Improved Prognostic and Predictive Accuracy Using Multiple Gene Assays in Luminal type Early Breast Cancer

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Abstract

We evaluated the prognostic and neoadjuvant chemotherapy (NAC) predictive performance of four multigene assays(MGAs), using public microarray CEL data in ER-positive, HER2-negative early breast cancer. Among 555 node-negative cases, all assays significantly stratified DRFS, and combining results improved prognostic precision.
In 550 NAC-treated cases, high-risk tumors showed higher pCR rates across all assays. The “high-risk number” (0–4 panels high) correlated with both recurrence and chemosensitivity, supporting integrated multigene analysis for personalized treatment.

Introduction

MGAs are useful for assessing recurrence risk in ER-positive, HER2-negative early breast cancer in addition to clinicopathological factors.
In Japan, the Japan-developed assay Curebest™ 95GC has been available for research-based clinical use since 2013, whereas Oncotype DX® became the only assay reimbursed by national insurance in 2023.
Although several MGAs are clinically informative, treatment decisions can be inconsistent when their results differ.
This limited reimbursement may restrict access to broader genomic evaluation.
Therefore, we aimed to compare and integrate the prognostic and NAC-predictive performance of multiple MGAs using microarray CEL data, to explore a more accessible and individualized approach to breast cancer management.

Materials and Methods ①(Prognostic Method)

Microarray CEL files containing genome-wide expression data were used to calculate four MGAs: PAM50, 21GC (Oncotype DX® surrogate), Curebest™ 95GC, and EndoPredict (EP).
We analyzed ER-positive, HER2-negative, node-negative breast cancers treated with endocrine therapy only. All data were normalized using MAS5, and distant recurrence-free survival (DRFS) was evaluated by Kaplan–Meier and Cox analysis.
The prognostic impact of combined high-risk number (0–4) was also examined.

Results①

	N=555
Age	
Median	62 (30-84)
Size	
≦2.0	208 (37.5%)
>2.0	170 (30.6%)
unknown	177 (31.9%)
PgR	
Positive	110 (19.8%)
negative	245 (44.1%)
unknown	200(36.1%)
Histopathological grade	
1	73 (13.2%)
2	121 (21.8%)
3	49 (8.8%)
unknown	312 (56.2%)

Table 1. Patient characteristics

Factor	N=555	Univariate analysis		Multivariate analysis		C-index*
		HR (95% CI), Univariable	p-value	HR (95% CI), Univariable	p-value	
Size ≦2cm >2cm	208 170	1.45 (0.87-2.42)	0.154			
PgR positive negative	110 245	1.09 (0.63-1.88)	0.772			
PAM50 LuminalA Others	304 251	3.32 (2.08-5.30)	4.67e-07	1.33 (0.66-2.67)	0.42	0.66
EP low high	311 244	3.08 (1.95-4.87)	1.38e-06	1.53 (0.85-2.73)	0.16	0.65
21GC low high	419 136	2.99 (1.94-4.59)	5.97e-07	1.20 (0.71-2.00)	0.51	0.65
95GC Low High	358 197	3.63 (2.33-5.65)	1.08e-08	1.43 (0.72-2.86)	0.31	0.68
High risk number of group 0 or 1, 2 or more	321 234	4.46 (2.77-7.21)	9.34e-10	1.90 (0.68-5.32)	0.22	0.70

Table2. Univariate and multivariable Cox regression analyses with C-index for prognostic performance
*C-index was calculated from univariable models. Higher values indicate better prognostic ability (0.5=random, 1=perfect).”

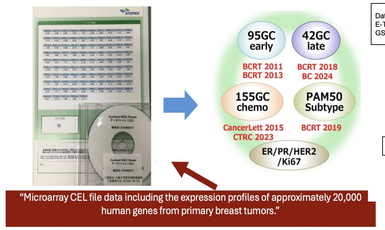


Figure 1. Conceptual overview of microarray CEL file data
The CEL file, provided as a research-use supplement after Curebest™ 95GC testing, contains the expression profiles of approximately 20,000 human genes from the primary tumor. Using this dataset, it is possible to capture mRNA expression comprehensively and retrospectively calculate other multigene assays.
(The CEL file is available for research purposes only and not intended for routine clinical use.) **(Ref. 1)**

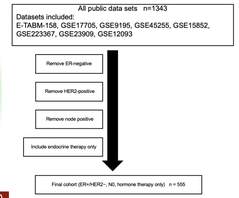


Figure 2. Study selection flowchart for prognostic analysis

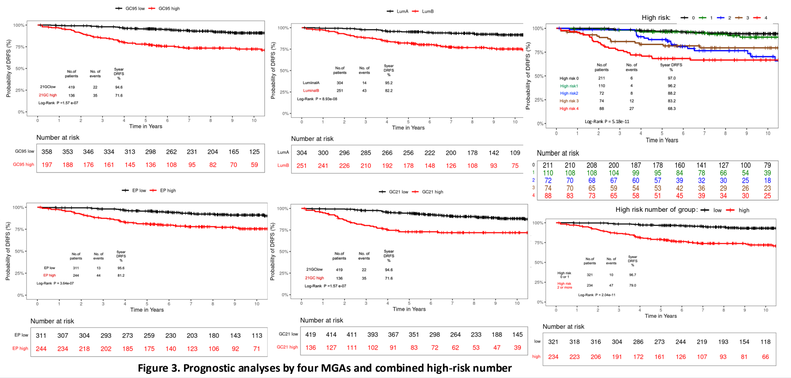


Figure 3. Prognostic analyses by four MGAs and combined high-risk number

Discussion①

Univariable analysis demonstrated significant differences in DRFS for each MGA and for the combined high-risk classification. However, in multivariable analysis, none remained significant, likely due to multicollinearity among assays, as correlations existed between each MGA and the high-risk group. Therefore, to objectively compare prognostic performance, the C-index was calculated from univariable models. All four MGAs showed similar C-index values, indicating comparable and favorable prognostic ability.
These findings suggest that integrating multiple MGAs may allow more accurate identification of patients who can achieve favorable outcomes without adjuvant chemotherapy. In particular, patients with only one high-risk result among the four panels showed an excellent prognosis, and those classified as low risk by all assays may be treated effectively with endocrine therapy alone after surgery.

Methods and Materials② (Predictive Method)

We extracted 550 ER-positive, HER2-negative early breast cancer cases treated with NAC from five public microarray cohorts (GSE25066, GSE42822, GSE63471, GSE140494, and GSE231629). Genome-wide expression data (CEL files) were normalized using MAS5, and four MGAs—PAM50, 21GC (Oncotype DX® surrogate), 155GC, and EndoPredict (EP)—were calculated. NAC sensitivity was evaluated using pCR as the endpoint. We also examined whether integrating the number of assays classified as high risk (0–4) could improve the accuracy of predicting chemosensitivity.

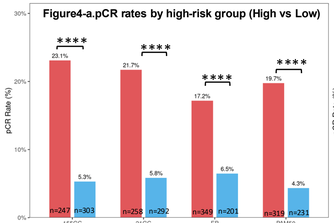
Results②

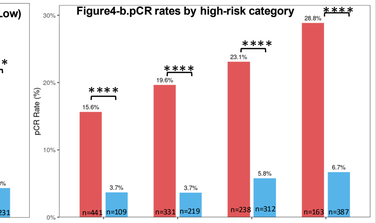
	N=550
age	
Median	49.2 (24-75)
cN	
negative	235 (42.7%)
positive	315 (57.3%)
cT	
0	3 (0.51%)
1	18 (3.27%)
2	237 (43.1%)
3	126 (22.9%)
4	54 (9.82%)
NA	112 (20.4%)
PgR	
Positive	95 (17.3%)
negative	345 (62.7%)
unknown	110 (20%)

Table 3. Patient characteristics

	n=550	pCR rate n(%)	Odds ratio (95%CI)	*p*-value
EP				
Low	201	13 (6.5)	3.00	
High	349	60 (17.2)	(1.60-5.62)	5.90e-04
155GC				
Low	303	16 (5.3)	5.38	
High	247	57 (23.1)	(3.00-9.65)	1.62e-08
21GC				
Low	292	17 (5.8)	0.223	
High	258	56 (21.7)	(0.126-0.395)	2.76e-07
PAM50				
LuminalA	231	10 (4.3)	5.44	
Others	319	63 (19.7)	(2.73-10.9)	1.55e-06
high risk				
0	109	4 (3.7)	4.87	
≥1	441	69 (15.6)	(1.74-13.6)	2.61e-03
high risk				
0–1	219	8 (3.7)	6.45	
≥2	331	65 (19.6)	(3.03-13.7)	1.37e-06
high risk				
0-2	312	18 (5.8)	4.91	
≥3	238	55 (23.1)	(2.79-8.62)	3.10e-08
high risk				
0-3	387	26 (6.7)	5.63	
4	163	47 (28.8)	(3.34-9.49)	9.37e-11

Table 4. Univariable logistic regression analysis for predictors





Discussion②

Tumors classified as high risk by MGAs are generally known to show higher chemosensitivity and higher pCR rates, while exhibiting an increased risk of recurrence and poorer prognosis(**Ref.1**). In this study, pCR rates increased as the number of assays classified as high risk increased, suggesting that integrating multiple MGAs may improve the accuracy of predicting NAC response. The ability to estimate NAC efficacy in advance may help optimize treatment intensity and avoid overtreatment.

Conclusions

Integrating multiple MGAs enhances both prognostic and NAC-response prediction, supporting more personalized treatment decisions. The use of CEL files enables simultaneous calculation of multiple assays, providing a practical pathway toward optimized precision medicine.

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References

1. Naoi Y, et al. Cancer Science(Review Article)2021;112(4):1369-1375

Conflict of Interest: The authors declare no conflicts of interest.

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