#312

Molecular qRT-PCR grade index: a new tool for breast cancer (BC) patient grading improvement.

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BACKGROUND

- Proliferation captured by the GGI (97 genes) is one of the most important prognostic indicators in BC.
- The majority of these genes were over-expressed in high grade tumours.
- The major impact of the GGI to the clinic is that tumours with intermediate histological grade and then unknown prognostic were assigned to two subgroups whose gene expression profiles ranged from those for low histological grade to those for high histological grade tumours.
- Therefore the three-category histological grading system could be replaced with a two-category gene expression grading system that may be clinically more relevant.

PURPOSE

The aims of this study were 1) to convert this microarray index to an index using qRT-PCR and 2) to assess its prognostic and predictive value for tamoxifen response.

PATIENTS AND METHODS

A qRT-PCR genomic grade index (PCR-GGI) was developed based on the expression of 4 genes selected from the GGI microarray signature and 4 reference genes.

PATIENTS & TUMORS CHARACTERISTICS

	Oxfd (n = 78)	*IJB95/96 (n = 212)*	*JNIadj (n = 141)*	*JNIadv (n = 279)*
	Patients and tumors characteristics			
Mean age at diagnosis (years) (range)	**64 (40-86)**	**58.5 (31-87)**	**64 (46-87)**	**58 (26-89)**
Menopausal status Premenopausal Postmenopausal *UK**	/ / **78**	**54 135 23**	**7 134 /**	**98 181 /**
Events free survival (means ; months) (range)	*DMFS:* **67 (0.26-129)**	*DMFS:* **78.70 (0.13-142.27)**	*RFS :* **49 (2-129)**	*Progression:* **13 (1-70)**
Death Yes No	/ /	**24 188**	**51 90**	**188 91**
Tumor size (mean) (range)	**3.14 (1-7)**	**2.29 (0.15-8)**	**3.2 (1-8)**	/
Histological grade 1 2 3 *UK**	**13 (16.7%) 40 (51.2%) 13 (16.7%) 12 (15.4%)**	**37 (17.5%) 90 (42.5%) 83 (39.2%) 2 (0.01%)**	**1 (0.01%) 16 (11.3%) 77 (54.6%) 47 (33.3%)**	**1 (0.003%) 34 (12.2%) 154 (55.2%) 90 (32.2%)**
Number of metastasis sites 0 1 2 ☒ 3	**51 24 3** /	**171 20 19 2**	/	/
Histo. Estrogen Receptor status Positive Negative *UK**	**78 0 0**	**114 62 36**	**all**	**all**
Histo. Progesterone Receptor status Positive Negative *UK**	**0 0 78**	**82 90 40**	/	/
Histo. Ki-67 Receptor status (>15%) Positive Negative *UK**	**0 0 78**	**42 42 128**	/	/
No Positive Lymph Nodes (at chirurgery) 0 1 - 3 ☒ 4 *UK**	**45 28 / 5**	**115 49 36 12**	**0 97 44 /**	**121 117 28 13**

*Legend: * :UK = Unknown.*

RESULTS

To assure the effectiveness of the qRT-PCR assay -even with partially degraded RNA from FFPE specimens- we compared the qRT-PCR index accuracy and concordance with the original GGI (97 genes) using a small set of breast cancers (IJBtest) from which frozen, FFPE tissues and microarray data were available (N=19).

Correlation between the original GGI index and the qRT-PCR index derived from frozen and FFPE samples.

genes	GGI vs GG RT-PCR (*Frozen*)			GGI vs GG RT-PCR (*FFPE*)			GG RT-PCR *Frozen* vs *FFPE*		
	Cor.coef	**CI95%**	**p.val.**	**Cor.coef**	**CI95%**	**p.val.**	**Cor.coef**	**CI95%**	**p.val.**
CDC20	0.933	[0.825-0.975]	1.70E-08	0.732	[0.403-0.893]	5.55E-04	0.775	[0.482-0.912]	1.60E-04
CDC2	0.644	[0.253-0.854]	3.93E-03	0.819	[0.57-0.93]	3.22E-05	0.694	[0.336-0.877]	1.39E-03
CCNB1	0.942	[0.848-0.979]	5.38E-09	0.808	[0.548-0.926]	4.92E-05	0.731	[0.401-0.893]	5.67E-04
KPNA2	0.762	[0.458-0.906]	2.37E-04	0.64	[0.247-0.852]	4.22E-03	0.73	[0.4-0.893]	5.78E-04
4genes	**0.95**	**[0.86-0.98]**	**3.6E-09**	**0.89**	**[0.72-0.96]**	**8.26E-07**	**0.85**	**[0.64-0.94]**	**7.7E-06**

Frozen (Oxfd)

To evaluate the performance of the qRT-PCR assay to consistently identify low-risk and high-risk patients for distant metastasis, the qRT-PCR signature was compared to the histological grade as well as the original GGI in predicting distant metastases free survival on an independent ER-positive tamoxifen-only treated breast cancer population (N=78) (OXFD).

Figure 1 :

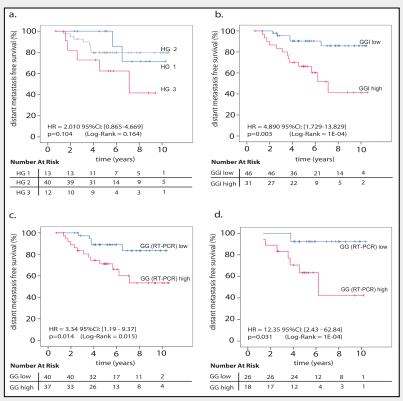


Figure 1: DMFS analysis for the Oxford (OXFD) ER + frozen population. (A) whole population by histological grade (HG1 (blue), HG2 (gray) and HG3 (red)). (B) Whole population by gene expression grade index (GGI) (GG low = blue and GG high = red). (C) Whole population by RT-PCR grading (GG(RT-PCR) low = blue and GG(RT-PCR) high = red). (D) Node negative (n=45) samples by qRT-PCR grading (GG(RT-PCR) low = blue and GG(RT-PCR) high = red). (E) Cross-tab for RT-PCR grading (GG(RT-PCR)), gene expression grade index (GGI) and histological grade (HG).

FFPE (IJB95/96)

To validate the performance of the RT-PCR grade index in predicting distant metastases free survival in FFPE tissues, the qRT-PCR assay was applied on an independent population of 212 primary breast cancer FFPE samples originated from patients consecutively diagnosed in our institution from 1995 to 1996 (IJB95/96).

Figure 2 :

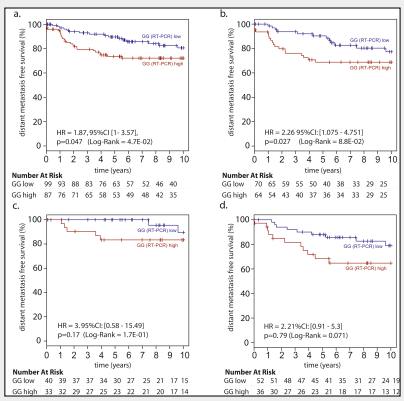
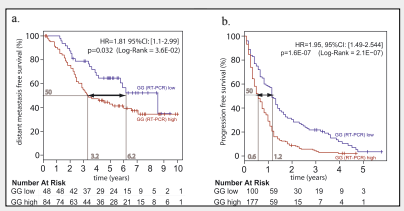


Figure 2: DMFS analysis for the IJB95/96 FFPE population by RT-PCR grading. (A) Whole population (GG(rt-PCR) low = blue and GG(rt-PCR) high = red) (B) ER+ samples (GG(rt-PCR) low = blue and GG(rt-PCR) high = red). (C) ER+ node negative samples (GG(rt-PCR) low = blue and GG(rt-PCR) high = red). (D) Patient of histological grade 2 (HG2) tumors by RT-PCR grading. The 90 patients with HG2 tumors were separated into low-and high-risk subsets by this signature as GG(rt-PCR) low = blue and high = red.

RT-PCR grade index in high-risk tamoxifen-only treated patients (JNI)

- Relapse free survival analysis for JNIadj ER+ node positive tamoxifen only treated population (N= 141) by RT-PCR grading. The low-risk patients recurring 3 years later compared to high-risk patients (difference observed at 50% survival)(Figure 3.A).
- Progression free survival (PFS) analyses for JNIadv ER+ advanced BC tamoxifen only treated patients (N= 279) by RT-PCR grading. The low-risk patients recurring 7.5 month later compared to high-risk patients (difference observed at 50% survival) (Figure 3.B)

Figure 3 :



CONCLUSIONS

The RT-PCR score index has the potential to improve the accuracy of grading for prognosis purposes as :

- The assay is not subject to the inter-observer variability,
- The assay assigned the patient with intermediate grade tumor to well defined prognostic group,
- The assay is a strong predictor for node negative ER positive patients avoiding unecessary treatment.

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