In early breast cancer, the ratios of neutrophils, monocyctes and platelets to lymphocytes significantly correlate with the presence of subsets of circulating tumor cells in blood and disseminated tumor cells in the bone marrow.

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Background / Purpose of the Study

Circulating tumor cells (**CTCs**) are the precursors of metastasis and while travelling through the peripheral blood, they crosstalk with different types of blood cells before they finally reach distant organs to settle down as disseminated tumor cells (**DTCs**). Proinflammatory markers include the neutrophil lymphocyte ratio (**NLR**), the platelet lymphocyte ratio (**PLR**) as well as the monocyte lymphocyte ratio (**MLR**) and the presence of tumor cells as well as specific proinflammatory markers are two independent predictors of worse outcome in breast cancer (**BC**). However, little is known about the correlation between NLR, PLR, MLR and subsets of CTCs as well as DTCs in early, non-metastatic BC.

Here we evaluated the correlation of NLR, PLR, MLR and the presence of epithelial CTCs (eCTCs), CTCs in epithelial mesenchymal transition (EMT-CTCs) as well as DTCs to better identify BC patients (pts) at risk, to monitor treatment reponse and probably adjust therapeutic options.

Patients and Methods

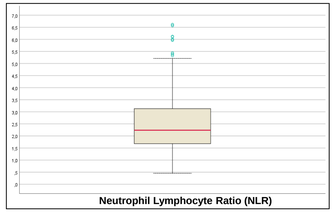
Patients: This retrospective study includes 171 early, non-metastatic BC pts who presented with first diagnosis of BC between July 2006 and December 2012. The majority of the pts were postmenopausal, had T1-T2 tumors, 59% were node-negative (38,6% = N1) and most of the patients presented with a poor or moderately differentiated tumor. Expression of the estrogen- (ER) and progesterone-receptor (PR) was observed in 78% and 70% of the tumors and HER2 was overexpressed in 16% of cases. 69% were ER- and or PR-positive and HER2-negative, 15% were triple-negative and 11% showed HER2 overexpression, respectively.

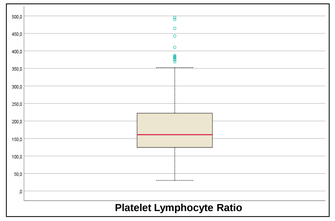
Blood counts: The counts of peripheral neutrophils, lymphocytes, monocytes and platelets to determine NLR, PLR and MLR were retrospectively recorded before the start of therapy. NLR, PLR and MLR were calculated from peripheral blood cell counts and their optimal cutoff levels were determined by the 75% percentile resulting in the following values: NLR: 3.13, MLR: 0.39 and PLR: 222.3, respectively (Figure 1).

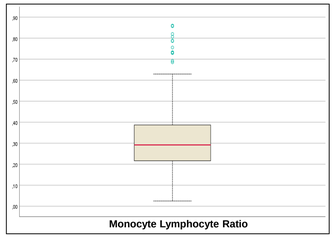
Evaluation of DTCs: 170/171 pts were analyzed for DTCs by immunocytochemistry using the pan-cytokeratin antibodyA45-B/B3 (Clodronate intake was recommended in case of DTC-positivity).

Evaluation of CTCs: CTCs were determined in 155/171 pts applying positive immunomagnetic selection using the AdnaTest BreastCancerSelect. The recovered cDNA was tested for the presence of eCTCs using the AdnaTest *BreastCancerDetect* (detection of EpCAM, MUC-1, HER2) and EMT-CTCs applying the AdnaTest *EMTDetect* (detection of PI3K, AKT, TWIST). Pts were considered CTC-positive if at least one of the two tests were positive.

Statistical analysis included descriptive reporting, U-test for group differences, Rho correlations for dependencies and Fisher's chi-square test for distributional differences. Rho correlations were used to assess the relationship between NLR, MLR, PLR and tumor cells as well as progression-free survival (PFS) and overall survival (OS).







	Mean	Median	Perc 25	Perc 75	SD	Min	Max
NLR	2,97	2,24	1,68	3,13	2,60	0,45	22,37
PLR	193,6	161,0	124,5	222,3	135,0	30,2	1492,7
MLR	0,33	0,29	0,22	0,39	0,17	0,03	0,99
Neutro [nl]	4,45	4,03	3,13	5,31	2,00	0,25	11,8
Lympho [nl]	1,81	1,70	1,34	2,20	0,71	0,41	4,3
Platelets [nl]	296,6	283,0	237,0	336,0	87,3	52	612
Mono [nl]	0,55	0,53	0,40	0,67	0,23	0,02	1,36

Figure 1: Distribution of blood values.
Optimal cutoff levels for blood values of all pts were determined by the 75% percentile resulting in the values highlighted in blue. Representative figures are shown for NLR, PLR and MLR.

Results

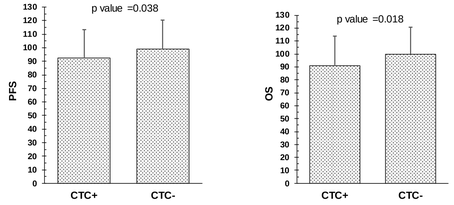


Figure 2: Prognostic Significance of CTCs.
DTCs were detected in 34% of the pts and at least one CTC-subtype (eCTCs and/or EMT-CTCs) was found in 28% of the pts. Whereas the presence of DTCs was not associated with PFS or OS (data not shown), the presence of CTCs was significantly correlated with a shorter PFS (p=0.046) and OS (p=0.018). However, neither eCTCs nor EMT-CTSs alone were of prognostic significance.

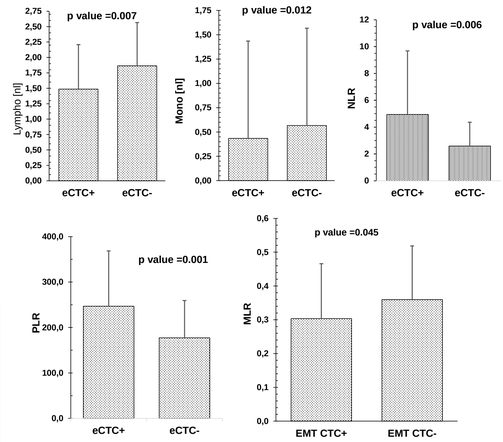


Figure 3: Mean differences of CTC-subtypes and blood values.
The presence of eCTCs significantly associated with reduced lymphocyte (p=0.007) and monocyte counts (p=0.012) as well as an an elevated NLR (p=0.003) and PLR (p=0.001). In contrast, the presence of EMT-CTCs only correlated with a significantly reduced MLR (p=0.045).

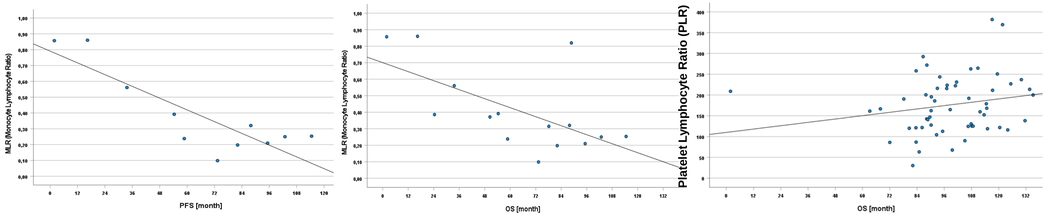
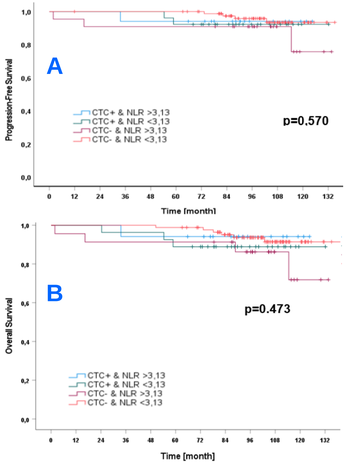


Figure 4: Significant correlations between blood cells, ratios and patients outcome.
Enhanced lymphocyte (p=0.025 / 0.011) and monocyte counts (p=0.039 / p=0.037) as well as a low PLR (p=0.032 / 0.023) significantly correlated with a reduced PFS / OS in pts still alive (data not shown) whereas an enhanced MLR showed a high correlation with a shorter PFS (p=0.007) and OS (p=0.021) in deceased pts.

Figure 5: Significant correlations for DTCs
No significant correlations were found for NLR, PLR and MLR with DTCs, however, DTC-positive pts, harboring a lower PLR, had a significant shorter OS (p=0.043).



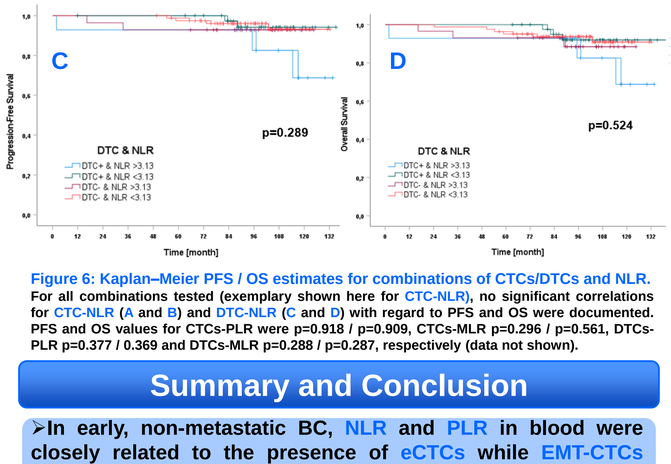


Figure 6: Kaplan–Meier PFS / OS estimates for combinations of CTCs/DTCs and NLR.
For all combinations tested (exemplary shown here for CTC-NLR), no significant correlations for CTC-NLR (A and B) and DTC-NLR (C and D) with regard to PFS and OS were documented. PFS and OS values for CTCs-PLR were p=0.918 / p=0.909, CTCs-MLR p=0.296 / p=0.561, DTCs-PLR p=0.377 / 0.369 and DTCs-MLR p=0.288 / p=0.287, respectively (data not shown).

Summary and Conclusion

- In early, non-metastatic BC, NLR and PLR in blood were closely related to the presence of eCTCs while EMT-CTCs associated with MLR (Figure 3).
- No direct correlations were found for inflammatory markers and tumor cell spread to the bone, however, DTC-positive pts, harboring a lower PLR, had a significant shorter OS (Figure 5).
- These findings might improve the prognostication of these pts and probably help to monitor response to therapy and adjust treatment options.

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