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Plasma Cell-Free DNA Integrity Assessed by Automated Electrophoresis Predicts the Achievement of Pathologic Complete Response to Neoadjuvant Chemotherapy in Patients With Breast Cancer

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abstract

PURPOSE The study of plasma cell-free DNA integrity (cfDI) has shown potential for providing useful information in neoplastic patients. The aim of this study is to estimate the accuracy of an electrophoresis-based method for cfDI evaluation in the assessment of pathologic complete response (pCR) in patients with breast cancer (BC) undergoing neoadjuvant chemotherapy (NACT).

PATIENTS AND METHODS Fifty-one patients with BC undergoing anthracycline-/taxane-based NACT were recruited. Plasma samples were collected from each patient at diagnosis (t0), after anthracycline administration (t1), and after NACT completion (t2). The concentration of differently sized cell-free DNA fragments was assessed by automated electrophoresis. cfDI, expressed as cfDI index, was calculated as the ratio of 321-1,000 bp sized fragment concentration to 150-220 bp sized fragment concentration assessed at t2. cfDI index was then used to build an exploratory classifier for BC response to NACT, directly comparing its sensitivity and specificity with magnetic resonance imaging (MRI), through bootstrapped logistic regression.

RESULTS cfDI index was assessed on 38 plasma samples collected from as many patients at t2, maintaining a 30/70 ratio between pCR and non-pCR patients. cfDI index showed an area under the receiver operating characteristic curve in predicting the achievement of pCR of 81.6, with a cutoff above 2.71 showing sensitivity = 81.8 and specificity = 81.5. The combination of cfDI index and MRI showed, in case of concordance, an area under the receiver operating characteristic curve of 92.6 with a predictive value of complete response of 87.5 and a predictive value of absence of complete response of 94.7.

CONCLUSION cfDI index measured after NACT completion shows great potential in the assessment of pCR in patients with BC. The evaluation of its use in combination with MRI is strongly warranted in prospective studies.

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ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Breast cancer (BC) represents a worldwide issue, ranking first among malignant neoplasms, involving approximately 13% of women in their lifetime.[1] Neoadjuvant chemotherapy (NACT) represents the standard of care for locally advanced BC, and its use is increasing even in early-stage tumors.[2,3] The best achievement of NACT is pathologic complete response (pCR) most commonly defined by the absence of invasive cancer in breast and in axillary nodes, without considering the possible presence of ductal carcinoma in situ (ypT0 ypN0 or ypT0/is ypN0).[4] The achievement of pCR with NACT is generally associated with an improved survival rate, and pCR is widely regarded as a good surrogate prognostic indicator for disease-free survival in BC management.[5]

To date, the presurgical assessment of clinical complete response (cCR) is mostly on the basis of magnetic resonance imaging (MRI). MRI is useful to guide the surgical management of breast and axillary lymph nodes. In particular, an incomplete axillary response in clinical N+ patients typically requires extensive nodal dissection, whereas clinical N0 patients undergo sentinel lymph node biopsy (SLB).[6] Several studies, however, have reported suboptimal results of MRI in predicting presurgery pCR, and many clinical experts refer to MRI as a useful, but not sufficient, instrument in guiding surgical decisions.[7,8] Given the increasing

CONTEXT

Key Objective

The assessment of the response to neoadjuvant chemotherapy (NACT) in patients affected by breast cancer (BC), currently on the basis of magnetic resonance imaging, presents suboptimal accuracy. In this work, we set to exploratively evaluate the sensitivity and specificity of an electrophoresis-based method for the assessment of cell-free DNA integrity aimed at the prediction of pathologic complete response to NACT in a cohort of patients with BC.

Knowledge Generated

Cell-free DNA integrity assessed at the end of NACT significantly correlates with the achievement of pathologic complete response at surgery.

Relevance

An accurate and noninvasive assessment of the response to NACT would allow the optimization of the surgical and systemic management of patients affected by BC, possibly reducing the rate of unnecessary axillary interventions for patients who undergo a complete response, and envisaging studies aimed at modifying and/or extending chemotherapy schemes for patients with noncomplete response.

rate of long-term survival in patients with BC,[9] the achievement of an efficient protocol for axilla-sparing surgery is particularly desirable to avoid chronic side effects of axillary dissection or SLB in a large section of the population. Moreover, an accurate assessment of cCR may allow the optimization of BC patients' systemic management, possibly personalizing current chemotherapy regimens upon noninvasive indicators of either complete or suboptimal response.

The evaluation of cell-free DNA (cfDNA) obtained through peripheral blood draw seems suitable for these intents and has already shown great potential in providing predictive and prognostic information in BC-affected patients.[10-12] In particular, the integrity of cfDNA (cfDI), a parameter typically used to measure cfDNA fragmentation, seems to be altered in patients affected by cancer. Several studies point out toward the potential role of cfDI as a useful marker for BC recurrence,[13] early diagnosis,[14] and progression,[10] although only few indicate its possible application for the evaluation of the response to NACT in BC. The underlying causes of cfDI alteration in neoplastic patients are still debated, although the release of highly fragmented DNA from apoptotic and/or necrotic tumor cells in the bloodstream is supposed to play a substantial role.[15] However, conflicting findings are reported in the literature, cfDI being reported either as increased or decreased in patients affected by cancer in comparison to healthy controls.[10,16] In this work, we compare the sensitivity and the specificity of an electrophoresis-based method for cfDI assessment and MRI in predicting the achievement of post-NACT pCR in a cohort of 38 BC-affected patients. cfDI, expressed as cfDI index, was assessed after cfDNA fragmentation profiling (DFP) performed by automated electrophoresis on cfDNA extracted from plasma samples collected before surgery after NACT completion from 38 patients with BC, by investigating the concentration of differently sized cfDNA fragments.

The aims of this study are (1) to estimate the sensitivity and the specificity of an electrophoresis-based method for cfDI assessment in predicting the achievement of post-NACT pCR in patients with BC in comparison to standard-of-care techniques such as MRI, and (2) to evaluate the accuracy of cfDI and MRI combined for the same task.

PATIENTS AND METHODS

Ethics

This study was approved by the local ethics committee (Comitato Etico Regione Liguria, reference number: 11484). Written informed consents were obtained from all study participants.

Patients

The study population was recruited between 2013 and 2019 from a basin of 70 BC-affected patients scheduled for four cycles of epirubicin-based NACT followed by 12 cycles of paclitaxel. Inclusion criteria included histologic diagnosis of invasive BC with indication to NACT as per clinical practice; completion of at least 85% of NACT[17]; and written informed consent. Exclusion criteria included death for non–cancer-related causes within 12 months from surgery; bilateral disease at diagnosis; metastatic disease within 6 months from diagnosis; contraindications to MRI; autoimmune disease; and refusal to participate or consent withdrawal.

Of the 70 patients of the population basin, 18 were ruled out (six because of metastatic disease at diagnosis, four for bilateral BC, four for autoimmune disease, two for early NACT interruption, one for death at 5 months from surgery for cerebral hemorrhage, one for contraindications to MRI), and one patient withdrew from the study.

Blood samples were collected from all patients at three specific time points:

- At baseline, before the first cycle of epirubicin-based NACT (t0);
- At the completion of the epirubicin-based cycles of NACT, before the first cycle of paclitaxel (t1),
- Before surgery after NACT completion (t2).

Blood samples were obtained at the specified time points from 49 of the 51 eligible patients.

pCR was defined, as proposed by CTNeoBC, as the complete absence of invasive tumor in the primary site and excised nodes, without considering in situ neoplasia as invasive disease.[4] Patients were divided in two distinct groups depending on the response to NACT at surgery. Complete responders were classified as pCR patients, whereas noncomplete responders were classified as noncomplete responders (nR). pCR patients amounted to 11 (22.45%), whereas nR patients amounted to 38 (77.55%). Blood samples were collected from six female healthy controls as well.

Materials and Methods

cfDNA isolation, extraction, and quantification were performed following established, well-documented protocols.[18-21] Quality check and cfDNA fragment sizing and quantification were performed by using High Sensitivity D1000 ScreenTape Assay kit (High Sensitivity D1000 ScreenTape with High Sensitivity D1000 Reagents) on a TapeStation 2200 (Agilent Technologies, Santa Clara, CA).

A detailed description of materials and methods, together with data processing and statistical analyses, is reported in the Data Supplement.

cfDNA Fragmentation Profiling

DFP was performed on cfDNA extracted from plasma samples by assessing the concentration of differently sized cfDNA fragments. DNA fragment size ranges investigated amounted to 90-150 bp, 150-220 bp, 100-300 bp, 221-320 bp, and 321-1,000 bp.

cfDI Index Calculation

cfDI was expressed as the ratio of long to short cfDNA fragment concentrations, namely cfDI index. cfDNA fragments sized from 150 to 220 bp were selected as short fragments. cfDNA fragments sized from 321 to 1,000 bp were selected as long fragments. cfDI index was therefore calculated as follows:

$$\text{cfDI index} = \frac{[321 - 1,000 \text{ sized cfDNA fragments}]}{[150 - 220 \text{ sized cfDNA fragments}]}.$$

Power Considerations

From our preliminary data, we observed a mean cfDI across patients of 2.6 with a standard deviation of 2.5. Assuming a mean difference between nR and pCR patients of 2, and further assuming a ratio of 2:1 between nR and pCR patients, with a two-sided $\alpha = .05$ and $1 - \beta = 0.90$, we could reject the null hypothesis of not finding significant differences in cfDNA fragmentation by analyzing 11 pCR patients and 22 nR patients (ie, at least 33 patients). Considering a 25% failure rate occurring in the analytic process (because of shortcomings either in obtaining or properly analyzing the required samples at all time points), and a 30% failure rate in the recruitment phase (either because of ineligibility refusal to participate), at least 70 patients needed to be screened to proceed to the required analyses.

RESULTS

Demographics

Patients' median age was 49.5 years (range, 32-76 years). Of the 38 analyzed patients, 36 were diagnosed with ductal BC, one with lobular BC, and one with metaplastic BC. Three patients were diagnosed with luminal A-like tumors, 14 with luminal B-like human epidermal growth factor receptor 2 (HER2)– tumors, 12 with luminal B-like HER2+ tumors, one with an HER2+ tumor, and eight with triple-negative tumors (Table 1). These patients underwent a median of four cycles of epirubicin-based chemotherapy and 12 cycles of paclitaxel. Trastuzumab was administered concomitantly with paclitaxel in case of HER2 positivity. The median interval from t0 to t1, from t1 to t2, and from t2 to surgery amounted, respectively, to 91 days (interquartile range [IQR]: 11.5 days), 92.5 days (IQR: 20.75 days), and 17.5 days (IQR: 12.75 days). Eleven patients underwent pCR to NACT. Of the remaining patients, 13 presented a stage I BC at surgery, 10 a stage II BC, and 4 a stage III BC (Table 1). Summary data of each patient are reported in the Data Supplement.

cfDNA Fragmentation Assessed Before Surgery After NACT Completion Significantly Differs From pCR Patients to nR Ones

DFP was first performed on samples collected at t0, t1, and t2 from 13 patients (four pCR and nine nR), along with the samples collected from the six healthy controls. No significant difference in the concentration of the investigated cfDNA fragments in samples collected at t0 and t1 was observed either between nR and pCR patients, or between patients with BC and the healthy controls (Data Supplement).

Conversely, at t2, the concentration of 100-300 bp (nR median: 499 pg/μL; 95% CI, 159.2 to 850.8; pCR median: 79.15 pg/μL; 95% CI, 54.95 to 256.53) and 150-220 bp (nR median: 385 pg/μL; 95% CI, 126.2 to 954.0; pCR median: 39.65 pg/μL; 95% CI, 24.95 to 57.40) sized fragments significantly differed from nR subjects to pCR ones ($P = .0014$ and $P = .0072$, respectively). Moreover, the concentration of 150-220 bp, 100-300 bp, and 221-320 bp sized fragments assessed at t2 was significantly higher in nR patients compared with the healthy controls ($P = .0039$, $P = .011$, and $P = .008$, respectively).

No significant difference in the concentration of cfDNA fragments assessed at t2 in pCR patients and in the healthy controls was observed, except for the concentration of 150-

TABLE 1. Summary Data of the Study Cohort

Characteristic	
Age, years	
Median	49.5
IQR	14.2
Range	32-76
Menopausal status, No. (%)	
Pre	21 (55.3)
Post	17 (44.7)
cT at diagnosis (MRI), No. (%)	
cT1	2 (5.3)
cT2	20 (52.6)
cT3	12 (31.6)
cT4	4 (10.5)
cN at diagnosis (MRI plus ultrasound), No. (%)	
cN0	22 (57.9)
cN+	16 (42.1)
IHC classification, No. (%)	
Luminal A-like	3 (7.9)
Luminal B-like	14 (36.8)
Luminal B-like, HER2+	12 (31.6)
HER2+	1 (2.6)
TNBC	8 (21.1)
Ki67 at diagnosis, No. (%), %	
≥ 20	34 (89.5)
< 20	4 (10.5)
Hystotype, No. (%)	
Ductal	36 (94.7)
Other	2 (5.3)
cT at surgery (MRI), No. (%)	
cT0	13 (34.2)
cT1	19 (50)
cT2	5 (13.2)
cT3	1 (2.6)
cT4	0
cN at surgery (MRI), No. (%)	
cN0	35 (92.1)
cN+	3 (7.9)
Pathologic stage at surgery, No. (%)	
0	11 (29)
I	13 (34.2)
II	10 (26.3)
III	4 (10.5)
IV	0
pT, No. (%)	
pT0	6 (15.8)
pTis	7 (18.4)
pTmic	2 (5.3)
pT1	17 (44.7)
pT2	4 (10.5)
pT3	2 (5.3)
pT4	0
pN, No. (%)	
pN0	23 (60.5)
pNmic	3 (7.9)
pN1a	9 (23.7)
pN2a	3 (7.9)
Response to NACT, No. (%)	
pCR	11 (28.9)
No pCR	27 (71.1)
cfDI index, median (IQR)	
pCR	1.31 (1.52)
nR	3.51 (3.5)

NOTE. Radiologic staging was performed by using MRI at baseline and in presurgical phase. Axillary lymph nodes were evaluated by using MRI and ultrasound at diagnosis and by using MRI alone before surgery. HER2 status together with estrogen and progesterone receptors, and Ki-67 were assessed via histologic examination as per SIAPEC/ASCO/CAP criteria.

Abbreviations: cfDI, cell-free DNA integrity; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; IQR, interquartile range; MRI, magnetic resonance imaging; NACT, neoadjuvant chemotherapy; nR, noncomplete responders; pCR, pathologic complete response; TNBC, triple-negative breast cancer.

220 bp sized fragments, which was significantly higher in the latter (median: 84.05; 95% CI, 49.875 to 146.750; $P = .043$). No significant variation was observed as well in the concentration of the investigated cfDNA fragments at the different time points either in pCR or nR patients (Data Supplement).

This first screening allowed us to select t2 as the most significant time point. No further analysis was performed on samples collected at t0 and t1.

cfDI Index Assessed Before Surgery Significantly Correlates With the Achievement of pCR to NACT

To reach adequate sample size, samples collected at t2 from further 25 patients were analyzed, reaching a total of 38 samples analyzed at this specific time point. Of these, 11 samples were collected from pCR patients, and 27 from nR patients (Data Supplement).

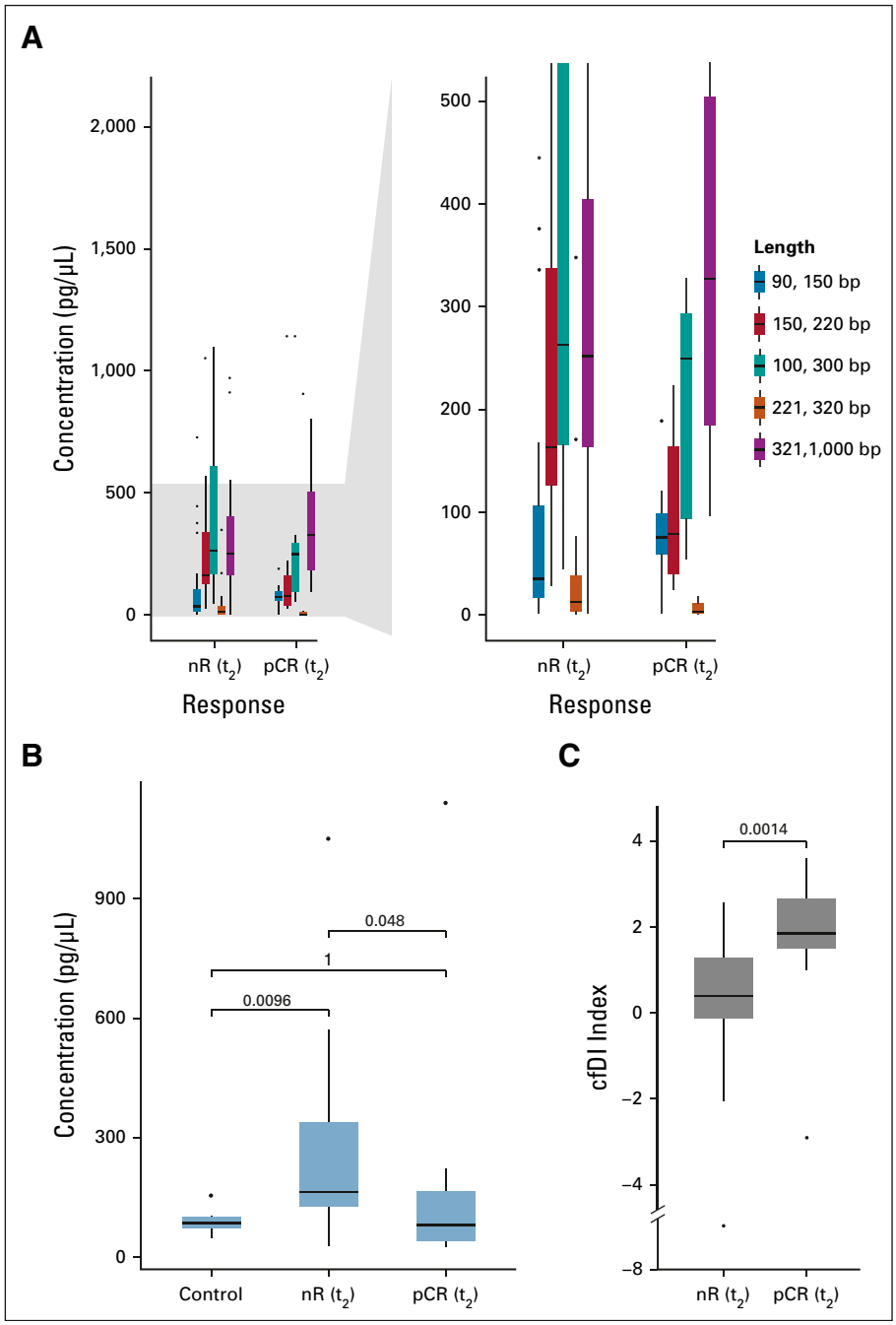

FIG 1. cfDI index correlates with the response to neoadjuvant chemotherapy. (A) Boxplots of cell-free DNA fragment concentration assessed on 38 samples collected at t2 from as many patients (11 pCR and 26 nR). (B) Boxplot of 150-220 bp sized fragment concentration in six healthy controls, 27 nR, and 11 pCR patients at t2. The concentration of 150-220 bp sized fragments assessed at t2 was significantly higher in nR patients compared with the pCR ones (P = .048) and the healthy controls (P = .0096). No significant difference in the same parameter was observed between pCR patients and the healthy controls (P = .93). (C) Boxplot of cfDI index in nR and pCR patients. A significant correlation (P = .0014) between cfDI index and the achievement of pCR was observed. cfDI, cell-free DNA integrity; nR, noncomplete responders; pCR, pathologic complete response.

As for the preliminary essay, a significant correlation between 150-220 bp sized fragment concentration and the response to NACT was observed (Fig 1A). The concentration of 150-220 bp sized fragments was significantly higher in nR patients compared with pCR ones (nR median: 163 pg/µL; 95% CI, 93.61 to 990.80; pCR median: 78.9 pg/µL; 95% CI, 57.4 to 937.0; P = .048) and the healthy controls (P = .0096; Fig 1B), whereas it did not significantly differ between pCR patients and the healthy controls (P = .93).

cfDI index showed an even stronger correlation with the achievement of post-NACT pCR (P = .0014; Fig 1C). No significant correlation between cfDI index and immunohistochemical parameters assessed at surgery was found, except for Ki-67 (P = .03; Data Supplement).

The Combination of cfDI Index and MRI Enhances the Accuracy of pCR Assessment

cfDI index was selected to build an exploratory classifier of the response to NACT, which achieved an accuracy comparable to MRI. As shown in Figure 2A, cfDI index area under the receiver operating characteristic curve amounted to 0.816, achieving an optimal specificity and sensitivity,

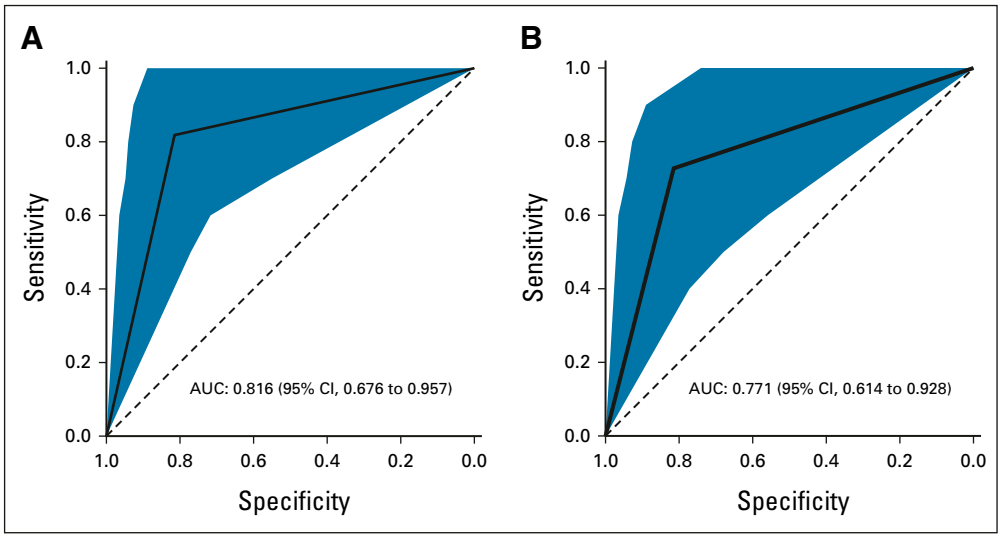

FIG 2. cfDI index achieves a comparable accuracy to MRI in predicting pathologic complete response. (A) cfDI index ROC curve. AUC amounted to 0.816 (95% CI, 0.676 to 0.957). (B) MRI ROC curve. AUC amounted to 0.771 (95% CI, 0.614 to 0.928). AUC, area under the ROC curve; cfDI, cell-free DNA integrity; MRI, magnetic resonance imaging; ROC, receiver operating characteristic.

respectively, of 0.815 and 0.818, for values above 2.71 in predicting pCR. MRI area under the receiver operating characteristic curve amounted to 0.771, with a specificity and a sensitivity of 0.815 and 0.727, respectively (Fig 2B).

As displayed in Figure 3, cfDI index and MRI concordantly classified 27 of 38 patients. Of these, eight were evaluated as cCR and 19 as nR by both methods. The assessment of cCR by both cfDI index and MRI achieved a predictive value of complete response of 0.875, whereas the assessment of suboptimal response to NACT by both techniques achieved a predictive value of noncomplete response of 0.947.

DISCUSSION

Clinical oncology has been moving toward an increasingly optimized approach to BC in regard to both diagnostic and therapeutic procedures. The study of cfDNA obtained through peripheral blood draw may represent a turning point in the pursuit of this intent, thanks to its low invasiveness and its manifold potential applications.

The effective application of cfDNA for clinical purposes, however, is still subject to severe limitations, because of the lack of standardized procedures for its evaluation. The assessment of cfDNA concentration in plasma mainly relies on quantitative polymerase chain reaction with the selection of genes such as *GAPDH, B2M, HBB,* or *ALU* repeats as targets.[10,22-24] Conflicting results reported in literature concerning this method may be due to the erratic DNA fragmentation in the bloodstream or during samples processing, with consequent heterogeneity in cfDNA fragments amplification. Moreover, amplification can be affected by passive fluorescein or DNA-labeling dyes. By

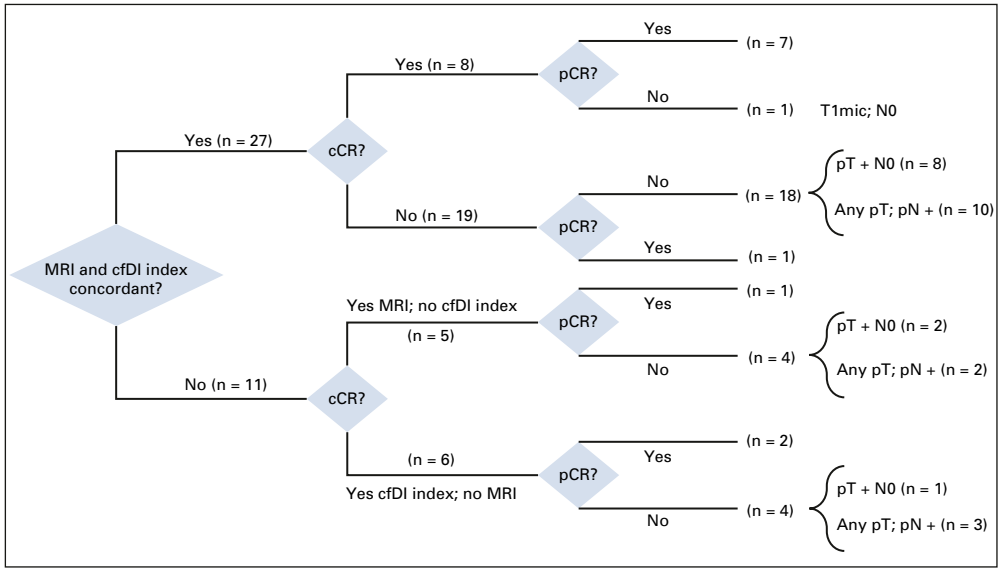

FIG 3. The combination of MRI and cfDI index augments the accuracy in pathologic complete response prediction. MRI and cfDI index concordantly classified 27 of 38 patients with breast cancer. Of these, eight were classified as complete responders, whereas 19 as nR. MRI and cfDI index were discordant in 11 of 38 patients. Of these, five were classified as complete responders by MRI and as nR by cfDI index, whereas six were classified as nR by MRI and as complete responders by cfDI index. When concordant, cfDI index and MRI achieved a predictive value of complete response of 0.875 and a predictive value of noncomplete response of 0.947. cCR, clinical complete response; cfDI, cell-free DNA integrity; MRI, magnetic resonance imaging; nR, noncomplete responders; pCR, pathologic complete response.

contrast, approaches on the basis of sequencing somatic mutations from cfDNA aimed at detecting circulating tumor DNA for diagnostic and predictive purposes, despite the potential, are still hardly applicable in real-life clinical settings because of the high costs and complex execution, besides presenting to date inadequate accuracy.[25]

Here, we display an alternative approach to cfDNA evaluation for a specific predictive purpose in patients with BC, which overcomes those limitations in terms of reproducibility and cost-effectiveness that affect current methods for cfDNA study, and is amenable to real-time decision making, given its short turnaround time (< 1 day). To date, there is no robust evidence indicating a potential role of cfDI in the evaluation of the response to NACT in patients with BC. An exploratory report by Wang et al[26] hinted indeed a correlation between cfDI and the achievement of post-NACT pCR, although the small case set and the in-house detection method used in that study limit the possibility to draw strong conclusions from those results.

Standard-of-care procedures typically used to assess BC response to NACT, such as MRI, present suboptimal accuracy in detecting post-NACT residual disease, with a recent meta-analysis estimating MRI sensitivity and specificity, respectively, at 0.80 (95% CI, 0.70 to 0.88) and 0.84 (95% CI, 0.79 to 0.88) in predicting pCR.[27] The lack of a specific technique for the assessment of nodal neoplastic involvement after NACT requires the execution of SLB even for patients assessed as with cCR. Moreover, MRI presents several contraindications, such as incompatible implanted devices or claustrophobia, which still consistently limit its usage.[28]

cfDI is typically expressed through cfDI index, defined as the ratio of longer fragment concentration to shorter fragment concentration. However, to date, there are no specifically sized cfDNA fragments definitely recognized as informative of cancer-related parameters. DFP performed before cfDI estimate was meant to select the most informative cfDNA fragments of BC response, independently from the underlying biology. Fragments ranging from 90 to 150 bp and from 221 to 320 bp have been reported to be enriched with the highest quantity of circulating tumor DNA in neoplastic patients,[29] whereas fragments ranging from 150 to 220 bp and from 100 to 300 bp include DNA derived from cellular apoptosis,[30] as confirmed by the electrophoretic analysis conducted on the cfDNA Reference Set (Data Supplement). Moreover, the use of automated electrophoresis instead of polymerase chain reaction–based methods allowed the assessment of the concentration of fragments > 321 bp.

DFP performed at the different time points on the preliminary case set (13 patients) showed a progressive increase of 150-220 bp sized fragment concentration alongside the administration of NACT in nR patients. This finding may seem counterintuitive as it suggests an active response to NACT in nR patients, with a significant impact on cfDI, rather than a normalization of short fragment concentration in complete responders. The meaning of such finding is currently unknown, and to the best of our knowledge, we report it for the first time.

DFP performed at t2 after sample size expansion confirmed a significant correlation between the concentration of 150-220 sized fragments and the achievement of post-NACT pCR ($P = .048$). cfDI index, calculated as the ratio of 321-1,000 bp sized fragment concentration to 150-220 bp sized fragment concentration, showed an even stronger correlation with the achievement of pCR ($P = .0014$), and the cfDI index–based response classifier achieved a comparable accuracy to MRI in predicting such outcome. The combination of cfDI index and MRI achieved even higher accuracy in case of concordance, with a predictive value of complete response of 0.875 and a predictive value of noncomplete response of 0.947 (Fig 3). Of note, the only misclassified patient by both MRI and cfDI index as complete responder (UPN 21) presented a residual T1mic breast neoplasia at surgery, with disease-free axilla. The assessment of cCR by both techniques resulted therefore in a predictive value of nodal complete response of 1, although in an exploratory case set (n = 8 patients).

An accurate assessment of cCR would have a significant impact on BC patients' surgical and systemic management. Patients assessed as complete responders to NACT may be spared from SLB, which could be replaced by radiologic monitoring. By contrast, studies could be envisaged to extend/modify NACT if biologic complete response has not been achieved by noninvasive assessments. This would indeed be in line with studies showing that additional treatment blocks upon missed achievement of pCR is beneficial in terms of disease-free survival in selected patients with BC.[31]

Further studies should also focus on investigating the mechanistic bases underlying cfDI alteration in patients with BC undergoing NACT, which was outside the scope of the present work.

This study presents with several limitations, including the limited sample size, the monoinstitutional accrual, and the variability of collection time points, which may have had an impact on the overall accuracy of pCR assessment by cfDI index. However, this is the first study of its kind, meant as hypothesis-generating in nature. We are now designing larger, prospective studies to tackle the issue of cfDI in NACT BC from a more systematic perspective. These will allow us to report on the potential medical utility of our present findings with greater accuracy and higher clinical transferability potential.

In conclusion, the study of cfDI shows great potential as a predictive marker of BC response to systemic treatment. The method here displayed for the assessment of cfDI yields potential as a novel approach to cfDNA evaluation, possibly implementing current sequencing-based workflows or radiologic procedures in the pursuit of a noninvasive and personalized medicine addressed to neoplastic patients.

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EQUAL CONTRIBUTION

G.C., L.F., and F.R. contributed equally to this work. P.F., A.B., and G.Z. contributed equally to this work.

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Final approval of manuscript: All authors
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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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