between node-negative tumors and DNA diploidy, compared with DNA aneuploidy ($p = 0.003$) and between node-positive tumors and DNA hyper-tetraploidy ($p = 0.002$) [28].

A study of 807 patients showed that an increasing number of positive lymph nodes correlated with DNA aneuploidy ($p < 0.01$) [25]. Dressler et al., seem to agree, because in their study, it became clear that node-negative tumors were less likely to be aneuploid (49%) vs. node-positive tumors (57%) ($p = 0.04$) [29]. Eskelinen et al. found that 50 % of patients with aneuploid tumors had lymph node involvement, compared to 33.3% of diploid ones ($p < 0.05$) [24]. In a study, it was shown that aneuploidy was significantly associated with tumors of greater size ($p = 0.018$) and a higher grade of differentiation ($p < 0.001$) [22]. Aneuploidy which was the 56.6% of all cases in another study, was significantly associated with low-grade carcinoma ($p < 0.001$). Also, there was an increasing aneuploidy rate among tumors with a short Doubling Time (DT), ($p = 0.009$). Of 11 tumors growing extremely slowly (indefinite DT), 27% were aneuploid [30]. However, Ottesen et al. studied four groups of patients with a DNA aneuploidy rate of 49-90% and observed a relationship between histological grade and ploidy, as tumors with high

histological grade associated with DNA diploidy ($p = 0.002$) and DNA hyperdiploidy ($p = 0.003$). An inverse association was found with DNA hyper-tetraploidy ($p < 0.0001$) [28]. Keyhani-Rofagha et al. also reported that tumors with an aneuploid pattern are more frequently of high histological grade [31]. DNA ploidy measured by FC can be used to predict the aggressiveness of the tumor and patients' survival. Premenopausal patients, had about the same number of diploid and aneuploid tumors, but more than twice as many of the postmenopausal patients had aneuploid tumors than had diploid ones [24]. Ploidy was an additional, independent prognostic factor in postmenopausal patients. Aneuploidy was associated with a significantly lower OS in postmenopausal but not in premenopausal patients. In a study of 114 patients, an association was found between ploidy and age, as significant differences were noted between mean ages for tetraploid compared to all other aneuploid tumors and for multiploid compared to all other tumors. Multiploidy might associate with the menopause [27].

Against

Ploidy status was not an independent prognostic factor in a study of 1831 breast samples in the multivariate analysis, although it reached statistical significance in the univariate analysis, as patients with near-hyperdiploid and diploid tumors had a somewhat similar prognosis, which was a good one. Patients with hypodiploid tumors had a tendency toward poorer prognosis than those with tetraploid, hyperdiploid, hypertetraploid or multiploid [3]. In a study by Bergers et al., among 932 breast cancer patients, DNA ploidy correlated significantly with Mitotic Activity Index, Mean Nuclear Area, steroid receptor status and tumor type, as Medullary and Ductal tumor types were more often DNA non-diploid. No significant correlation was shown with tumor size, lymph node

status, age, and hormonal status. This study suggests that DNA ploidy and DI, as markers of genetic instability, mainly correlate with differentiation and proliferation markers but correlate less with lymph node status as a marker of metastatic potential [4]. In another study of 158 patients, 56.6% of them had aneuploid tumors. Doubling Time (DT) and DNA ploidy correlated well with each other but did not have a correlation at all with axillary node metastasis, or periglandular growth [30].

Taylor et al. showed that there was no significant correlation between ploidy and histological type, tumor size, lymph node involvement or steroid receptor status, in a study of 114 patients, with a 79% of aneuploid tumors [27]. Ploidy had no significant relationship between ER status and DNA content. Also, ploidy by itself yields no significant prognostic information regardless of age, in node-negative breast carcinoma [31].

Noguchi et al. studied the lymph node metastasis versus DNA ploidy as prognostic factors for IDC, among 121 patients, of which 60% had aneuploid tumors. They suggested that DNA ploidy was not an independent prognostic factor in small number of patients [16]. Ploidy status did not predict DFS or OS, maybe because of the small number of patients in their study. All tumors were axillary node-negative, and 56% were aneuploid [15]. DNA ploidy was also not a strong prognostic factor for survival, as there were no statistical difference in survival among breast cancer patients with diploid, or aneuploid tumors after a mean follow up of 4.1 years, in 122 patients [32].

DNA ploidy by itself was not a significant prognostic factor in another study, although all patients with multiploid and hypertetraploid tumors had a recurrence. Ploidy status was correlated significantly with tumor size, histological grade, nuclear grade and mitotic grade [17].

The role of SPF in breast cancer, as a significant prognostic factor

In favor

In a study of 1985 patients, SPF was a prominent prognostic factor, even after multivariate analysis. SPF, when combined with mitotic activity, had the same prognostic impact as the lymph node status, as both of them correlated with every type of survival [12]. A study of 211 premenopausal node-negative breast cancer patients found that S+G2/M phase fraction was the only predictor of OS in the univariate analysis. Patients with S+G2/M greater than 9.3% had shorter survival than patients with an S+G2/M equal or less than 9.3%, ($p=0.039$), suggesting that S+G2/M in premenopausal node-negative carcinoma could be an additional valuable prognostic factor to classify high-risk patients needing adjuvant chemotherapy [33]. In a study amongst 327 breast cancers, SPF had been calculated in 245 of cases in univariate analysis and ranged from 1.0% to 35% (median=5%). Cancers with SPF larger than the median (8.3%) were associated with 65% 5-year survival rate, compared with 86% in those with SPF below or equal to 5%, ($p=0.0002$) [34].

In a study of 393 patients with IDC, it was found that the SPF had a range of 1.0-27.8 % (median 6.9 %), and was significantly higher ($p<0.001$) in aneuploid (median 10.8 %; range 3.7–27.8 %) than in diploid tumors (median 4.3 %; range 1.0–12.0 %). Higher SPF values were correlated with advanced disease stage. High SPF exhibited only statistical significance for DSS, but this parameter did not reach statistical significance in the Kaplan-Meier survival, neither in the univariate Cox Analysis [22].

The fraction and percentage of SPF in another study was higher in the group with patients with hypoploid tumors ($DI<0.95$), which was the group characterized by the worst prognosis with no patients alive after a 10-year follow up.

SPF retained statistical significance in the univariate analysis, however not in the multivariate one [8]. Eskelinen et al. seem to agree that SPF has a prognostic value, as in their study of 117 patients, SPF values greater than 7% were associated more closely with distant metastases or death [24]. SPF correlated significantly with tumor size, histologic grade, nuclear grade, and mitotic rate. SPF was related significantly to the recurrence of disease. However, in the multivariate analysis peritumoral lymphovascular invasion was the most important variable [17]. In a study among 158 patients according to the chi-square test, there was a significant correlation between SPF and pathologic stage of the disease and SPF and tumor size. SPF higher than 7.5% was correlated weakly to axillary lymph node metastasis ($p=0.046$) but correlated strongly with low histologic grade ($p=0.001$) and short DT ($p=0.02$). Also, a highly significant association was observed between SPF and ploidy ($p<0.001$), as 23% of the tumors with SPF, less than 7.5% were aneuploid, compared to 74% of the tumors with higher proliferation rates [30].

In a study of 807 frozen breast cancer samples, SPF was the only independent factor that was significantly related to nodal status. After a multiple regression analysis it became clear that DNA ploidy, ER status, PR status, lymph node status and tumor size, were all independently related to SPF [25]. According to a study with four groups of patients by Ottesen et al., DNA aneuploid tumors had a median SPF of 11%, compared to 5% for diploid tumors. Testing for difference among DNA diploid and DNA aneuploid SPF showed a significantly higher value ($p<0.0001$), for the latter. Also, there was a statistical difference between DNA aneuploid SPF in small clinical cancers and DNA aneuploid SPF in screening cancers, 10% and 4% respectively, ($p<0.0001$) [28]. Significantly lower SPF values in diploid tumors (median=2.6%), as compared to aneuploid tumors (median=10.3%, $p<0.0001$)

were also observed in another study. Receptor-negative tumors had the highest median SPF value (median=12.7%), and receptor-positive tumors had the lowest median SPF value (median=4.6%, $p<0.0001$). Tumors with ER+/PgR- had intermediate values. When they examined further the relationship between SPF values and receptor status in the two ploidy groups separately, it was again clear that receptor-negative tumors had more often high SPF values, and that the difference was especially in the aneuploid group. Significantly higher SPF values were observed in younger and premenopausal patients, and when these groups were divided by ploidy status, greater SPF differences were found within the aneuploid tumors. When patients were examined by nodal status, node-positive patients with diploid tumors were more likely to have a high SPF tumor, than node-negative patients with diploid tumors, whereas in aneuploid tumors, high SPF was frequent independent of nodal status.

In node-negative patients exclusively, among diploid tumors, there was no a difference in the SPF values in ER- compared to ER+ tumors, but a highly significant difference in aneuploid tumors. For PgR they observed that in both diploid and aneuploid tumors, PgR- tumors were more likely to have a high SPF value. When the node-negative patients were subgrouped according to age or menopausal status, it was only within the aneuploid group that a significantly higher frequency of high S-phase was found in the younger or premenopausal patients [29]. Association of a low (< 10%) S phase with 81% of all diploid and near-diploid tumors compared to only 22% of single aneuploid and tetraploid tumors were highly significant in another study [27].

In a study of 50 patients, patients with grade 3 tumors had significantly higher SPF results in comparison to patients with grade 1 or grade 2 tumors. Also, patients with grade 3 tumors

with a high SPF (equal or less than 15%) were almost more likely to relapse compared to the rest of the group [15]. In a study of 1831 samples of breast cancer, SPF values showed a significant positive correlation with the number of lymph nodes involved ($p < 0.001$), tumors size (< 0.001), DNA ploidy ($p < 0.001$), cathepsin D content ($p = 0.003$), erbB2 ($p < 0.001$) and c-myc amplification ($p = 0.004$). SPF also had a significant negative association with age ($p < 0.001$), ER- ($p < 0.001$) and PgR content ($p < 0.001$). SPF values had no significant correlation with int2 amplification, however when diploid and near-hyperdiploid samples were examined on their own, a significant positive correlation was found ($p = 0.05$). SPF greater than 12% was associated with the lowest rate of recurrence-free survival. SPF remained an independent prognostic factor even in the multivariate analysis [3].

Against

SPF of the tumor was not a significant prognostic factor because it didn't associate with survival in a study among 122 patients. However, the follow-up time was limited in this study [32]. In a study of 58 patients with invasive breast cancer, no significant correlation was found between the number of stemlines and intra-tumor variability and SPF [35]. In a study of 106 women who underwent treatment for invasive breast cancer, neither SPF nor DNA index proved to be statistically significant in determining axillary node status. Also, neither SPF nor the DNA index could predict the presence of distant metastasis [36].

Of all the studies that have been taken into account, in 13 of them fresh/frozen samples were used in order to determine the ploidy and SPF fraction of the tumors. Also, paraffin-embedded tissues were used in 11 of the studies, whereas 4 of the total studies used both fresh/frozen and paraffin-embedded specimens and in 2 of the total studies the sample's kind was not

mentioned. According to a study by Bergers et al. measurement of DNA ploidy, DNA Index and SPF may be more reliable in paraffin wax sections because the thick slices of specimens provide a more representative sample [35]. Although S-phase measurements were not obtainable in a number of tumors ranging from around 5% from fresh specimens to up to 25-40% in the case of paraffin-embedded ones [37-40]. In a study by Alanen et al., it is concluded that all three types of samples (fresh, ethanol-preserved, formalin-fixed and paraffin-embedded samples) are suitable for the determination of DNA ploidy, DI, and S-phase fraction, although uninterpretable histograms were most often obtained from fresh samples [41]. According to another study by Chen et al., there was 89% agreement in the detection of DNA aneuploidy by flow cytometry in fresh and paraffin-embedded, formalin-fixed tissue; the coefficients of variation of the DNA diploid G0/G1 peaks were much wider in the latter [42].

Intraoperative flow cytometry

During the last few years two research groups, one from Tokyo, Japan and the other from Ioannina, Greece working independently have investigated the possible role of flow cytometry for intraoperative usage in brain tumor surgery [43-45]. Shioyama et al. developed a flow cytometry protocol that could evaluate the tumor DNA content within 10 minutes. The authors calculated the malignant index (MI) of the analyzed cells and used thereafter in all analyses [45]. Researchers from Ioannina, developed a quite similar protocol for rapid cell cycle analysis, named Ioannina Protocol. Based on cell cycle fractions, namely G0/G1, S and G2/M phase fraction, brain tumors could be categorized intraoperatively in low and high-grade both in adults and children, glioma margins could be identified and primary central nervous system lymphoma could be identified within 5 minutes

[43,44,46,47]. Cell cycle analysis by propidium-iodine staining of CD56+ (gated) cells could assess the malignancy of pediatric brain tumors [48]. Furthermore, quantification of CD56 expression in pediatric brain tumors can be an indicator of tumor's grade and aggressiveness [49]. In patients with head and neck lesions intraoperative flow cytometry allowed the identification of neoplastic lesions within 6 minutes with high sensitivity and specificity and when surgical margins were assessed a complete concordance with pathology was reported [50]. Promising results have been reported for other solid masses as breast cancer [51]. Intraoperative flow cytometry provides new horizons during surgical resection of solid tumors in general and could be a novel adjunct to pathology.

CONCLUSION

Breast cancer is the most common cancer and also the primary cause of mortality due to cancer in female around the world. A major part of the literature has been dedicated to defining the long-term outcome of patients, suffering from this type of carcinoma.

Flow cytometry analysis of the DNA pattern of the carcinoma does correlate with well established prognostic factors and has a lot to offer in shaping the prognosis of patients, according to the literature. Flow cytometry analysis provides information as regards to the ploidy of cancer and the percentage of cells in the S-phase, with the last one being a hallmark of cancer. According to many studies, aneuploidy appears to be in a significant relationship with long-term prognosis. Also, aneuploidy correlated significantly with the presence of distant metastases and decreased survival. Intraoperative flow cytometry is a promising novel application and is expected to have a significant impact in breast cancer surgery.

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Abbreviations

N/A: not available

S/A: significantly associated

A: associated

S/R: significantly related

N/S: not significant

IDC: invasive or infiltrating ductal carcinoma

DCIS: ductal carcinoma in situ

ILC: invasive or infiltrating lobular carcinoma

IMC: invasive medullary carcinoma

DFS: disease-free survival

DSS: disease-specific survival

RFS: recurrence-free survival

OS: overall survival

CIN: chromosomal instability

DNAs: DNA copy number aberrations

PF: prognostic factor

HSPF: highly significant prognostic factor

E/P REC: estrogen/progesterone receptors

UP: unfavorable prognosis

MAI: mitotic activity index

MNA: mean nuclear area

IDH: intratumoral DNA heterogeneity

AXM: axillary lymph-node metastases

*IMM: internal mammary lymph
node metastases*

RR: recurrence rate

DM: distant metastases



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Table 2 Studies included

Study	No of patients	Mean age	Cancer type	Study variable	DNA aneuploidy	SPF	Cut off	Sample type	Prognostic value
Pinto et al (2012)	393	59 (23-88)	IDC	DNA ploidy, SPF	58.8 % (43.0 % hyperdip, 6.1 % tetra, 5.1% hypertetra and 4.6 % multi)	median 6.9 % (1-27.8%)	6.1%	Fresh/ Frozen	DNA ploidy is an independent PF in breast IDC
Kawauchi et al (2010)	46	57.6	Invasive breast cancer, sporadic tumors	DNA ploidy (CIN), DCNAs	67.4% 69.4% (of 36) were CIN+, and 30.6% were CIN-. 23 Aneu/CIN, and 1 Aneu/CIN-.	N/A	N/A	Frozen	DNA ploidy was likely to determine the beginning of carcinogenesis
Chavez-Uribe et al (2007)	584	59 (25-85)	88.1% Ductal type, 9% Lobular type, 2.8% Medullary type, 1% other	DNA ploidy, SPF	<u>GROUP I:</u> (diploid+ near-diploid) with DI =0.96-1.15, <u>GROUP II:</u> (22.9% hyperploid, 8.2% multiploid, 9.4% diploid populations), DI >1.16, <u>GROUP III:</u> 5,5% hypoploid with DI<0.95.	GROUP I =5.5% GROUP II =8.5%, GROUP III =10.1%	N/A	Frozen	DNA hypoploidy (DI<0.95) is an independent PF in long-term prognosis.
Michels et al (2003)	1984	58	81% IDC, 16% ILC, 3% miscellaneous tumors. 50% G1, 50% G2 80% SR+	DNA ploidy, SPF	50% 10.8% multiploid. (aneuploid: 1.8% hypoploid and 5.8% tetraploid), 10.8%	33% <3% 70.3% <4%	Med CV =3.5%.	Frozen	SPF is a HSPF
Martinez-Aribas et al (2002)	181	N/A	152 IDC, 17 ILC, 12 other	DNA ploidy, SPF, Ki67	47%, (DI>1)		Aneuploid +15.8% diploid =9.9%	Fresh and paraffin	FC provides additional indirect info on aggressiveness ass with DNA ploidy.
Schmidt et al (1998)	106	57	IDC majority, 6 ILC, 1 inflam., 1 colloid type. 3 in situ exclude	DNA ploidy, SPF, axillary-node status	56% 56% node-negative (66% of those had elevated SPF) 0.01% G1, 40.2% G2, 59.8% G3	High SPF in 72%	SPF =9% Mean SPF =14.1%	NA	DI is a poor PF SPF N/S in AX node status or DM

Wong et al (1998)	50-46 suitable	50	92% IDC, 2% ILC, 2% tubular, 4% medullary, 33% G1, 22% G2, 39% G3, missing data for 6%	DNA ploidy, SPF, in node-negative	56%	SPF range 0-30%	SPF High >15%	Fresh Frozen	Ploidy N/S with DFS or OS. SPF S/A with relapse
Wyss-Desserich et al (1997)	57	45	39 DC, 12 LC, 6 other	DNA ploidy, DI, SPF, S+(G2+M)-phase fraction	60%		DI =1.2 SPF =3% S+G2+M-PF =9.3%	Paraffin	S+(G2+M)PF in premenopausal node(-) is an additional PF
Bergers et al (1996)	932	60	86% DC, 9% LC, 5% other	DNA ploidy, DI, SPF	62%		SPF =8%, first D =1.8 second DI =2.3	Frozen	DI and DNA ploidy N/S
Bergers, Diest, Baak et al (1996)	58	N/A	Invasive breast cancer	DNA ploidy, DI, SPF	Intra-tumor heterogeneity in 53% of frozen and 38% of paraffin cases.	Fresh: range = 9.5-31.6 and 4.5-67.3% (paraffin range = 0-62.7%.	N/A	Fresh frozen and paraffin	N/S
Ottesen et al (1995)	148, in 4 groups	53 (38-76) 58 (39-85) 62 (39-88) 61 (50-70)	33 IC/ predominance of DCIS, 52 Clinical IC<15mm, 40 Node-negative IC, 41 Screening IC <15 mm	DNA ploidy, DI, IDH, SPF	74% (18 Hypo, 61 Hyper, 13 Triplo, 52 Hypotetra, 8 Tetra, 14 Hypertetra), 3% tetraploid	ANEUPLOID Med SPF =11% (2-31%)	N/A	Frozen	SPF A with Ploidy. Ploidy S/A with grade and node status.
Brotheric et al (1995)	110	59+-0,86	8 DCIS, 37 GII, 35 GII, 29 GIII	c-erbB-2 by ΦΨ+ immunocytochemistry. Relationship c-erbB-2 and ploidy		N/A	+threshold for (FC) c-erbB2 expression =3300-3600 molecules /cell	Frozen	C-erbB2 N/S if assayed by FC.
Ottesen, Christensen et al (1995)	48	/	IC with DCIS. 15 only DCIS, 17 only IC, 16 cases separate samples from DCIS and IC available.	DNA ploidy, cancer type corr in each case	<u>31 cases with DCIS:</u> 10% tetra, 67% aneuploid (1 Hypo, 5 Hyper, 13 Hypotetra, 7Tetra, 5 Hypertetra) <u>33 cases with IC:</u> 6% 82% aneuploid (1 Hypo, 18 Hyper, 2 Triplo, 14 Hyportetra, 3 Tetra, 4 Hypertetra).	Med SPF for DCIS = 8% (2-38%) AND Med IC=11% (2-28%) Aneuploid SPF= 13% (3-38%).	N/A	Frozen	DNA ploidy pattern, as detected by FC is established at the preinvasive stage of carcinogenesis.

Stal et al (1992)	807	50 (40-74)	N/A	DNA ploidy, SPF, nodal status, ER status	60%, 73% receptor negative	mean =8.4% (1-36%)	SPF =5% and 10%	Frozen	SPF is an independent PF
Joensuu et al (1992)	327	62.2	Unilateral invasive	DNA ploidy, SPF	33-49% 8-21% tetraploid, 2-6% multiploid	8.3% (range 1-35%)	SPF =5%	Paraffin	DNA Ploidy and SPF S/A with survival.
Arnerlov et al (1992)	158	65 (42-87)	125 DC, 6 LC, 6 Papillary, 3 Medullary, 12 Mucinous, 7 Tubular	DT, DNA ploidy, SPF	56.6% 6.7% tetraploid	mean SPF =10.7%, med =8.2% 43% patients had <7.5%, and 57% had >7.5%	SPF =7.5% DT = 9 months	Paraffin	Ploidy SA with grade, size and DT, not with AXM. SPF SA stage, size, grade, and ploidy..
Ferno et al (1992)	1831	61+-14	N/A	DNA ploidy, DI	60% (1.8% Hypo, 4.4% near-hyper, 35.8% hyper, 4.9% tetra, 7% hypertetra, 5.7% multi)	3 groups by SPF values (<7.0%; 7.0--11.9%, >12%)	N/A	N/A	SPF S/A with lymph node metastases, age, size, ploidy, E/R REC status, SPF and ploidy N/S in RFS in multivariate analysis.
Bosari et al (1992)	158	N/A	Axillary node-negative breast carcinoma	DNA ploidy, SPF	33% 19% tetra	SPF in 136: mean =7.3. Diploid: 4,6 aneuploid: 11, tetra: 8.1.	SPF =7%	Paraffin	Ploidy and SPF N/S alone. SPF S/R to recurrence.
Collan et al (1992)	116	N/A	N/A	DNA ploidy, DI, SPF	Lab1: 55% Lab2: 62% Lab3: 54%		DI =1.3 Aneu: Lab+Lab3 =>1G0/G1 peak, Lab2 =DI>1.0	Paraffin	DI = more reproducible variable than ploidy.
Noguchi et al (1991)	121	50.5	Invasive ductal carcinoma, 28 Stage I, 63 Stage II, 30 Stage III	DNA ploidy, regional lymph node metastasis	60% aneuploid	N/A	DI =1.0	Paraffin	DNA poidy N/S in small series.
Uyterlinde et al (1991)	PS=70, H=225	50	Invasive ductal	DI, MAI, MPI, MNA,	33.3% 23.3% tetra	N/A		Paraffin	PS group: DI had additional prognostic value to MAI . DNA ploidy S/A both in the H and PS group.
Keyhani-Rofagha et al (1990)	165	58 (27-81)	150 IDC , 6 LC, 2 MC, 6 colloid, 1tubular	DNA ploidy	57% aneuploid, Mean DI =1.3 (0.73-2.59)	N/A	N/A	Paraffin	Ploidy alone N/S in node-negative carcinoma.

Lewis et al (1990)	155	50	74% IDC, 11 LIC, 7 intraductal, 6 papillary, 9 MC, 7 mucinous	DNA ploidy, DI	41% aneuploid. 45% of IDC were aneuploid, 78% of medullary were aneuploid.	N/A	DI =1.0	Paraffin	<p>Aneu SA with grade and size.</p> <p>Ploidy N/S with age.</p> <p>Ploidy SA with relapse and survival.</p> <p>DNA ploidy by FC is an powerful PF in node negative patients.</p>
Eskeli/Nordling et al (1989)	122	NA	92.% IDC	DNA ploidy, DI, SPF	55% (32% Hyperdip, 20% near-tetra, 3% Hyper-tetra), 10% Multi	Higher in aneuploid than in diploid	DI =1.0-1.049 diploid SPF cut off =8.5%	paraffin	<p>Ploidy N/S in survival.</p> <p>SPF N/S in survival.</p>
Eskeli N/Pajarinen et al (1989)	119, 2 excluded	55.7 in diploid, 60.3 in aneupl	N/A	DNA ploidy, DI, SPF	45% (27% Hyperdip, 14% near-tetra, 4% Hyper-tetra), 17% multiploid	In 54 cases, Sing higher in aneu than diploid	DI=1.0-1.049 diploid SPF cut off =4.8% AND 7%	Paraffin	<p>Ploidy S/A with metastasis and survival.</p> <p>SPF S/A with distant metastasis and size.</p> <p>Ploidy can predict aggressiveness and survival.</p>
Dressler et al (1988)	1331	50	N/A	DNA ploidy, DI, SPF	57% [55% Hyperdip, 3.7% Hypodipl, 25% tetra, 7.4% Hypertetra, 8.8% muti]	Med SPF in aneu =10.3%, med SPF in dipl =2.6% (p<0.0001)	DI =1.0 diploid SPF med =5.8%	Frozen	<p>Ploidy and SPF S/A with receptor and nodal status.</p> <p>SPF is an important PF.</p>
Cornelisse et al (1986)	565	57.5 +-14.5	Primary	DNA ploidy, DI	61.6% 9.7% multiploid, 118 p. stage I, 301 p. stage II, 119 p. stage III	N/A		Frozen and Paraffin	<p>Ploidy is an additional PF in postmenopausal patients.</p>
Taylor et al (1983)	114	N/A	103 IDC, 18 LC, 2 Papillary, 2 MC, 1 Colloid, 1 Pagets disease +intraductal, 1infiltr mucoid	DNA ploidy, SPF	79% (12% near diploid 42% single aneuploid, 9% tetraploid, 16% multiploid)		SPF =10%	Frozen	<p>N/S between ploidy and histologic type, tumor size, lymph node involvement or receptor status.</p> <p>Ploidy S/A with SPF.</p> <p>Ploidy A with age.</p>
Olszewski et al (1981)	92	NA	75 DC, 6 MC, 5 LC, 2 colloid, 2 Tubular, 2 papillary	DNA ploidy, ER and PgR status	92%	N/A	N/A	Fresh	<p>Ploidy S/A with grade and ER status.</p>