30 Comprehensive evaluation of methodology to assess abundance of immune infiltrates in breast cancer

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Background: The immune system is critical in modulating therapeutic sensitivity and tumor progression in breast cancer patients. A plethora of methods exists to evaluate the amount and composition of tumor immune infiltrates and are used interchangeably, based on the assumption that they provide similar information. A systematic comparison of these methods, including microscopy evaluations and algorithms based on transcriptomic and methylation data, is still lacking.

Methods: We characterized stromal (str) and intra-tumoral tumor infiltrating lymphocytes (TIL) and 6 immune cell-types ($CD3^+$, $CD4^+$, $CD8^+$, $CD20^+$, $CD68^+$, $FOXP3^+$) using immunohistochemistry in the 560 genomes cohort from the International Cancer Genomics Consortium (Nik-Zainal Nature 2016). The same traits were computed using deconvolution methods (CIBERSORT, methylCIBERSORT, quanTIseq, EPIC), as well as published transcriptomic or methylation-based immune signatures. We first studied the associations of immune cells as continuous variables and further categorized tumors as hot (strTIL $\geq$60%) or cold (strTIL $\leq$10%).

Results: The immune infiltrate was reproducibly assessed by pathologists for all cell types and concordance correlation coefficients ranged from 0.63 (for strCD4) to 0.84 (for strTIL). The correlations between all methods to assess global immune infiltration were extremely variable, ranging from 0.08 to 0.95 (Table). The correlations between methylation or transcriptomic estimates and histopathology were weak to moderate and did not exceed 0.56. Several transcriptomic estimates were strongly correlated with each other (>0.85). ROC analyses showed that most methods can more accurately identify hot tumors as compared to cold tumors. Comparison regarding the specific immune cell types further highlighted heterogeneity between the different methods. Table. Spearman rank (r) between methods to assess absolute presence of tumor infiltrating lymphocytes.

Conclusions: This study highlights important differences between the currently existing methods in the assessment of global immune infiltration in breast cancer and raises therefore extreme caution when assessing immune infiltrates in the clinical context.

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Table: 30

PATHOLOGY				METHYLATION		TRANSCRIPTOME				
IntrTIL	TMA	Digital TMA	Whole Slide	methylTIL	methylCBS	Absolute CBS	quanTIseq	EPIC	TIL gene expression	
0.75	0.63	0.62	0.61	0.44	0.53	0.43	0.09	0.35	0.4y4	StrTIL
	0.44	0.47	0.57	0.35	0.43	0.39	0.15	0.31	0.35	IntrTIL
		0.87	0.64	0.34	0.49	0.46	0.16	0.32	0.49	TMA
			0.64	0.34	0.52	0.53	0.21	0.41	0.56	Digital TMA
				0.24	0.49	0.41	0.08	0.37	0.40	Whole Slide
					0.77	0.65	0.15	0.37	0.70	MethylTIL
						0.75	0.20	0.45	0.76	MethylCBS
							0.18	0.52	0.95	Absolute CBS
								0.40	0.20	quanTIseq
									0.55	EPIC

CBS, CIBERSORT; TMA, Tissue Micro Array; Whole Slide, Whole Slide Tissue Section; MethylTIL by Jeschke et al. 2017; TIL gene expression by Smid et al. 2016.