SCIENTIFIC REPORTS

Received: 10 August 2016 Accepted: 25 January 2017 Published: 24 March 2017

OPEN Circulating tumor cell status monitors the treatment responses in breast cancer patients: a metaanalysis

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Whether circulating tumor cells (CTCs) can be used as an indicator of treatment response in breast cancer (BC) needs to be clarified. We addressed this issue by a meta-analysis. PubMed, EMBase and Cochrane library databases were searched in June 2016. Effect measures were estimated as pooled risk ratio (RR), odds ratio (OR) or mean difference by fixed- or random-effect models, according to heterogeneity of included studies. In total, 50 studies with 6712 patients were recruited. Overall analysis showed that there was a significant reduction of CTC-positive rate (RR = 0.68, 95% CI: 0.61–0.76, P < 0.00001) after treatment. Subgroup analyses revealed that neoadjuvant treatment, adjuvant treatment, metastatic treatment or combination therapy could reduce the CTC-positive rate, but surgery could not; moreover, the reduction was only found in HER2+ or HER2- patients but not in the triple-negative ones. Reduction of CTC-positive rate was associated with lower probability of disease progression (OR = 0.54, 95% CI: 0.33-0.89, P = 0.01) and longer overall survival period (mean difference = 11.61 months, 95% CI: 8.63–14.59, P < 0.00001) as well as longer progression-free survival period (mean difference = 5.07 months, 95% CI: 2.70–7.44, P < 0.0001). These results demonstrate that CTC status can serve as an indicator to monitor the effectiveness of treatments and guide subsequent therapies in BC.

Metastasis is the major cause of cancer-related death in patients with breast cancer (BC)¹. Despite the improvements in treatment, metastatic relapse may occur in about 30% of BC patients with lymph node-negative axilla and about 50% of BC patients with positive axilla within 5 years². During the process of metastasis, cancer cells shed from primary tumors and migrate to distal sites through the blood or lymphatic system. Those migrating cells found in peripheral blood are known as circulating tumor cells (CTCs). CTCs are the metastatic precursors, which may have potential roles not only in predicting the risk of metastatic relapse and monitoring the treatment efficacy, but also in acting as a therapeutic target for preventing metastasis of cancers, including BC³⁻⁹.

Up to now, CTCs have been well studied and are currently being used in the clinical setting¹⁰. However, there are controversial results about the effectiveness of different treatments to reduce CTCs. For example, Martin M. et al. analyzed the change of CTCs in 117 BC patients and observed a substantial decline in CTC-positive rate after chemotherapy¹¹, but Rack B. et al. conducted a larger perspective study with 2026 BC patients and found that the detection rate of CTCs after chemotherapy (22.1%) was even a bit higher than the baseline condition (21.5%)¹². In addition, it needs also to be clarified whether molecular subtypes of BC affect CTC status under same treatments¹³. Hence, we conducted a meta-analysis of the published researches with measurement of CTCs before and after treatment in BC patients, and estimated the CTC-reducing effect of the current anti-tumor therapies. The CTC-reducing effects by different treatments were investigated separately in subgroup analyses, so were the effects in patients with different molecular subtypes. Then we also analyzed the relationship between reduction of CTCs and disease progression probability as well as survival period. This study followed the PRISMA criterions.

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Figure 1. Flowchart for article search.

Results

Study characteristics. We initially retrieved 1004 articles through database searching. 846 articles were excluded based on the title and abstract. Further, 108 articles were excluded after reviewing full-texts, in which the results of CTC status before and after treatment were not completely contained, or sample size was less than 20, and or duplicated data with other studies was reported. Finally, 50 studies were eligible for meta-analysis (Fig. 1)^{4,5,9,11-57}.

The eligible studies were conducted between 2009 and 2016 in America (USA), Asia (China, Japan and Korea) and Europe (Belgium, Czech, Denmark, France, Germany, Greece, Italy, Norway, Russia, Slovakia, Spain, and Turkey). The sample sizes of studies ranged from 21 to 2026 patients, accumulating 8019 patients before treatments and 6712 patients after treatments in total. Some studies reported the results in patients with particular molecular subtype BC, such as the HER2-positve or the triple-negative ones. The treatments performed in the studies included surgery, neoadjuvant treatment, adjuvant treatment, metastatic treatment, etc., either alone or in combination. The main characteristics of these studies are listed in Table S1. The quality of the studies was also estimated (Table S2).

Overall CTC-positive rate is significantly decreased after therapies. The CTC status was detected with different platforms or methods and presented with different indicators in the 50 studies with 6712 patients. The CTC-positive rate was reported in all of the studies, in which the different cut-off values of CTC count for CTC-capturing methods were used, such as \geq 5 CTCs/7.5 mL, \geq 1 CTCs/7.5 mL and so on, and the different expression thresholds of epithelial genes (EpCAM, CK18, CK19) were used for RT-PCR method. Some studies also presented the CTC status as CTC count. We first performed an overall analysis of the 50 studies with 6712 patients with CTC-positive rate by the random-effects model, and found that the CTC-positive rate was significantly decreased after treatment compared to the baseline (RR = 0.68, 95% CI: 0.61 to 0.76, *P* < 0.00001; I² = 73%, *P* < 0.00001) (Fig. 2).

We then analyzed the change of CTC counts in the 7 studies (2324 cases) that simultaneously reported CTC-positive rate and CTC count before and after treatments. No significant change of CTC counts was observed (mean difference = -1.17 CTCs/7.5 mL, 95% CI: -3.17 to 0.84, P = 0.25; $I^2 = 65\%$, P = 0.009) (Fig. 3), while a significant reduction was observed with CTC-positive rate in the 7 studies investigated after treatment (RR = 0.65, 95% CI: 0.48 to 0.87, P = 0.004; $I^2 = 85\%$, P < 0.00001) (Figure S1). This inconsistent results between CTC-positive rate and CTC count might attribute to the heterogeneity among the studies, because the CTC count, a continuous variable, could be more susceptible to the variance caused by individual study than the CTC-positive rate, a dichotomized variable. So we made a sensitivity analysis by removing each study, and found that one of them (Bidard FC 2012) substantially affected the result. Once it was excluded, the significant decrease of CTC level after treatment was observed compared to pre-treatment (mean difference = -0.94, 95% CI: -1.49 to -0.38, P = 0.0010 by fixed-effects model). These results confirmed the promising application of CTCs in monitoring the effectiveness of treatments for BC.

In addition, different methods as well as different cut-off values for a method were used in the studies. Hence, we also investigated the CTC-positive rate before and after treatment in the subgroups of different CTC-measuring methods. A significant reduction of CTC-positive rate after treatment was observed no matter how the CTC positivity was defined as \geq 5CTCs/7.5 mL, \geq 1CTCs/7.5 mL, or other threshold including a threshold for RT-PCR technique (Fig. 4).

After treatment		atment	Before tre	eatment		Risk Ratio	Risk Ratio
Study	Events	Total	Events	Total	Weight	M-H, Random, 95% 0	CI M-H, Random, 95% CI
Aurilio G 2012	21	56	16	56	2.0%	1.31 [0.77, 2.24]	
Azim HA Jr 2013	4	41	5	46	0.7%	0.90 [0.26, 3.12]	
Barnadas A 2014	15	100	42	122	2.0%	0.44 [0.26, 0.74]	
Bian L 2014	31	233	88	227	2.6%	0.34 [0.24, 0.50]	
Bidard FC 2010	5	42	35	65	1.2%	0.22 [0.09, 0.52]	
Bidard FC 2012	75	203	174	267	3.2%	0.57 [0.46, 0.69]	-
Bidard FC 2013	15	85	22	95	1.8%	0.76 [0.42, 1.37]	
Boutrus RR 2013	12	46	18	55	1.7%	0.80 [0.43, 1.48]	
Cabinakova M 2015	4	32	10	32	0.9%	0.40 [0.14, 1.14]	
Cristofanilli M 2004	49	163	100	177	3.0%	0.53 [0.41, 0.70]	-
Cristofanilli M 2009	21	102	51	102	2.3%	0.41 [0.27, 0.63]	
Daskalakis M 2011	7	104	2	104	0.5%	3.50 [0.74, 16.45]	
Hartkopf AD 2011	16	58	31	58	2.1%	0.52 [0.32, 0.84]	
Hayashi N 2012	21	49	31	52	2.5%	0.72 [0.49, 1.07]	
Horn P 2014	5	39	22	47	1.1%	0.27 [0.11, 0.66]	
Jiang ZF 2013	39	233	115	294	2.8%	0.43 [0.31, 0.59]	- -
Karaba M 2013	10	23	33	124	1.9%	1.63 [0.94, 2.83]	
Kasimir-Bauer S 2016	14	41	19	48	1.9%	0.86 [0.50, 1.50]	
Lavrov AV 2014	1	21	12	30	0.3%	0.12 [0.02, 0.85]	
Magbanua MJ 2015	29	89	42	95	2.5%	0.74 [0.51, 1.07]	
Maltoni R 2015	9	43	13	48	1.4%	0.77 [0.37, 1.63]	——————————————————————————————————————
Martín M 2013	28	99	47	99	2.5%	0.60 [0.41, 0.87]	
Mathiesen RR 2013	9	84	15	82	1.3%	0.59 [0.27, 1.26]	
Mego M 2012	9	21	6	21	1.2%	1.50 [0.65, 3.47]	
Mikulová V 2014	6	54	17	54	1.2%	0.35 [0.15, 0.83]	
Müller V 2005	17	43	14	43	1.9%	1.21 [0.69, 2.14]	-
Nadal R 2012	29	73	31	73	2.5%	0.94 [0.63, 1.38]	
Nakayama Y 2013	2	29	3	29	0.4%	0.67 [0.12, 3.70]	
Neugebauer JK 2013	109	392	144	392	3.2%	0.76 [0.62, 0.93]	-
Nolé F 2008	23	79	49	80	2.5%	0.48 [0.32, 0.70]	
Peeters DJ 2014	17	73	70	154	2.3%	0.51 [0.33, 0.80]	
Pierga JY 2008	15	86	22	97	1.8%	0.77 [0.43, 1.39]	
Pierga JY 2013	7	38	20	41	1.4%	0.38 [0.18, 0.79]	_
Pierga JY 2015	5	38	18	52	1.1%	0.38 [0.15, 0.93]	
Rack B 2014	330	1493	435	2026	3.4%	1.03 [0.91, 1.17]	t
Reinholz MM 2011	25	47	53	86	2.8%	0.86 [0.63, 1.18]	
Roop RP 2013	11	19	12	22	1.9%	1.06 [0.62, 1.82]	
Serrano MJ 2009	47	71	57	92	3.1%	1.07 [0.85, 1.35]	+
Serrano MJ 2012	12	24	17	24	2.2%	0.71 [0.44, 1.14]	
Smerage JB 2013	18	61	38	66	2.3%	0.51 [0.33, 0.80]	
Tokudome N 2011	5	23	9	28	1.0%	0.68 [0.26, 1.74]	
Tryfonidis K 2013	15	62	39	68	2.1%	0.42 [0.26, 0.69]	
Turker I 2013	7	34	4	34	0.8%	1.75 [0.56, 5.43]	
Ušiaková Z 2014	20	180	57	180	2.2%	0.35 [0.22, 0.56]	
van Dalum G 2015	66	367	75	403	2.8%	0.97 [0.72, 1.30]	+
Wallwiener M 2014	57	201	133	393	3.0%	0.84 [0.65, 1.09]	
Wang HY 2015	25	203	31	221	2.1%	0.88 [0.54, 1.43]	
Xenidis N 2009	143	437	179	437	3.3%	0.80 [0.67, 0.95]	-
Xenidis N 2013	190	545	237	545	3.4%	0.80 [0.69, 0.93]	-
Zhang JL 2015	16	33	21	33	2.3%	0.76 [0.49, 1.18]	-+
Total (95% CI)		6712		8019	100.0%	0.68 [0.61, 0.76]	•
Total events	1666		2734				
Heterogeneity: Tau ² = 0.0	9; Chi ² = 178	3.58, df =	49 (P < 0.0	0001); l²	= 73%	-	0.02 0.1 1 10 50
Test for overall effect: Z =	6.83 (P < 0.	00001)					After treatment lower Before treatment lower

Figure 2. Forest plot for the comparison of CTC-positive rate before and after treatment: overall analysis. The black diamond and its extremities indicate the pooled risk ratio center and 95% confidential interval.

	Afte	r treatn	nent	Before treatment				Mean Difference	Mean Difference
Study	Mean	SD	Total	Mean SD Total			Weight	IV, Random, 95% CI	IV, Random, 95% CI
Bidard FC 2010	0	17.8	42	7	124	65	0.4%	-7.00 [-37.62, 23.62]	
Bidard FC 2012	6	33.7	203	81.7	324.8	267	0.3%	-75.70 [-114.93, -36.47]	· · · · · · · · · · · · · · · · · · ·
Horn P 2014	6.4	37.3	39	353	3,450.8	47	0.0%	-346.60 [-1333.22, 640.02]] ← →
Pierga JY 2008	1	1.5	86	2	2.7	97	38.6%	-1.00 [-1.62, -0.38]	•
Rack B 2014	0.2	5.2	1493	0.2	34.5	2026	32.5%	0.00 [-1.53, 1.53]	•
Serrano MJ 2012	1.4	2.1	24	3.4	4.9	24	27.5%	-2.00 [-4.13, 0.13]	•
Xenidis N 2009	0	166.7	437	0.3	185.8	437	0.7%	-0.30 [-23.70, 23.10]	
Total (95% CI) 2324 2963 Heterogeneity: Tau ² = 2.60; Chi ² = 17.01, df = 6 (P = 0.009); l ² = 65%							100.0%	-1.17 [-3.17, 0.84]	
Test for overall effect: Z = 1.14 (P = 0.25)									After treatment lower Before treatment lower

Figure 3. Forest plot for the comparison of CTC count before and after treatment: overall analysis. The black diamond indicates the difference of CTC counts (cells/7.5 mL peripheral blood; the post-therapeutic counts minus the pre-therapeutic counts). Its center indicates the mean and the extremities indicate the 95% confidential interval.

CTC-positive rates are decreased after neoadjuvant treatment, adjuvant treatment, metastatic treatment and combination therapy, but not after surgery. Clinically, many therapeutic methods are employed in treatment of BC. The therapeutic methods involved in the pooled 50 studies could be roughly classified into neoadjuvant setting, adjuvant setting, metastatic setting, surgery and combination therapy. To clarify the efficacy of various therapies on decreasing CTC-positive rate, we performed a subgroup analysis. Compared to pre-treatment, CTC-positive rate were decreased after treatment in the neoadjuvant setting

	After tre	atment	Before tr	eatment		Risk Ratio	Risk Ratio
itudv	Evente	Total	Evente	Total	Weight	M-H. Random 95% (Cl M-H. Random 95% Cl
CTCe/7 5ml	_701103	· Jul	2.0113		maight		
urilio G 2012	21	56	16	56	1 9%	1 31 [0 77 2 24]	
arnadas A 2014	15	100	42	122	1.9%	0 44 [0 26, 0 74]	_
ian 2014	31	233	88	227	2.4%	0.34 [0.24, 0.50]	
idard FC 2010	5	42	35	65	1.2%	0.22 [0.09, 0.52]	
idard FC 2012	26	198	117	267	2.4%	0.30 [0.20, 0.44]	
ristofanilli M 2004	49	163	100	177	2.7%	0.53 [0.41, 0.70]	
ristofanilli M 2009	21	102	51	102	2.2%	0.41 [0.27, 0.63]	
artkopf AD 2011	16	58	31	58	2.1%	0.52 [0.32, 0.84]	
ayashi N 2012	9	49	18	52	1.5%	0.53 [0.26, 1.07]	
orn P 2014	5	39	22	47	1.1%	0.27 [0.11, 0.66]	
ang ZF 2013	39	233	115	294	2.6%	0.43 [0.31, 0.59]	-
agbanua MJ 2015	29	89	42	95	2.4%	0.74 [0.51, 1.07]	
artín M 2013	28	99	47	99	2.4%	0.60 [0.41, 0.87]	
ego M 2012	2	21	3	21	0.4%	0.67 [0.12, 3.59]	
olé F 2008	23	79	49	80	2.4%	0.48 [0.32, 0.70]	
eeters DJ 2014	17	73	70	154	2.2%	0.51 [0.33, 0.80]	
ierga JY 2013	3	38	9	41	0.7%	0.36 [0.11, 1.23]	
merage JB 2013	18	61	38	66	2.2%	0.51 [0.33, 0.80]	—
okudome N 2011	5	23	9	28	1.0%	0.68 [0.26, 1.74]	
rytonidis K 2013	5	62	20	68	1.1%	0.27 [0.11, 0.69]	
urker I 2013	7	34	4	34	0.8%	1.75 [0.56, 5.43]	
alwiener M 2014	57	201	133	393	2.8%	0.84 [0.65, 1.09]	▲ 1
abiotal (95% CI)	404	2053	4050	2040	40.3%	0.51 [0.43, 0.61]	•
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et for overall offoot: 7	= 7 50 /D -	0.10, 01 =	21 (F < 0.0	001), I* = t	J++ 70		0.02 0.1 1 10 5
sation overall effect. Z	- 1.00 (P <	0.00001)					After treatment lower Before treatment lower
	After treat	nent	Refore tree	tment		Risk Patio	Risk Patio
udy	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% Cl
TC/7.5mL						,	
dard FC 2012	75	203	174	267	46.6%	0.57 [0.46, 0.69]	T
idard FC 2013	15	85	22	95	6.4%	0.76 [0.42, 1.37]	-+
ayashi N 2012	21	49	31	52	9.3%	0.72 [0.49, 1.07]	
altoni R 2015	9	43	13	48	3.8%	0.77 [0.37, 1.63]	—- + —
ego M 2012	9	21	6	21	1.9%	1.50 [0.65, 3.47]	-+
erga JY 2008	15	86	22	97	6.4%	0.77 [0.43, 1.39]	
ierga JY 2013	7	38	20	41	6.0%	0.38 [0.18, 0.79]	
ierga JY 2015	5	38	18	52	4.7%	0.38 [0.15, 0.93]	
oop RP 2013	11	10			2 49/	1 06 10 62 1 821	
		13	12	22	3.470	1.00 [0.02. 1.02]	
rvfonidis K 2013	15	62	12 39	22 68	11.5%	0.42 [0.26, 0.69]	- - -
ryfonidis K 2013 ubtotal (95% CI) otal events	15 182	62 644 (P = 0.07	12 39 357 '): 1 ² = 44%	68 763	11.5% 100.0%	0.42 [0.26, 0.69] 0.61 [0.53, 0.70]	
ryfonidis K 2013 ubtotal (95% CI) otal events eterogeneity: Chi ² = 16 est for overall effect: Z ;	15 182 6.05, df = 9 = 6.83 (P <	62 644 (P = 0.07 0.00001	12 39 357 '); ² = 44%)	22 68 763	11.5% 100.0%	0.42 [0.26, 0.69] 0.61 [0.53, 0.70]	0.02 0.1 1 10 5 After treatment lower Before treatment lower
ryfonidis K 2013 ubtotal (95% CI) otal events leterogeneity: Chi ² = 16 est for overall effect: Z	15 182 6.05, df = 9 = 6.83 (P <	62 644 (P = 0.07 0.00001	12 39 357 '); ² = 44%) Before 1	22 68 763	11.5% 100.0%	0.42 [0.23, 0.69] 0.61 [0.53, 0.70]	0.02 0.1 1 10 5 After treatment lower Before treatment lower
ryfonidis K 2013 ubtotal (95% CI) otal events leterogeneity: Chi ² = 16 est for overall effect: Z	15 182 6.05, df = 9 = 6.83 (P < <u>After tr</u> Events	62 644 (P = 0.07 : 0.00001 <u>eatment</u> Total	12 39 357 '); I ² = 44%) <u>Before 1</u> Events	22 68 763 treatment Total	11.5% 100.0%	0.42 [0.52, 1.62] 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% CI	0.02 0.1 1 10 5 After treatment lower Before treatment lowe Risk Ratio M-H, Fixed, 95% Cl
ryfonidis K 2013 ubtotal (95% CI) otal events eterogeneity: Chi ² = 16 est for overall effect: Z : tudy ositive by other thres	15 182 5.05, df = 9 = 6.83 (P < <u>After tr</u> <u>Events</u> shold	62 644 (P = 0.07 0.00001 reatment	12 39 357 '); ² = 44%) <u>Before 1</u> Events	22 68 763 treatment Total	11.5% 100.0% Weight	No (10.2, 1.82) 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] - Risk Ratio M-H, Fixed, 95% CI	0.02 0.1 1 10 5 After treatment lower Before treatment lowe Risk Ratio M-H, Fixed, 95% Cl
ryfonidal (35% CI) otal events eterogeneity: Chi ² = 16 est for overall effect: Z ; tudy ositive by other thres zim HA Jr 2013	15 182 5.05, df = 9 = 6.83 (P < <u>After tr</u> <u>Events</u> shold 4	62 644 (P = 0.07 : 0.00001 <u>eatment</u> Total	12 39 357 '); I ² = 44%) <u>Before 1</u> <u>Events</u>	22 68 763 treatment Total 46	11.5% 100.0% Weight 0.6%	0.42 [0.26, 0.69] 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% CI 0.90 [0.26, 3.12]	0.02 0.1 1 10 5 After treatment lower Before treatment lowe Risk Ratio M-H, Fixed, 95% Cl
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ryfonidis K 2013 ubtotal (95% CI) total events eterogeneity: Chi ² = 16 est for overall effect: Z : tudy ositive by other thres zim HA Jr 2013 abinakova M 2015 axrov AV 2014	15 182 6.05, df = 9 = 6.83 (P < <u>After tr</u> Events shold 4 4 1	62 644 (P = 0.07 0.00001 eeatment Total 41 32 21	12 39 357 (); ² = 44%) <u>Before 1</u> <u>Events</u> 5 10 12	22 68 763 treatment Total 46 32 30	0.6% 1.3% 100.0% Weight 0.6% 1.3% 1.3%	No (10.62, 1.62) 0.42 (0.26, 0.69) 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% Cl 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85]	0.02 0.1 1 10 5 After treatment lower Risk Ratio M-H, Fixed, 95% Cl
ryfonidis K 2013 ubtotal (95% CI) otal events eterogeneity: Chi ² = 16 est for overall effect: Z : tudy ositive by other thres zim HA Jr 2013 abinakova M 2015 avrov AV 2014 lathiseen RR 2013	15 182 5.05, df = 9 = 6.83 (P < <u>After tr</u> <u>Events</u> shold 4 4 1 9	62 644 (P = 0.07 0.00001 eeatment Total 41 32 21 84	12 39 357 '); I ² = 44%) Before 1 Events 5 10 12 15	22 68 763 treatment Total 46 32 30 82	.11.5% 100.0% Weight 0.6% 1.3% 1.3% 2.0%	No (10.62, 1.62) 0.42 (0.26, 0.69) 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% Cl 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.59 [0.27, 1.26]	0.02 0.1 1 10 5 After treatment lower Before treatment lower Risk Ratio M-H, Fixed, 95% Cl
yfondis K 2013 ubtotal (95% CI) stal events eterogeneity: Chi ² = 16 sst for overall effect: Z utdy ositive by other thres zim HA Jr 2013 avrov AV 2014 athlesen RR 2013 ikulová V 2014	15 182 6.05, df = 9 = 6.83 (P < <u>After tr</u> <u>Events</u> shold 4 4 1 9 6	62 644 (P = 0.07 0.00001 <u>eatment</u> Total 41 32 21 84 54	12 39 357 '); ² = 44%) Before t Events 5 10 12 15 17	22 68 763 treatment Total 46 32 30 82 54	.14.5% 11.5% 100.0% Weight 0.6% 1.3% 2.0% 2.2%	No (10.62, 1.62) 0.42 (0.26, 0.69) 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.59 [0.27, 1.26] 0.59 [0.27, 1.26]	0.02 0.1 1 10 5 After treatment lower Before treatment lower M-H, Fixed, 95% Cl
yfonidis K 2013 ubtotal (95% CI) tal events eterogeneity: Chi ² = 16 est for overall effect: Z udy ositive by other thres zim HA Jr 2013 abinakova M 2015 abinakova M 2015 atvirov AV 2014 lathiesen RR 2013 likulovà V 2014	15 182 8.05, df = 9 = 6.83 (P < <u>After tr</u> <u>Events</u> shold 4 4 1 9 6 17	62 644 (P = 0.07 0.00001 <u>eatment</u> Total 41 32 21 84 54 54	12 39 357); l ² = 44%) Before 1 Events 5 10 12 15 17 14	22 68 763 treatment Total 46 32 30 82 54 43		No (10.62, 1.62) 0.42 (0.26, 0.69) 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% Cl 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14]	0.02 0.1 1 10 5 After treatment lower Risk Ratio M-H, Fixed, 95% Cl
yfondis K 2013 ubtotal (95% CI) stal events eterogeneity: Chi ² = 16 esst for overall effect: Z utuby tudy toty tabinakova M 2015 avrov AV 2014 athiesen RR 2013 ikulová V 2014 ülter V 2005 adal R 2012	15 182 6.05, df = 9 = 6.83 (P < After tr Events shold 4 4 1 9 6 17 29	62 644 (P = 0.07 0.00001 eatment Total 41 32 21 84 54 43 73	12 39 357 (); ² = 44%) Before Events 5 10 12 15 17 14 31	22 68 763 treatment Total 46 32 30 82 54 43 73	Weight 0.6% 1.3% 2.0% 2.2% 1.9% 4.1%	Risk Ratio M-H, Fixed, 95% Cl 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.94 [0.63, 1.38]	0.02 0.1 1 10 5 After treatment lower Before treatment lower Risk Ratio M-H, Fixed, 95% Cl
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yfonidis K 2013 ubtotal (95% CI) tal events eterogeneity: Chi ² = 16 est for overall effect: Z udy ositive by other thres zim HA Jr 2013 abinakova M 2015 abinakova M 2015 abinakova M 2014 uthiesen RR 2013 ikulová V 2014 uthies RR 2012 adal R 2012 akayama Y 2013 eugebauer JK 2013	15 182 6.05, df = 9 6.83 (P < After tr Events shold 4 4 1 9 6 17 29 2 109	(P = 0.07 0.00001 eatment Total 41 32 21 84 54 43 73 29 392	12 39 357 (); ² = 44%)) Before (+ Events 5 10 12 15 17 14 31 3 144	22 68 763 treatment Total 46 32 30 82 54 43 73 29 392	Weight 0.6% 1.3% 2.2% 1.9% 4.1% 0.4% 19.0%	Risk Ratio M-H, Fixed, 95% Cl 0.90 [0.26, 3.12] 0.42 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 0.59 [0.27, 1.26] 0.36 [0.12, 3.70] 0.94 [0.63, 1.38] 0.67 [0.12, 3.70]	0.02 0.1 1 10 5 After treatment lower Before treatment lower Risk Ratio M-H, Fixed, 95% Cl
yfondis K 2013 ubtotal (95% CI) stal events eterogeneity: Chi ² = 16 esst for overall effect: Z utuby tuby toty the by other thres proved by the threse stim HA Jr 2013 abinakova M 2015 avrov AV 2014 lathiesen RR 2013 akilavlová V 2014 ušiler V 2005 adal R 2012 akayama Y 2013 eugebauer JK 2013 ack B 2014	15 182 5.05, df = 9 = 6.83 (P < After tr Events shold 4 4 1 9 6 17 29 2 109 330	(P = 0.07 62 644 (P = 0.07 0.00001 eatment Total 41 32 21 84 54 43 73 29 392 1493	12 39 357); I ² = 44%)) Before I Events 5 10 12 15 5 17 14 31 44 31	22 68 763 treatment Total 46 32 30 82 54 43 73 29 392 2026	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 2.2% 1.9% 4.1% 0.4% 19.0% 48.8%	Risk Ratio M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.26, 3.12] 0.40 [0.14, 1.14] 0.15 [0.27, 0.28] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.94 [0.63, 1.38] 0.67 [0.12, 3.70] 0.76 [0.62, 0.93] 1.03 [0.91, 1.17]	0.02 0.1 1 10 5 After treatment lower Before treatment lower M-H, Fixed, 95% Cl
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ryfonidis K 2013 ubtotal (95% CI) total events eterogeneity: Chi ² = 11 east for overall effect: Z : tudy ositive by other thres zim HA Jr 2013 abinakova M 2015 avrov AV 2014 lathiesen RR 2013 likulová V 2014 Lithiesen RR 2013 adal R 2012 akayama Y 2013 eugebauer JK 2013 ack B 2014 errano MJ 2009 errano MJ 2012 an Dalum G 2015	15 182 3.05, df = 9 = 6.83 (P < After tr Events shold 4 4 1 9 6 17 29 2 109 3300 47 12 66	62 644 (P = 0.07 0.00001 eatment Total 41 32 21 84 43 73 29 392 392 392 392 392 392 392 392 392	12 39 357); l ² = 44%) Before t Events 5 10 12 15 17 14 31 3 144 435 57 17 75	22 68 763 treatment Total 46 32 30 82 54 43 73 29 392 2026 92 2026 92 24 403	Weight 11.5% 100.0% Weight 0.6% 1.3% 1.3% 1.3% 2.0% 2.2% 1.9% 4.1% 0.4% 19.0% 48.8% 6.6% 2.2% 9.5%	No (10.62, 1.62) 0.42 (0.26, 0.69) 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.59 [0.27, 1.26] 0.59 [0.27, 1.26] 0.50 [0.27, 1.26] 0.50 [0.27, 1.26] 0.50 [0.26, 0.35] 1.07 [0.85, 1.35] 0.77 [0.44, 1.14] 0.97 [0.72, 1.30]	0.02 0.1 1 10 5 After treatment lower Before treatment lower M-H, Fixed, 95% Cl
yfondis K 2013 ubtotal (95% CI) stal events eterogeneity: Chi ² = 16 sst for overall effect: Z utdy ositive by other thres zim HA Jr 2013 abinakova M 2015 avrov AV 2014 uthiesen RR 2013 athia X 2014 uthiesen RR 2013 adal R 2014 eugebauer JK 2013 adak 2014 errano MJ 2009 errano MJ 2009 errano MJ 2009 errano MJ 2009 errano MJ 2015 ubtotal (95% CI)	15 182 5.05, df = 9 = 6.83 (P < After tr Events shold 4 1 9 6 6 17 29 2 109 330 47 12 66	(P = 0.07 62 644 (P = 0.07 0.00001 eatment 41 32 21 84 54 43 73 29 392 1493 71 24 367 2724	12 39 357); l² = 44%) Before t Events 5 10 12 15 17 14 31 3 144 435 57 17 75	22 68 763 763 763 763 46 32 30 82 54 43 73 29 392 2026 92 24 403 3326	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 2.2% 1.9% 4.1% 0.4% 19.0% 48.8% 6.6% 2.2% 9.5% 100.0%	Risk Ratio M-H, Fixed, 95% CI 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.40 [0.14, 1.14] 0.42 [0.02, 0.85] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.94 [0.63, 1.38] 0.67 [0.12, 3.70] 0.76 [0.62, 0.93] 1.03 [0.91, 1.17] 1.07 [0.65, 1.35] 0.71 [0.44, 1.14] 0.97 [0.72, 1.30] 0.92 [0.24, 1.00]	0.02 0.1 1 10 5 After treatment lower Before treatment lower M-H, Fixed, 95% Cl
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ryfonidis K 2013 ubtotal (95% CI) total events eterogeneity: Chi ² = 16 east for overall effect: Z : tudy ositive by other thres zim HA Jr 2013 abinakova M 2015 avrov AV 2014 lathiesen RR 2013 akkuová V 2014 lathiesen RR 2013 akkuová V 2014 eagebauer JK 2013 akk 2014 errano MJ 2009 errano MJ 2009 errano MJ 2012 an Dalum G 2015 ubtotal (95% CI) otal events eterogeneity: Chi ² = 23	15 182 3.05, df = 9 = 6.83 (P < After tr Events shold 4 4 4 4 1 9 6 17 29 09 2 109 3300 47 12 66 3.325, df = 12	(P = 0.07 eatment Total 41 32 21 44 43 73 392 1493 392 1493 367 2724 (P = 0.0	12 39 357); l ² = 44%) Before Events 5 10 12 15 17 14 31 3 144 435 57 17 75 835 3); l ² = 48%	22 68 763 Total 46 32 30 82 54 43 73 29 392 2026 92 24 403 3326	Weight 11.5% 100.0% Weight 0.6% 1.3% 1.3% 2.0% 1.9% 4.1% 0.4% 19.0% 48.8% 6.6% 2.2% 9.5% 100.0%	Note [0.62, 1.62] 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.94 [0.63, 1.38] 0.67 [0.12, 3.70] 0.76 [0.62, 0.93] 1.21 [0.71 [0.46, 1.14] 0.97 [0.72, 1.30] 0.92 [0.84, 1.00]	0.02 0.1 1 10 5 After treatment lower Risk Ratio M-H, Fixed, 95% Cl
ryfonidis K 2013 ubtotal (95% CI) otal events eterogeneity: Chi ² = 16 esst for overall effect: Z tudy tudy tudy ositive by other thres zim HA Jr 2013 avrov AV 2014 Iathiesen RR 2013 Iikulová V 2014 Iathiesen RR 2013 Iikulová V 2014 Iathiesen RR 2013 Iikulová V 2014 Lither 2015 Iadal R 2012 Iadal R 2012 Iadal R 2012 Iadayama Y 2013 leugebauer JK 2013 leugebauer JK 2013 ack B 2014 errano MJ 2009 errano MJ 2012 ubtotal (95% CI) otal events leterogeneity: Chi ² = 23 esst for overall effect: Z	15 182 6.05, df = 9 = 6.83 (P < After tr Events shold 4 4 4 1 9 6 17 29 2 109 3300 47 12 66 636 632, 5, df = 12 = 1.87 (P =	(P = 0.07 0.00001 eatment 411 32 211 84 43 73 29 367 2724 433 71 24 1493 71 24 1493 71 2724 44 26 1493 71 2724 45 2772 45 2772 45 2772 45 2772 45 2772 45 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772 2772 4772	12 39 357); l² = 44%) Before t Events 5 10 12 15 17 14 31 3 144 435 57 7 7 8 5 5 3); l² = 48%	22 68 68 763 763 763 46 32 30 82 54 43 73 29 392 2026 92 24 403 3326	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 1.9% 4.1% 0.4% 19.0% 48.8% 6.6% 2.2% 9.5% 100.0%	No (10.62, 1.62) 0.42 (0.26, 0.69) 0.61 [0.53, 0.70] M-H, Fixed, 95% Cl 0.90 (0.26, 3.12) 0.40 (0.14, 1.14) 0.12 (0.02, 0.85] 0.59 (0.27, 1.26) 0.59 (0.27, 1.26) 0.76 (0.62, 0.93) 1.03 (0.91, 1.17] 1.07 (0.65, 1.35] 0.71 (0.44, 1.14) 0.97 (0.72, 1.30) 0.92 [0.84, 1.00]	0.02 0.1 1 10 5 After treatment lower Before treatment lower
ryfondis K 2013 ubtotal (95% CI) otal events eterogeneity: Chi ² = 16 est for overall effect: Z tudy ositive by other thress zim HA Jr 2013 abinakova M 2015 avrov AV 2014 lathiesen RR 2013 akayama Y 2013 ack B 2014 eurano MJ 2012 ack B 2014 eurano MJ 2012 ack B 2014 eurano MJ 2013 ack B 2014 eurano MJ 2019 errano MJ 2019 arrano MJ 2019 ubtotal (95% CI) otal events eterogeneity: Chi ² = 22 est for overall effect: Z	15 182 5.05, df = 9 = 6.83 (P < After tr Events shold 4 4 1 9 6 17 29 2 109 330 47 12 66 63 22, df = 12 = 1.87 (P =	(P = 0.07 0.00001 eatment 41 32 21 44 43 73 392 1493 372 392 1493 367 2724 (P = 0.0 0.006)	12 39 357); ² = 44%) Before t Events 5 10 12 15 17 14 31 3 144 435 57 75 835; ² = 48%	22 68 68 763 763 46 32 30 82 54 43 73 29 392 2026 92 24 403 3326	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 2.2% 1.9% 4.1% 0.4% 19.0% 48.8% 6.6% 2.2% 9.5% 100.0%	No (10.62, 1.62) 0.42 (0.26, 0.69) 0.61 [0.53, 0.70] M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.94 [0.63, 1.38] 0.76 [0.62, 0.93] 1.03 [0.91, 1.17] 1.07 [0.65, 1.35] 0.71 [0.44, 1.14] 0.97 [0.72, 1.30] 0.92 [0.84, 1.00]	0.02 0.1 10 5 After treatment lower Risk Ratio M-H, Fixed, 95% CI
ryfonidis K 2013 ubtotal (95% CI) otal events eterogeneity: Chi ² = 16 est for overall effect: Z tudy ositive by other thres zim HA Jr 2013 abinakova M 2015 avrov AV 2014 Iathiesen RR 2013 Italiaesen RR 2013 Italiaesen RR 2013 Italiaesen RR 2014 Italiaesen RR 2013 Italiaesen RR 2014 Italiaesen RR 2013 Italiaesen RR 2014 Italiaesen RR 2013 Italiaesen RR 2013 Italiaesen RR 2014 Italiaesen RR 2015 Italiaesen RR 2015	15 182 5.05, df = 9 = 6.83 (P < After tr Events shold 4 4 4 1 9 6 6 17 29 2 109 330 47 12 66 636 3.25, df = 12 = 1.87 (P = After tree	(P = 0.07 0.00001 eatment 41 32 21 44 43 37 392 392 1493 392 1493 392 1493 392 1493 392 1493 392 1493 392 1493 392 1493 392 1493 392 1493 392 1493 1493 1493 1493 1493 1493 1493 1493	12 39 357); l ² = 44%) Before tere 5 10 12 15 17 14 31 3 144 435 57 75 835 3); l ² = 48%	22 68 68 763 763 763 46 32 30 82 54 43 73 29 392 2026 920 2026 920 920 920 920 920 920 920 920 920 920	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 2.2% 4.1% 0.4% 19.0% 48.8% 6.6% 9.5% 100.0%	Risk Ratio M-H, Fixed, 95% Cl 90,02 [0.26, 0.69] 0.61 [0.53, 0.70] M-H, Fixed, 95% Cl 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.36 [0.12, 3.70] 0.76 [0.62, 0.93] 1.03 [0.91, 1.17] 1.07 [0.85, 1.35] 0.71 [0.44, 1.14] 0.97 [0.72, 1.30] 0.92 [0.84, 1.00]	0.02 0.1 1 0 5 After treatment lower Before treatment lower Risk Ratio M-H, Fixed, 95% Cl 0.02 0.1 1 10 5 After treatment lower Before treatment lower Risk Ratio
ryfonidis K 2013 ubtotal (95% CI) total events eterogeneity: Chi ² = 16 est for overall effect: Z : tudy tudy ositive by other threes zim HA Jr 2013 abinakova M 2015 avrov AV 2014 Lithiesen RR 2013 likulová V 2014 Lithiesen RR 2013 likulová V 2014 Lithiesen RR 2013 akayama Y 2013 ack B 2014 errano MJ 2019 errano MJ 2019 ubtotal (95% CI) total events eterogeneity: Chi ² = 22 est for overall effect: Z hudy	15 182 5.05, df = 9 = 6.83 (P < After tr Events shold 4 4 1 9 6 6 17 29 2 109 330 47 12 66 636 63.25, df = 12 = 1.87 (P = After treation of the short	(P = 0.07 0.00001 eatment 41 32 21 44 34 43 73 99 93 92 1493 367 7274 2724 2724 167 2724 1772	12 39 357); ² = 44%) Before ! Events 5 10 12 15 17 14 31 3 144 435 57 17 15 17 14 835 3); ² = 48% Before tre Events	22 68 68 763 763 763 46 32 30 82 54 43 73 29 392 2026 92 24 403 3326 84 82 54 73 29 82 2026 92 24 403 3326	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 2.2% 9.5% 100.0% Weight	Risk Ratio M-H, Fixed, 95% CI 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.94 [0.63, 1.38] 0.67 [0.12, 3.70] 0.76 [0.62, 0.93] 1.03 [0.91, 1.17] 1.07 [0.85, 1.35] 0.71 [0.44, 1.14] 0.97 [0.72, 1.30] 0.92 [0.84, 1.00]	0.02 0.1 10 5 After treatment lower Before treatment lower Risk Ratio 0.02 0.1 10 5 M-H, Fixed, 95% CI 0.02 0.1 10 5 Before treatment lower Before treatment lower
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yfonidis K 2013 ubtotal (95% CI) stal events teterogeneity: Chi ² = 16 ast for overall effect: Z utudy csitive by other threes zim HA Jr 2013 abinakova M 2015 arvor AV 2014 athiesen RR 2013 ikulová V 2014 athiesen RR 2013 ack B 2014 errano MJ 2009 arvan V 2013 ack B 2014 errano MJ 2019 ubtotal (95% CI) otal events eterogeneity: Chi ² = 22 est for overall effect: Z utudy TC positive by RT-PC ourtus RR 2013 exelative X 2014	15 182 5.05, df = 9 = 6.83 (P < After tr Events shold 4 4 4 1 9 6 6 17 29 2 109 330 47 12 66 6325, df = 12 = 1.87 (P = After tree Events R 12 7	(P = 0.07 0.00001 eatment Total 41 32 21 44 43 37 392 392 1493 392 1493 392 1493 392 1493 392 1493 392 1493 392 10.0006 10.	$\frac{12}{39}$ $\frac{357}{3}; ^2 = 44\%$) $\frac{Before i}{Events}$ $\frac{5}{10}$ 12 15 17 14 31 144 435 57 17 75 835 $3); ^2 = 48\%$ $Before tree$ $Events$ $\frac{18}{2}$	22 68 763 Treatment Total 46 32 30 82 54 43 73 29 392 2026 92 24 403 3326 24 403 3326 25 104	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 2.2% 9.5% 19.0% 48.8% 6.6% 9.5% 100.0% Weight 18%	Risk Ratio M-H, Fixed, 95% CI 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.14, 1.14] 0.12 [0.02, 0.85] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.35 [0.15, 0.83] 1.21 [0.69, 2.14] 0.40 [0.43, 1.38] 0.67 [0.12, 3.70] 0.76 [0.62, 0.93] 1.03 [0.91, 1.17] 1.07 [0.85, 1.35] 0.71 [0.44, 1.14] 0.97 [0.72, 1.30] 0.92 [0.84, 1.00] Risk Ratio M-H, Random, 95%	0.02 0.1 10 5 After treatment lower Before treatment lower Risk Ratio M-H, Fixed, 95% Cl 0.02 0.1 10 5 After treatment lower Before treatment lower Risk Ratio Cl M-H, Random, 95% Cl
ryfonidis K 2013 ubtotal (95% CI) total events eterogeneity: Chi ² = 16 east for overall effect: Z tudy tudy tudy tudy tudy tudy tudy tulier V 2005 akayama Y 2014 akinisen RR 2013 akik 2012 akayama Y 2014 akinisen RR 2013 akik 2012 akayama Y 2013 ack B 2014 errano MJ 2009 errano MJ 2009 errano MJ 2019 eterogeneity: Chi² = 23 est for overall effect: Z tudy tudy TC positive by RT-PC ourus RR 2013 askalakis M 2011 askabis M 2011 askabis M 2011 askalakis M 2011	15 162 3.05, df = 9 = 6.83 (P < After trr Events shold 4 4 4 1 9 6 17 29 0 3300 47 12 66 636 63.25, df = 12 = 1.87 (P = After tree Events R 12 7 10 10 10 10 10 10 10 10 10 10	41 32 41 32 41 32 41 32 21 84 43 73 71 21 84 43 73 392 392 343 71 2724 41 367 2724 367 100 0.06) 46 104 23 23	12 39 357); ² = 44%) Before t Events 5 10 12 15 17 14 31 3 144 435 57 17 75 835 835 835; ² = 48% Before tre Events 18 2 33	222 68 763 763 763 763 763 763 764 704 705 705 705 705 705 705 705 705 705 705	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 1.9% 4.1% 0.4% 19.0% 48.8% 0.4% 19.0% 48.8% 100.0%	Noise 1.06 1.082 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] Risk Ratio M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.34, 1.14] 0.12 [0.20, 0.85] 0.59 [0.27, 1.26] 0.35 [0.15, 0.83] 1.21 [0.90, 2.14] 0.94 [0.34, 1.38] 0.67 [0.12, 3.70] 0.76 [0.52, 0.93] 1.03 [0.91, 1.17] 1.07 [0.85, 1.35] 0.71 0.92 [0.84, 1.00] - - Risk Ratio M-H, Random, 95% 0.80 [0.43, 1.48] 3.50 [0.43, 1.48] 3.50 [0.42, 2.83]	0.02 0.1 1 10 5 After treatment lower Before treatment lower Risk Ratio M-H, Fixed, 95% Cl
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ryfonidis K 2013 ubtotal (95% CI) total events eterogeneity: Chi ² = 16 east for overall effect: Z : tudy total vents eterogeneity: Chi ² = 17 abinakova M 2015 arrox AV 2014 ubinakova M 2015 arrox AV 2014 athisean RR 2013 akayama Y 2013 eugebauer JK 2013 akayama Y 2014 eterogeneity: Chi ² = 23 est for overall effect: Z tudy TC positive by RT-PC outrus RR 2013 askatkis M 2011 araba M 2013 asimir-Bauer S 2016	15 182 5.05, df = 9 = 6.83 (P < After tr Events shold 4 4 4 1 9 9 2 109 3300 47 12 66 6.365, df = 12 = 1.87 (P = Events R 12 7 10 11 1 1	19 62 62 644 (P = 0.07 0.00001 41 32 41 34 54 54 43 37 292 392 31433 71 24 367 2724 367 2700.066 104 46 104 23 21	$\frac{12}{39}$ $\frac{357}{3}; ^{2} = 44\%$) $\frac{Before + 44\%}{2}$ $\frac{5}{10}$ $\frac{12}{15}$ $\frac{17}{14}$ $\frac{31}{31}$ $\frac{31}{144}$ $\frac{435}{37}$ $\frac{7}{17}$ $\frac{75}{835}$ $\frac{835}{3}; ^{2} = 48\%$ $\frac{Before tre}{Events}$ $\frac{18}{2}$ $\frac{2}{33}$ $\frac{32}{12}$	22 68 763 763 763 763 763 70 82 54 40 30 82 54 40 332 2026 92 24 403 3326 92 24 403 3326 92 24 403 3326	Weight 11.5% 100.0% Weight 0.6% 1.3% 1.3% 2.0% 1.9% 4.1% 0.4% 19.0% 48.8% 6.6% 2.2% 100.0% Weight 7.4% 1.8% 100.0%	Note [0.62, 1.62] 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] Risk Ratio	0.02 0.1 10 5 After treatment lower Before treatment lower Risk Ratio M-H, Fixed, 95% Cl 0.02 0.1 10 5 Before treatment lower Before treatment lower Risk Ratio Cl M-H, Random, 95% Cl
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ryfonidis K 2013 ubtotal (95% CI) otal events eterogeneity: Chi ² = 11 east for overall effect: Z tudy ositive by other thres zim HA Jr 2013 abinakova M 2015 avrov AV 2014 lathiesen RR 2013 ilkulová V 2014 aklaviesen RR 2013 akulová V 2014 aklaviesen RR 2013 akulová V 2014 errano MJ 2012 akayama Y 2013 eugebauer JK 2013 akk B 2014 errano MJ 2012 atal erros en J ubtotal (95% CI) otal events eterogeneity: Chi ² = 23 est for overall effect: Z tudy TC positive by RT-PC outrus RR 2013 askalakis M 2011 askalakis M 2011 askalakis M 2011 askalakis M 2011 askalakis M 2011 álaková Z 2014 'ang HY 2015 enholz MN 2011 šlaková Z 2014 'ang HY 2015 enholz NN 2019	15 182 3.05, df = 9 = 6.83 (P < After tr Events 109 330 44 4 1 9 6 6 3.25, df = 12 7 109 330 47 12 66 6 3.25, df = 12 F R 12 7 10 11 1 20 25 143 199 7 10 10 10 10 10 10 10 10 10 10	Image: Second	12 39 357); ² = 44%) Before te 12 15 17 14 31 3 144 435 57 17 75 835; ² = 48% Before tre Events 18 2 33 32 12 57 17 9 237 31 179 237 24 24 24 24 24 24 24 24 24 24	222 68 763 763 763 763 763 46 32 30 82 54 43 73 29 392 2026 92 24 403 3326 92 224 403 3326 92 24 403 3326 92 24 403 3326 92 224 403 3326 92 224 403 3326 92 224 403 3326 92 224 403 3326 92 224 403 3326 92 224 403 3326 92 224 403 3326 92 224 403 3326 92 224 403 3326 92 224 403 3326 3326 3326 3326 3326 3326 3326 33	Weight 11.5% 100.0% Weight 0.6% 1.3% 2.0% 1.9% 4.1% 0.4% 19.0% 48.8% 6.6% 2.2% 9.5% 100.0% Weight 7.4% 1.8% 8.4% 12.8% 9.8% 9.8% 9.8% 9.8% 10.7% 10.0%	1.06 [0.62, 1.62] 0.42 [0.26, 0.69] 0.61 [0.53, 0.70] M-H, Fixed, 95% CI 0.90 [0.26, 3.12] 0.40 [0.41, 1.41] 0.12 [0.20, 0.85] 0.50 [0.27, 1.26] 0.50 [0.27, 1.26] 0.50 [0.27, 1.26] 0.50 [0.12, 3.70] 0.76 [0.62, 0.93] 1.03 [0.11, 1.37] 1.07 [0.55, 1.35] 0.71 [0.44, 1.14] 0.97 [0.72, 1.30] 0.92 [0.84, 1.00] - Risk Ratio M-H, Random, 95% CI 0.80 [0.43, 1.48] 3.50 [0.74, 16.45] 0.63 [0.8, 0.61] 0.43 [0.43, 1.48] 3.50 [0.74, 16.45] 0.63 [0.63, 1.18] 0.58 [0.54, 1.43] 0.68 [0.63, 1.18] 0.68 [0.67, 0.95] 0.80 [0.69, 0.33] 0.80 [0.69, 0.33] 0.80 [0.69, 0.33] 0.80 [0.69, 0.33] 0.80 [0.69, 0.33] 0.80 [0.69, 0.33] 0.80 [0.69, 0.33] 0.80 [0.69, 0.33] 0.80 [0.69, 0.33]	After treatment lower Risk Ratio M-H, Fixed, 95% Cl
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Figure 4. Forest plot for the comparison of CTC-positive rate before and after treatment: subgroup analysis of different CTC determination methods (threshold). The results of ≥5 CTCs/7.5 mL as positive (A) ≥ 1 CTCs/7.5 mL as positive (B) other threshold as positive (C) and RT-PCR method (D) are shown, respectively. The center of black diamond and its extremities indicate the pooled risk ratio and 95% confidential interval.

SCIENTIFIC REPORTS | 7:43464 | DOI: 10.1038/srep43464

 $(RR = 0.65, 95\% \text{ CI: } 0.48 \text{ to } 0.88, P = 0.006; I^2 = 40\%, P = 0.08)$, and adjuvant setting $(RR = 0.89, 95\% \text{ CI: } 0.76 \text{ to } 1.02, P = 0.10; I^2 = 62\%, P = 0.007)$, the metastatic setting $(RR = 0.59, 95\% \text{ CI: } 0.50 \text{ to } 0.70, P < 0.00001; I^2 = 66\%, P < 0.0001)$ and the combination therapy $(RR = 0.78, 95\% \text{ CI: } 0.62 \text{ to } 0.97, P = 0.03; I^2 = 29\%, P = 0.20)$, but not in the surgery $(RR = 1.27, 95\% \text{ CI: } 0.71 \text{ to } 2.27, P = 0.42; I^2 = 45\%, P = 0.16)$ (Fig. 5). These results indicate that surgery as a local treatment can not eliminate CTCs timely, because CTCs can survive in peripheral blood for a certain amount of time⁵⁸, suggesting that patients with CTC positive should be further treated with other therapies after surgery, in order to decrease the risk of metastasis and recurrence.

CTC-positive rates are decreased after therapies in the HER2 -positive or -negative patients, but not in the triple-negative patients. Currently, the clinical management of breast cancer mainly relies on the molecular subtypes based on the expression of estrogen receptor, progesterone receptor and HER2 in primary tumors. It is well known that different molecular subtypes of BC are associated with distinct malignant nature and drug response. Therefore, we further assessed the effects of therapies on reduction of CTCs in different subgroups, including HER2-positive, HER2-negative and triple-negative BC. Compared to pre-therapy, CTC-positive rates were significantly decreased after treatment in HER2-positive patients (RR = 0.68, 95% CI: 0.57 to 0.82, P < 0.0001; $I^2 = 0\%$, P = 0.59) and HER2-negative patients (RR = 0.52, 95% CI: 0.31 to 0.86, P = 0.01; $I^2 = 66\%$, P = 0.01), but not in the triple-negative ones (RR = 0.38, 95% CI: 0.06 to 2.33, P = 0.29; $I^2 = 72\%$, P = 0.06) (Fig. 6). These results indicate that different molecular subtypes of BC affect the efficacy of therapeutics on reducing CTCs. The poor reduction of CTC-positive rate in triple-negative BC is consistent with clinical outcome, implying that current therapies should be further optimized and the new therapeutic methods should be developed for this specific molecular subtype.

Correlation between status of CTCs after treatment and prognosis of the patients. Because CTCs are shed from the primary tumor and serve as the metastatic precursors, the changes of CTC status after therapies may associate with the risk of metastasis as well as the outcome of patients. We compared the prognosis of the CTC-reduced patients with that of the CTC-unchanged or -elevated patients after treatment. The overall survival (OS) of patients after treatment was available in 2 studies (71 cases), in both of which the patients received metastatic setting. The CTC-reduced patients had a longer overall survival period compared to the CTC-unchanged or -elevated patients (mean difference = 11.61 months, 95% CI: 8.63 to 14.59, P < 0.00001; $I^2 = 69\%$, P = 0.07) (Fig. 7). The progression-free survival (PFS) of patients after treatment were available in 3 studies (125 patients), in which all the patients received metastatic setting. The CTC-reduced patients had a longer PFS than the CTC-unchanged or -elevated patients (mean difference = 5.07 months, 95% CI: 2.70 to 7.44, P < 0.0001; $I^2 = 96\%$, P < 0.00001) (Fig. 7). The disease progression of patients after treatments was available in 11 studies with 1363 patients. A significantly lower probability of disease progression was observed in the CTC-reduced patients (OR = 0.54, 95%CI: 0.33 to 0.89, P = 0.01; $I^2 = 45\%$, P = 0.05) (Fig. 7). The 11 studies could be divided into 3 subgroups, namely metastatic setting (6 studies with 244 patients), adjuvant setting (3 studies with 1095 patients), neoadjuvant setting (2 studies with 24 patients). Significantly lower probability of disease progression in CTC-reduced patients was observed in the metastatic setting subgroup (OR = 0.37, 95% CI: 0.20 to $0.66, P = 0.0008; I^2 = 13\%, P = 0.33$) (Fig. 8). But there was no significant difference of probability of disease progression in patients with or without CTC reduction in the adjuvant setting subgroup (OR = 0.73, 95% CI: 0.44 to 1.22, P = 0.23; $I^2 = 54\%$, P = 0.11) and in the neoadjuvant setting subgroup (OR = 0.30, 95% CI: <0.01 to 77.67, P = 0.67; $I^2 = 80\%$, P = 0.03) (Fig. 8). There was a significant heterogeneity in the adjuvant-setting subgroup. When a study (Rack B 2014) was excluded, the heterogeneity was relieved (P = 0.68, $I^2 = 0\%$) and the correlation of CTC status and disease progression became significant (OR = 0.57, 95%CI: 0.37 to 0.86, P = 0.008 by fixed-effect model). In the neoadjuvant setting subgroup, the sample size was extremely small, implying that did not have adequate statistic power. The results showed that the reduction of CTCs was significantly associated with decreased probability of disease progression, increased overall survival and progression-free survival period.

Sensitivity Analysis and Publication Bias. Among the 50 studies included for the pooled RR estimation, no single one contributed substantial influence. When we analyzed the change of CTC counts post-treatments with 7 studies reported CTC status both in CTC-positive rate and CTC counts, the sensitivity analysis was tested by removal of each study. One of them (Bidard FC 2012) was found to substantially affect the heterogeneity and the significance of overall effect: when it was excluded, the heterogeneity was relieved (P = 0.69, $I^2 = 0\%$) and the change of CTC counts after treatment became significant (mean difference = -0.94, 95%CI: -1.49 to -0.38, P = 0.0010 by fixed-effect model). In the adjuvant-setting subgroup analysis of disease progression (Fig. 8), one of the three recruited studies (Rack B 2014) was found to substantially affect the heterogeneity and the significance of overall effect: when it was excluded, the heterogeneity was relieved (P = 0.68, $I^2 = 0\%$) and the correlation of CTC status and disease progression became significant (OR = 0.57, 95%CI: 0.37 to 0.86, P = 0.008 by fixed-effect model). No substantial publication bias was found according to the Funnel plot (Fig. 9).

Discussion

It has been well known that even localized tumors without clinically apparent metastasis give rise to CTCs. Because generation of CTCs is an indispensable step of the metastatic process of tumors, the promising application as a noninvasive blood biomarker in prognosis and response to therapy are very attractive. Although the actual utility of CTCs remains largely academic⁵⁹, many studies have reported the detection of CTCs to facilitate early diagnosis of relapse or metastasis and improve the treatment decisions. In present meta-analysis, we analyzed the changes of CTC status after therapies compared to that of before therapies in pooled 50 studies with 6712 BC patients, and demonstrated that CTC status was a useful indicator to monitor the treatment response, and predict the outcome of patients.

Α Risk Ratio **Risk Ratio** After treatment Before treatment Study Events Total Events Total Weight M-H, Random, 95% Cl M-H, Random, 95% Cl neoadiuvant setting Bidard FC 2013 13.2% 0.76 [0.42, 1.37] Boutrus RR 2013 12 4 46 18 55 12.5% 0 80 [0 43 1 48] Cabinakova M 2015 21 5 29 5.3% 1.10 [0.34, 3.63] Kasimir-Bauer S 2016 11 133 32 135 12.0% 0.35 [0.18, 0.66] Lavrov AV 2014 21 40 12 30 2.2% 0.12 [0.02, 0.85] Mathiesen RR 2013 4.5% 3 6 39 0.49 [0.13, 1.81] Nadal R 2012 16 2 35 29 15 35 29 14.6% 1.07 [0.63, 1.80] Nakayama Y 2013 3 2.8% 0.67 [0.12, 3.70] 0.77 [0.43, 1.39] 0.71 [0.44, 1.14] Pierga JY 2008 15 12 86 24 22 17 97 24 13.1% Serrano MJ 2012 15.8% Ušiaková Z 2014 2 38 13 38 3.9% 0.15 [0.04, 0.64] 0.65 [0.48, 0.88] Subtotal (95% CI) 558 606 100.0% Total events 03 165 Heterogeneity: Tau² = 0.10; Chi² = 16.76, df 10 (P = 0.08); I² = 40% 0.02 0.1 10 50 Test for overall effect: Z = 2.75 (P = 0.006) After treatment lower Before treatment lower в **Risk Ratio Risk Ratio** After treatment Before treatment Total Tota Weigh om, 95% Cl M-H, Rar n, 95% Cl Study Events Events M-H Rando adjuvant setting Karaba M 2013 10 23 33 124 5.4% 1.63 [0.94, 2.83] 0.81 [0.46, 1.45] 0.76 [0.62, 0.93] Nadal R 2012 13 38 16 38 5.0% 144 15.9% Neugebauer JK 2013 109 392 392 Pierga JY 2015 6 29 5 38 17% 1 57 [0 53 4 65] Rack B 2014 330 1493 435 2026 19.7% 1.03 [0.91, 1.17] Serrano MJ 2009 27 42 35 57 11.5% 1.05 [0.77, 1.42] Ušiaková Z 2014 13 143 100 26 100 4.7% 0.50 [0.27, 0.92] Xenidis N 2009 437 179 437 17.4% 0.80 [0.67, 0.95] 18.6% 100.0% 0.80 [0.69, 0.93] 0.89 [0.76, 1.02] Xenidis N 2013 190 545 545 237 Subtotal (95% CI) 3099 3757 841 1110 Total events Heterogeneity: Tau² = 0.02; Chi² = 21.21, df = 8 (P = 0.007); l² = 62% .02 0.1 After treatment lowe 0.02 10 50 Test for overall effect: Z = 1.63 (P = 0.10) Before treatment lower C After treatment Events Total Before treatmen **Risk Ratio Risk Ratio** M-H, Random, 95% CI Study M-H. Random, 95% C Events Total Weight metastatic setting Bian I 2014 31 233 88 227 6 1% 0 34 10 24 0 501 Bidard FC 2010 0.22 [0.09, 0.52] 42 35 65 2.6% ÷ 174 Bidard FC 2012 75 203 267 7.7% 0.57 [0.46, 0.69] Cristofanilli M 2004 49 177 7.0% 0.53 [0.41, 0.70] 163 100 Hartkonf AD 2011 16 21 58 31 31 58 5.0% 0.52 [0.32, 0.84] Hayashi N 2012 49 52 5.8% 0.72 [0.49, 1.07] Horn P 2014 5 39 22 47 2.6% 0.27 [0.11, 0.66] Jiang ZF 2013 39 233 115 294 6.5% 0.43 [0.31, 0.59] Magbanua MJ 2015 Martín M 2013 0.74 [0.51, 1.07] 0.60 [0.41, 0.87] 29 28 89 42 95 6.0% 99 47 99 6.0% Mego M 2012 Müller V 2005 9 21 6 11 21 24 2.7% 1.50 [0.65, 3.47] 9 3.6% 0.82 [0.42, 1.61] 24 5.3% 3.2% 0.51 [0.33, 0.80] 0.38 [0.18, 0.79] Peeters DJ 2014 17 7 73 38 70 20 154 41 Pierga JY 2013 6.6% 4.7% Reinholz MM 2011 25 12 47 53 12 86 22 0.86 [0.63, 1.18] 19 Serrano MJ 2009 1.16 [0.69, 1.93] 5.4% 2.3% Smerage JB 2013 18 5 61 23 38 9 66 28 0.51 [0.33, 0.80] Tokudome N 2011 0.68 [0.26, 1.74] Turker I 2013 6 22 42 3 22 42 1.5% 2.00 [0.57, 7.01] 2.5% Ušiaková Z 2014 18 5 0.28 [0.11. 0.68] Wallwiener M 2014 57 201 133 393 2280 7.1% 100.0% 0.84 [0.65, 1.09] 0.59 [0.50, 0.70] Subtotal (95% CI) 1779 ٠ 468 1058 Total events Heterogeneity: Tau² = 0.08; Chi² = 58.45, df = 20 (P < 0.0001); l² = 66% 0.02 0.1 10 50 Test for overall effect: Z = 6.20 (P < 0.00001) Before treatment lowe After treatment lower D After treatment Before treatment **Risk Ratio Risk Ratio** M-H, Fixed, 95% CI Study Weight M-H, Fixed, 95% CI **Events** Total Events Total surgery Daskalakis M 2011 3 50 [0 74 16 45] 104 2 104 11 7% Maltoni R 2015 43 13 48 71.7% 0.77 [0.37, 1.63] 9 Pierga JY 2015 Subtotal (95% CI) 5 38 3 42 16.6% 1.84 [0.47, 7.19] 1.27 [0.71, 2.27] 185 194 100.0% 21 18 Total events Heterogeneity: Chi² = 3.65, df = 2 (P = 0.16); l² = 45% 0.02 0.1 10 50 Test for overall effect: Z = 0.80 (P = 0.42) Before treatment lower After treatment lower Е After treatment Before treatment **Risk Ratio Risk Ratio** Study M-H. Fixed, 95% CI M-H. Fixed. 95% C Events Total Events Total Weight combination therapy Azim HA Jr 2013 3.5% 0.90 [0.26, 3.12] 41 5 46 Cabinakova M 2015 32 32 7.5% 0.40 [0.14, 1.14] 10 4 Mikulová V 2014 6 54 19 17 54 19 12.8% 0.35 [0.15, 0.83] Müller V 2005 3 8 6.0% 0.38 [0.12, 1.20] Turker I 2013 12 12 0.8% 1.00 [0.07, 14.21] van Dalum G 2015 66 367 75 403 53.7% 0.97 [0.72, 1.30] Zhang JL 2015 16 33 21 33 15.8% 0.76 [0.49, 1.18 Subtotal (95% CI) 558 599 100.0% 0.78 [0.62, 0.97] 100 137 Total events Heterogeneity: Chi² = 8.48, df = 6 (P = 0.20); I² = 29% 0 02 01 10 50 Test for overall effect: Z = 2.22 (P = 0.03) After treatment lowe Before treatment lo Figure 5. Forest plot for the comparison of CTC-positive rate before and after treatment: subgroup

Figure 5. Forest plot for the comparison of CTC-positive rate before and after treatment: subgroup analysis of different therapy strategies. The results of neoadjuvant setting (A), adjuvant setting (B), metastatic setting (C), surgery (D) and combination therapy (E) are shown, respectively. The center of black diamond and its extremities indicate the pooled risk ratio and 95% confidential interval.

А



Heterogeneity: Tau² = 1.34; Chi² = 3.57, df = 1 (P = 0.06); l² = 72%

Test for overall effect: Z = 1.05 (P = 0.29)

Figure 6. Forest plot for the comparison of CTC-positive rate before and after treatment: subgroup analysis of different molecular subtypes. The results of HER2-positive subtype (**A**) HER2-negative subtype (**B**) and triple-negative subtype (**C**) are shown, respectively. The black diamond and its extremities indicate the pooled risk ratio center and 95% confidential interval.

0.02

0.1

After treatment lower

10

Before treatment lower

50

The actual application of CTCs in clinical setting relies on the progression of detection technologies. In the past decades, a number of technically diverse platforms have been developed for CTC assay⁶⁰. However, for any technology to be used in the clinic, demonstration of analytic validity, clinical validity, and clinical utility is required⁶⁰. Up to now, the only system approved by the Food and Drug Administration (FDA) as an aid in monitoring patients with metastatic breast, colorectal, or prostate cancer is CellSearch[®] (Veridex, Raritan, NJ, USA). Also, CellSearch[®] is the only semi-automated system and has contributed considerably to the development of standards for CTC enumeration. Nevertheless, there are disadvantages to be perfect for it. Its enrichment/capture technology is based on epithelial marker EpCAM, which is usually with low sensitivity and efficiency, due to the lost expression of EpCAM in CTCs by EMT process^{61,62}. In our meta-analysis, other CTC detection methods are employed in some studies, including RT-PCR, which determined the CTC status by detecting the mRNA expression of epithelial markers, such as EpCAM or CKs. There is a probability that the CTC detection methods based on different labels or rationales would find different counts of CTCs in the same individual patients. Given the CTC rarity, especially in non-metastatic breast cancer, continuous training and central image review is required in order to gain best inter-reader agreement. A recent study evaluated the inter-reader agreement in 22 readers from 15 academic laboratories and 8 readers from two Veridex laboratories with non-metastatic (M0) and metastatic (M1) breast cancer samples. For CTC definition (No CTC vs. CTC), the median agreement between academic readers and VC was 92% (range 69 to 97%) with a median κ of 0.83 (range: 0.37 to 0.93). The inter-reader agreement for CTC definition was high. Reduced agreement was observed in M0 patients with low CTC counts⁶³. In addition, the inconsistence of the cut-off value to determine CTC-positive amongst studies would be a limitation for actual application of CTCs in clinical setting (Fig. 4). Standard or uniform protocol for CTC measurement would be required before this indicator could be clinically adopted.

There were several limitations in the present research. First, most of studies included in this meta-analysis were consisted of patients receiving certain therapy alone without an appropriate negative control. Second, some studies with substantial sample heterogeneities, which were caused by the complexity of patient characteristics (race, age), therapeutic details (drug, dose, treatment periodicity, etc.) or other factors, were excluded in our

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в

•	CTC-re	duced	d CTC-unchanged/-elevated			Odds Ratio	Odds Ratio
Study	Events	Total	Events	Total	Weight	M-H, Random, 95%	CI M-H, Random, 95% CI
artkopf AD 2011	5	24	8	10	5.6%	0.07 [0.01, 0.41]	
lorn P 2014	14	27	7	8	4.1%	0.15 [0.02, 1.43]	
avrov AV 2014.	0	12	1	1	1.2%	0.01 [0.00, 0.95]	<
/lüller V 2005	2	5	4	7	3.7%	0.50 [0.05, 5.15]	
lolé F 2008	16	26	16	23	10.3%	0.70 [0.21, 2.30]	
Peeters DJ 2014	16	18	17	17	2.3%	0.19 [0.01, 4.23]	·
Rack B 2014	21	238	24	329	18.6%	1.23 [0.67, 2.27]	
Serrano MJ 2012	4	7	1	4	2.9%	4.00 [0.27, 60.32]	
Vallwiener M 2014	11	32	24	47	13.5%	0.50 [0.20, 1.27]	
(enidis N 2009	21	92	46	143	18.8%	0.62 [0.34, 1.14]	
(enidis N 2013	20	103	60	190	19.2%	0.52 [0.29, 0.93]	
otal (95% CI)		584		779	100.0%	0.54 [0.33, 0.89]	•
otal events	130		208				
leterogeneity: Tau ² = est for overall effect:	= 0.24; Chi² Z = 2.45 (F	= 18.19, P = 0.01)	df = 10 (P = 0.05)); l² = 45%			Image: Non-Structure Image: No

	CTC-reduced CTC-unchange				TC-unchanged/-elevated Mean Difference				e Mean Difference				
Study	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% C	I	IV, Random, 95% CI			
Magbanua MJ 2015	3.65	0.99	13	1.45	0.23	24	39.0%	2.20 [1.65, 2.75]					
Tokudome N 2011	16	5	9	4	1	6	22.1%	12.00 [8.64, 15.36]			+		
Wallwiener M 2014	8.8	1.7	32	4.8	0.6	41	38.8%	4.00 [3.38, 4.62]					
Total (95% CI)			54			71	100.0%	5.07 [2.70, 7.44]			•		
Heterogeneity: Tau ² = 3	² = 45.	50, df =	2 (P < 0.000	001); l² =	96%			-100	-50	0	50	100	
Test for overall effect: Z = 4.19 (P < 0.0001)									CTC-redu	uced worse	CTC-un	changed/-elev	ated wors
c	стс-	reduce	ed C	TC-unchan	ged/-elev	ated		Mean Difference		Mear	n Difference		

								-	Sector A management							
Study	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV,	Random, 95%	СІ				
Hartkopf AD 2011	17.67	5.9	24	4.53	0.54	10	49.7%	13.14 [10.76, 15.52]								
Magbanua MJ 2015	15.5	4.2	13	5.4	1.2	24	50.3%	10.10 [7.77, 12.43]								
Total (95% CI)			37			34	100.0%	11.61 [8.63, 14.59]			•					
Heterogeneity: Tau ² = 3.17; Chi ² = 3.19, df = 1 (P = 0.07); l ² = 69%									-100	-50	Ó	50	100			
Test for overall effect:	Z = 7.64	(P < 0	.00001)						CTC-redu	iced worse	CTC-unc	hanged/-elevat	ed worse			

Figure 7. Forest plot for the comparison of prognosis between the CTC-reduced patients and those without CTC-unchanged or -elevated. The diamond indicated the odds ratio of disease progression (A) the difference of progression-free survival (B) or overall survival period (C). The centers of the diamonds indicated the pooled odds ratio (A) or the mean difference (B,C) and the extremities indicated the 95% confidential interval.

subgroup analyses. Third, the sample sizes of some subgroups were relatively small, which might affect the detection of potential difference. Fourth, CTCs were thought to be a set of cells with different characteristics, so it would be more meaningful to investigate the correlation of change of the CTC subpopulations after treatment with patient prognosis. However, so far few data are available to perform an analysis.

In summary, the present meta-analysis demonstrated that the status of CTCs is a useful indicator of the efficacy of therapies for BC, which may help clinicians make a decision for further personalized therapy of patients. However, it is on the way for application of CTCs in clinical setting because there are still challenges presented in analytic validity, clinical validity, and clinical utility of CTCs.

Methods

Search strategy. A comprehensive literary search for potential studies was searched in June 2016 without time or language restrictions. The electronic databases include PubMed, EMBase and Cochrane library. The keywords and MeSH terms were variably combined: "circulating tumor cell (s)", "breast cancer", "therapy". The search strategy was intended to exclude reviews, comments, letters and editorials, which have irrelevant study data, by screening the titles and abstracts of publications.

Eligibility criteria. Inclusion criteria: (i) enrolled patients with BC were pathologically diagnosed; (ii) CTCs were detected by any method, including cell capture and quantitative PCR; (iii) the patients' CTC status both preand post-therapy was reported.

Exclusion criteria: (i) cell-line experiments or animal models; (ii) a small size <20 patients; (iii) when information of the same patients was reported in different studies, only the latest and most informative one was included; (iv) CTC status was reported pre- or post-therapy alone; (v) CTC status pre- and post-therapy was reported in different cohort of patients; (vi) Studies which reported CTCs only in continuous style were excluded, as their results could not be integrated with the majority of studies which reported the results in dichotomous pattern (positive vs. negative).

Data extraction. Studies were reviewed and extracted independently by two reviewers (WT Yan and Q Chen). The primary data of the included studies were following: the general information (i.e., the first author, the year of publication, the nationality of studies), sample size, the patients' characteristics (i.e., ages, tumor stage,

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Figure 8. Forest plot for the comparison of prognosis between the CTC-reduced patients and those without CTC-unchanged or –elevated: subgroup analysis of disease progression. The diamond indicated the odds ratio of disease progression in the metastatic setting (A), the adjuvant setting (B) or the neoadjuvant setting (C). The centers of the diamonds indicated the pooled odds ratio and the extremities indicated the 95% confidential interval.



Figure 9. Funnel plot for the studies included for comparison of CTC-positive rate before and after treatment.

molecular subtypes of tumor), assessment of CTCs (i.e., methods of CTC detection, blood volume, the cut-off value of CTCs, the count of CTCs and/or the positive rate of CTCs) and the type of the treatments. If CTCs at more than one phase of follow ups were reported in a study, the latest phase with follow-up rate \geq 75% was chosen; if no phase met the criteria, the earliest phase was chosen. The prognosis (progression-free survival, overall survival) was also extracted for assessing prognostic value of reducing CTCs.

Quality assessment of primary studies. The quality of each included study was evaluated by a scale based on the Newcastle–Ottawa Quality Assessment Scale.

Statistical analysis. The pooled RR (relative risk) and mean difference were calculated to analyze the difference of CTCs between pre-therapy and post-therapy by fixed model or random-effects model according to the heterogeneity of the studies, which was estimated by the Cochran's Q test and the I² index (P value < 0.10 or I² over 50% was define as substantial heterogeneity). RR less than 1 or mean difference less than 0 indicated declined CTCs in the peripheral blood. Pooled OR (odds ratio) and mean difference were calculated to analyze

the difference of progression between the patients with different dynamic conditions of CTCs (decreasing vs. increasing or persistent elevated). Subgroup analyses were performed for CTC determination methods, treatments and molecular subtypes of primary tumor as long as two or more studies were available to be included. Forest plot was used to illustrate the pooled RR mean difference. All analyses were run by Review Manager Version 5.3 (Cochrane Collaboration, Copenhagen, Denmark).

Sensitivity analysis and estimation of publication bias. To evaluate the influence of individual study on the pooled RR or mean difference, sensitivity analysis was performed by removing each eligible study separately. Funnel plot developed by Begg was used to detect potential publication bias which might affect the validity of the results.

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Acknowledgements

This work was supported by Major Project of Chongqing (Grant No. cstc2015shms-ztzx10005), P. R. China.

Author Contributions

J.J. and C.Y.H. conceived the study, participated in drafting the final manuscript. Y.W.T., C.Q. and C.X. analyzed the data and completed the final draft of the manuscript. L.Y.F. and W.Y. prepared all the figures. All authors reviewed the manuscript.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Yan, W.-T. *et al.* Circulating tumor cell status monitors the treatment responses in breast cancer patients: a meta-analysis. *Sci. Rep.* **7**, 43464; doi: 10.1038/srep43464 (2017).

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