

Analysis of A Real-world Cohort of Metastatic Breast Cancer Patients Shows Circulating Tumor Cell Clusters (CTC-clusters) as A Predictor of Patient Outcomes

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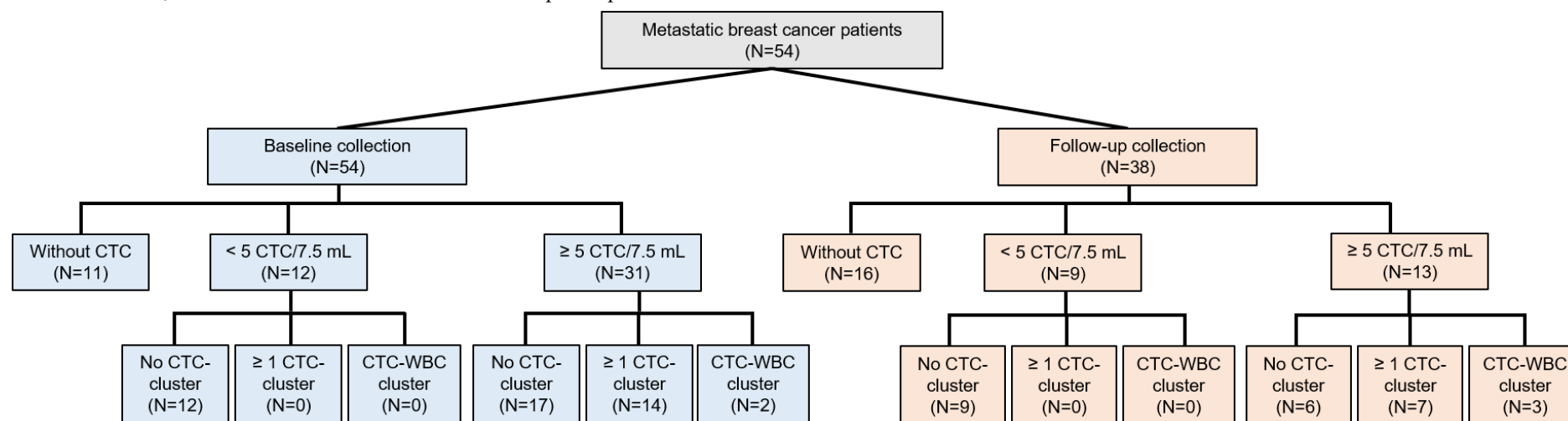


Figure S1. Study design. CONSORT flow diagram of study cohort and patient distribution at times of blood draw according to the presence or not of CTCs, CTC-clusters, and CTC-WBC clusters (N indicates the number of patients).

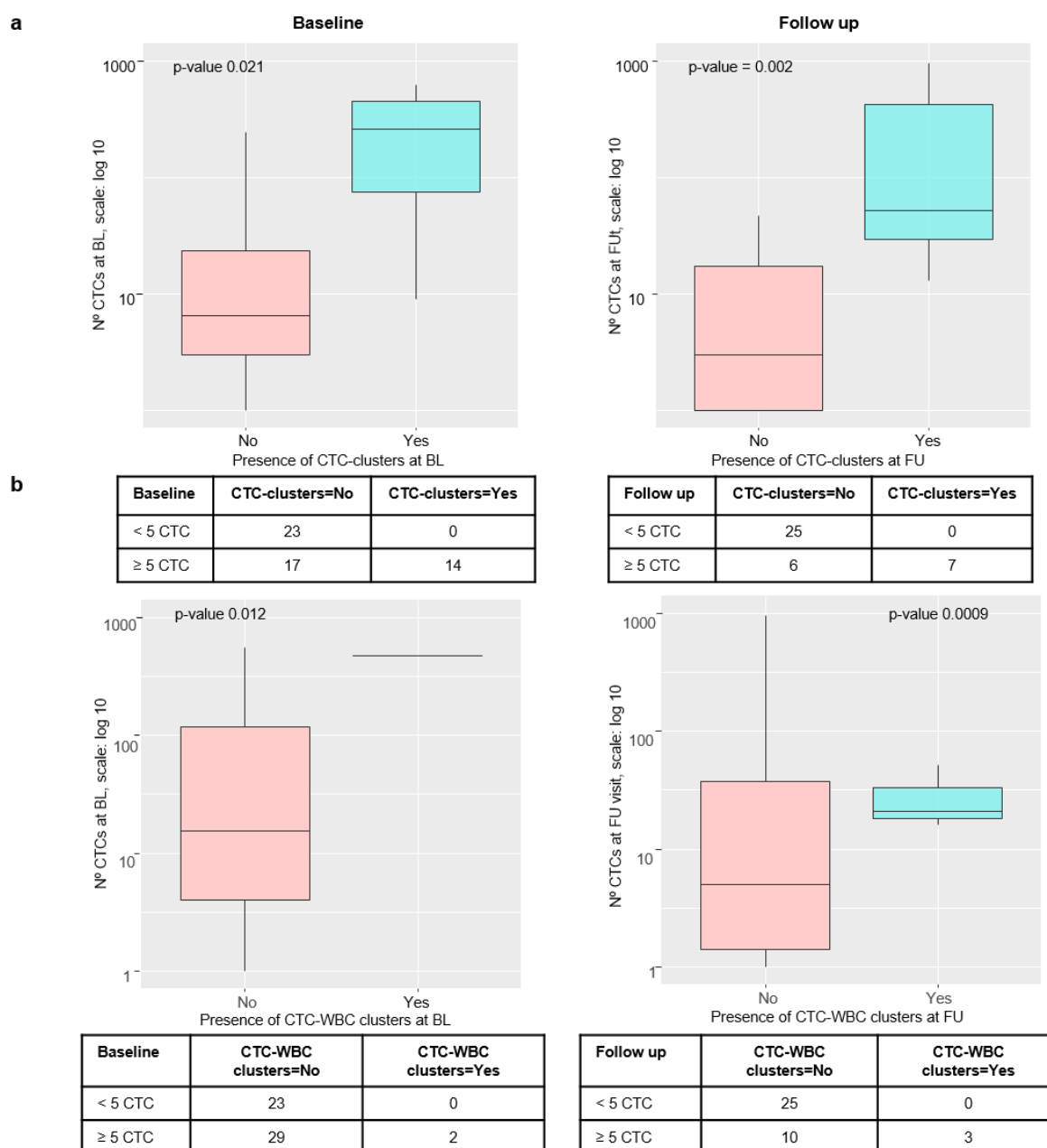


Figure S2. Association between CTC count and the presence of CTC-clusters or CTC-WBC clusters at baseline and follow up. Box plots showing the association between the number of CTCs and the presence or absence of CTC-clusters at baseline (BL) and follow up (FU) (a). Box plots showing the association between the number of CTCs and the presence or absence of CTC-WBC clusters at baseline (BL) and follow up (FU) (b). Associations were assessed using Pearson's chi-squared test.



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Table S1. Study cohort analysis of CTCs and CTC-clusters.

Baseline	n= 54	Percent (%)	Mean	Min-Max
CTCs	43	79.2	183.1	1-1970
CTCs ≥ 5	31	57.4	253.1	5-1970
CTC-cluster ≥ 1	14	25.9	4.28	1-14
CTC-WBC-cluster ≥ 1	2	3.7	7.5	2-13
Follow up	n= 38			
CTCs	22	57.9	95.6	1-969
CTCs ≥ 5	13	34.2	160.6	5-969
CTC-cluster ≥ 1	7	18.4	12.71	1-74
CTC-WBC-cluster ≥ 1	3	7.9	2.0	2-2

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Table S2. Distribution of CTCs and CTC-clusters in different BC subtypes.

Variable	Total	BC subtypes				<i>p</i> Value
		HR+HER2- (<i>n</i> = 27)	HER2+ (<i>n</i> = 11)	HR-HER2- (<i>n</i> = 15)	Undet. (<i>n</i> = 1)	
Baseline CTCs						
< 5 CTCs/7.5 ml	23 (100.0)	12 (52.2)	4 (17.4)	7 (30.4)	0 (0)	0.861
≥ 5 CTCs/7.5 ml	31 (100.0)	15 (48.4)	7 (22.6)	8 (25.8)	1 (3.2)	
Baseline CTC-clusters						
No	40 (100.0)	21 (52.5)	7 (17.5)	11 (27.5)	1 (2.5)	0.668
Yes	14 (100.0)	6 (42.9)	4 (28.6)	4 (28.6)	0 (0)	
2-3 cells	12 (100.0)	6 (50)	3 (25)	3 (25)	0 (0)	
≥ 4 cells	2 (100.0)	0 (0)	1 (50)	1 (50)	0 (0)	
Baseline CTC-WBC clusters						
No	52 (100.0)	26 (50)	10 (19.2)	15 (28.8)	1 (1.9)	0.488
Yes	2 (100.0)	1 (50)	1 (50)			
		(<i>n</i> = 19)	(<i>n</i> = 9)	(<i>n</i> = 9)	(<i>n</i> = 1)	
Follow up CTCs						
< 5 CTCs/7.5 ml	25 (100.0)	11 (44)	7 (18.4)	6 (24)	1 (4)	0.583
≥ 5 CTCs/7.5 ml	13 (100.0)	8 (61.5)	2 (15.4)	3 (23.1)	0 (0)	
Follow up CTC-clusters						
No	31 (100.0)	15 (48.4)	9 (29.0)	6 (19.4)	1 (3.2)	0.184
Yes	7 (100.0)	4 (57.1)	0 (0)	3 (42.9)	0 (0)	
2-3 cells	5 (100.0)	3 (60)	0 (0)	2 (40)	0 (0)	
≥ 4 cells	2 (100.0)	1 (50)	0 (0)	1 (50)	0 (0)	
Baseline CTC-WBC clusters						
No	35 (100.0)	17 (48.6)	8 (22.9)	9 (25.7)	1 (2.9)	0.590
Yes	3 (100.0)	2 (66.7)	1 (33.3)	0 (0)	0 (0)	
Abbreviations: CTCs circulating tumor cells, HR hormone receptors, HER2 human epidermal growth factor receptor 2, Undet. undetermined.						

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Table S3. Multivariable Cox regression analysis of prognostic variables included in the clinicopathological model.

Variable	PFS		OS	
	HR (95% CI)	<i>p</i> Value	HR (95% CI)	<i>p</i> Value*
Age at baseline				
< 65	1.0		1.0	
≥ 65	0.54 (0.24-1.2)	0.15	0.59 (0.23-1.5)	0.27
ECOG				
Ordinal scale (0. 1. 2)	0.73 (0.4-1.3)	0.36	0.82 (0.4-1.6)	0.56
Number of metastatic sites				
< 3	1.0		1.0	
≥ 3	1.7 (0.8-3.5)	0.18	2.0 (0.58-4.7)	0.12
Site of metastasis				
Non-visceral	1.0		1.0	
Visceral	2.5 (0.94-6.6)	0.067	5.4 (1.2-23)	0.026
Subtype				
HR+HER2-	1			
HER2+	1.38 (0.48-3.9)	0.547	0.85 (0.25-3.5)	0.94
HR-HER2-	1.63 (0.7-3.8)	0.25	1.9 (0.7-4.7)	0.16
Treatments				
Chemotherapy	1		1	
Hormone	0.13 (0.03-0.57)	0.006	0.10 (0.01-0.79)	0.028
Targeted	1.41 (0.48-4.16)	0.53	1.14 (0.3-3.9)	0.829
Abbreviations: HR hazard ratio and hormone receptors, PFS progression-free survival, OS overall survival, CI confidence interval, ECOG Eastern Cooperative Oncology Group, HER2 human epidermal growth factor receptor 2.				
* Adjusted for age, ECOG, subtype, number of metastatic sites, site of metastasis and treatments.				

23 **Table S4.** Cox regression analysis for CTC count ≥ 20 versus < 20 , and presence versus
 24 absence of CTC-clusters.

Variables	Total	Events, <i>n</i> (%)	HR (95% CI)	<i>p</i> Value	HR (95% CI)*	<i>p</i> Value*
Baseline						
Associated with OS						
≥ 5 vs < 5 CTCs	31	17 (54.8)	3.15 (1.16-8.55)	0.024	3.33 (1.14-9.73)	0.027
≥ 20 vs < 20 CTCs	21	13 (57.1)	3.53 (1.50-8.31)	0.0037	11.92 (2.79-50.93)	0.0008
Joint effect of baseline CTC and CTC-clusters						
Associated with OS						
< 20 CTCs	33	10 (30.3)	1.0		1.0	
≥ 20 CTCs without CTC-cluster	8	3 (37.5)	1.61 (0.43-6.04)	0.478	11.9 (1.5-94.7)	0.019
≥ 20 CTCs, ≥ 1 CTC-cluster	13	10 (76.9)	5.5 (2.19-13.76)	0.0002	11.9 (2.69-52.6)	0.0010
Abbreviations: CTC circulating tumor cell, OS overall survival, HR hazard ratio, CI confidence interval.						
* Adjusted for age, ECOG, subtype, number of metastatic sites, site of metastasis and treatments.						

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Table S5. Longitudinal changes in CTCs and CTC-clusters on relation to PFS and OS.

Variable	Total	Events, n (%)	HR (95% CI)	p Value	HR (95% CI) [#]	p Value [#]
Associated with PFS						
No CTC	28	7 (25.0)	1.00		1.00	
1-4 CTC without CTC-cluster	21	10 (47.6)	1.39 (0.52-3.72)	0.50	1.53 (0.56-4.61)	0.45
≥ 5 CTC without CTC-cluster	25	14 (56.0)	1.82 (0.71-4.61)	0.21	2.00 (0.73-5.43)	0.17
≥ 5 CTC ≥ 1 CTC-cluster	22	16 (72.7)	3.99 (1.64-9.73)	0.0022	4.65 (1.79-12.61)	0.0024
1-4 CTC without CTC-cluster			1.00		1.00	
≥ 5 CTC without CTC-cluster			1.31 (0.57-3.01)	0.52	1.227 (0.40-3.41)	0.69
≥ 5 CTC ≥ 1 CTC-cluster			2.88 (1.26-6.55)	0.0115	2.863 (1.00-8.15)	0.0489
Associated with OS						
No CTC	28	3 (10.7)	1.00		1.00	
1-4 CTC without CTC-cluster	21	6 (28.7)	1.62 (0.38-6.79)	0.51	3.32 (0.62-17.62)	0.16
≥ 5 CTC without CTC-cluster	25	12 (48.0)	4.01 (1.12-14.34)	0.0324	7.38 (1.68-32.43)	0.0081
≥ 5 CTC ≥ 1 CTC-cluster	22	15 (68.1)	7.91 (2.28-27.37)	0.0010	13.93 (3.22-60.25)	0.0004
1-4 CTC without CTC-cluster			1.00		1.00	
≥ 5 CTC without CTC-cluster			2.45 (0.86-6.99)	0.093	2.22 (0.64-7.68)	0.2051
≥ 5 CTC ≥ 1 CTC-cluster			4.84 (1.75-13.41)	0.0023	4.42 (1.25-15.63)	0.0209
Abbreviations: CTC circulating tumor cell, PFS progression-free survival, OS, overall survival, HR hazard ratio, CI confidence interval.						
[#] Adjusted for age, ECOG, subtype, number of metastatic sites, site of metastasis and treatments.						

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