BioMarQ – a novel approach to automated HER2-analysis of circulating tumor cells (CTCs)

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Background

Targeted treatment approaches guided by phenotypic characteristics of CTCs might improve the outcome of breast cancer patients. Therefore, accurate and reproducible assessment of the CTC phenotype is required to use CTCs as treatment targets. The aim of this study was to test the suitability of the semi-automated BioMarQ System (Veridex, USA) – a Research Use Only Application used together with the CELLSEARCH System (Veridex USA) for CTC detection– for assessing the HER2-status of CTCs based on fluorescence and cell-morphology data.

Methods

Data on classification of the HER2-status divided into four groups (0, +, ++, +++) obtained by visual inspection of CTCs after immunocytochemical staining with the CellSearch System, and of BioMarQ-generated automatic measures on fluorescence and cell morphology via image clips generated from raw images were available for 329 CTCs from 17 metastatic breast cancer patients. A discriminant analysis was performed to test whether linear combinations of the fluorescence and/or cell morphology measures allow a separation of CTCs into four groups according to the visual classification of their HER2-status.

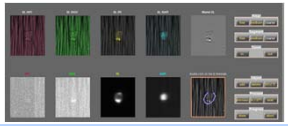

Figure 1: Manual segmentation and selection of CTCs

Results

The highest concordance with the visual classification was obtained by a set of three discriminant functions based on the five variables mean-, maximum- and integrated fluorescence intensity, cell area (µm²), and coefficient of variation for the pixel fluorescence values within a cell. All five variables differed significantly among the 17 patients (Kruskal-Wallis-test, $p < 0.001$ each). Based on the three discriminant functions, 74.5% of all grouped cases were correctly assigned to the four visually obtained categories (6 out of 8 CTCs classified as +++; 57 out of 80 as ++; 17 out of 65 as +, 165 out of 176 classified as 0; see Table 1). Assignment of the HER2 status of CTCs to the visual classification was most accurate for HER2 negative and +++ positive CTCs. Analysis of CTCs which are + or ++ HER2 positive also include assignment of false HER2 +++ positive CTCs. In seven cases (i.e. 4.8%) CTCs visually classified as HER2 + or HER2 ++ were assigned to the HER2 +++ group. The correlation between image parameters and visual classificators is shown in Figures 2-4.

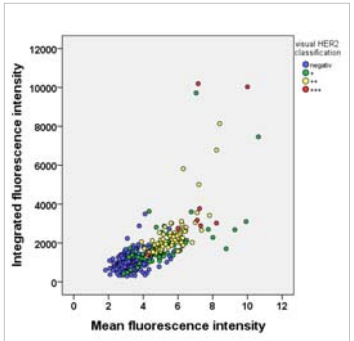

Fig. 2: Scatter diagram showing the correlation between mean fluorescence intensity and integrated fluorescence intensity for the four visually classified HER2 categories.

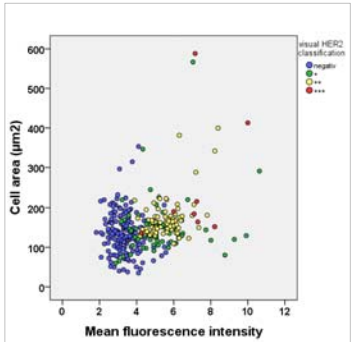

Fig. 3: Scatter diagram showing the correlation between mean fluorescence intensity and cell area for the four visually classified HER2 categories.

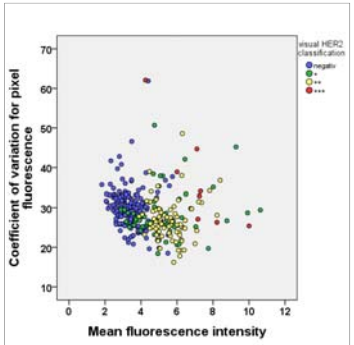

Fig. 4: Scatter diagram showing the correlation between mean fluorescence intensity and coefficient of variation for the four visually classified HER2 categories.

		visual HER2 classification	Predicted Group Membership				Total
			negativ	+	++	+++	
Original	Count	negativ	165	3	8	0	176
		+	23	17	20	5	65
		++	10	11	57	2	80
		+++	0	0	2	6	8
	%	negativ	93,8	1,7	4,5	,0	100,0
		+	35,4	26,2	30,8	7,7	100,0
		++	12,5	13,8	71,3	2,5	100,0
		+++	,0	,0	25,0	75,0	100,0

Table 1: Discriminant analysis classification results.

Conclusions

Our preliminary analysis shows that discriminant functions derived from measurements on fluorescence and cell morphology generated by BioMarQ correctly assigned the HER2-status of CTCs to the four visually obtained categories in 75% of all cases. The BiomarQ system is most accurate for the assignment of HER2 negative and HER2 +++ positive CTCs. Further analysis based on a larger number of HER2-positive CTCs, and cross validation by FISH-analysis will provide deeper insight into the suitability of BioMarQ for objectively assessing the HER2-status of CTCs.

Acknowledgments

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