Characterization of the cytokine profile of metastatic Breast Cancer, from mediators of chronic inflammation to potential biomarkers

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Introduction and Hypothesis

- Chronic inflammation is a canonical hallmark of cancer and promotes tumour progression.
- It offers potential for new therapeutic strategies, particularly immunotherapy.
- Cytokines are key regulators of inflammation, influencing cell communication and immune responses within the tumour microenvironment (1,2).
- Current clinical classification of breast cancer (BC) often neglects the immune landscape.

Our **hypothesis** is that the cytokine profile of metastatic breast cancer possesses prognostic and predictive value and that cytokines can be potential biomarkers in breast cancer.

Materials and Methods

- We prospectively collected serum samples from early and metastatic BC patients (n = 52) and from early and advanced cancer patients from other solid tumor types (n=160) from Donostia, Basurto and Txagorritxu University Hospitals (Table 1).
- We also collected fresh tumor and serum samples from 6 early TNBC patients from Hospital Donostia (Table 2), before and after (only serum) treatment with neoadjuvant chemotherapy and pembrolizumab (KEYNOTE-522 treatment scheme, ref.3).
- Cytokine levels in serum samples were measured by the Olink Target 48 Cytokine Panel, which quantitatively assess 45 cytokines.
- Cytokine levels were correlated with clinical data, including tumor type, stage and treatment .
- Statistical analysis was done using specified statistical tests using R and GraphPad.

Cancer type	Total number	Early		Metastatic		Female		Male		Cohorts	(n)
	(n)	(n)	(%*)	(n)	(%*)	(n)	(%*)	(n)	(%*)		
Breast	52	7	13	45	87	52	100	0	0	COHORT 4 COHORT 1 COHORT 3 COHORT 2	6 5 11 30
Prostate	40	29	73	11	28	0	0	40	100	COHORT 5 COHORT 1	29 11
Gastrointestinal (no colon)	31	7	23	24	77	9	29	22	71	COHORT 1	31
Lymphoma	23	6	26	17	74	15	65	8	35	COHORT 1	23
Lung	21	4	19	17	81	6	29	15	71	COHORT 1	21
Genitourinary	20	4	20	16	80	4	20	16	80	COHORT 1	20
Colon	11	5	45	6	55	3	27	8	73	COHORT 1	11
Head and neck	6	1	17	5	83	2	33	4	67	COHORT 1	6
Gynecological	2	1	50	1	50	2	100	0	0	COHORT 1	2
Brain	1	0	0	1	100	0	0	1	100	COHORT 1	1
Melanoma	1	0	0	1	100	0	0	1	100	COHORT 1	1
Skin	1	1	100	0	0	0	0	1	100	COHORT 1	1
Skin sarcoma	1	0	0	1	100	0	0	1	100	COHORT 1	1
Genitourinary + Lung	1	1	100	0	0	0	0	1	100	COHORT 1	1
Others	1	0	0	1	100	1	100	0	0	COHORT 1	1
Total	212	66	31	146	69	94	44	118	56		

***Table 1**: Table summarizing the tumor type, sex and stage of the patients' samples analyzed.*

Results 1

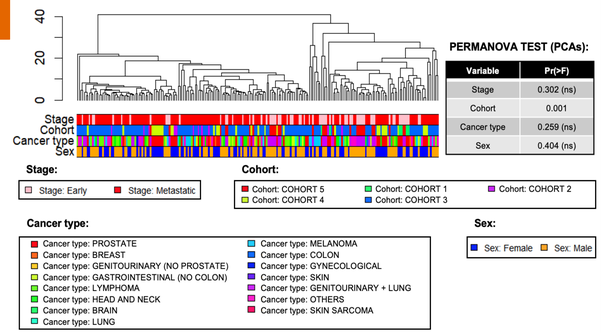


***Figure 1**: Unsupervised dendrogram of all serum samples analyzed.*

The cytokine profile of serum samples from cancer patients does not cluster according to stage, sex and cancer type.

Results 2

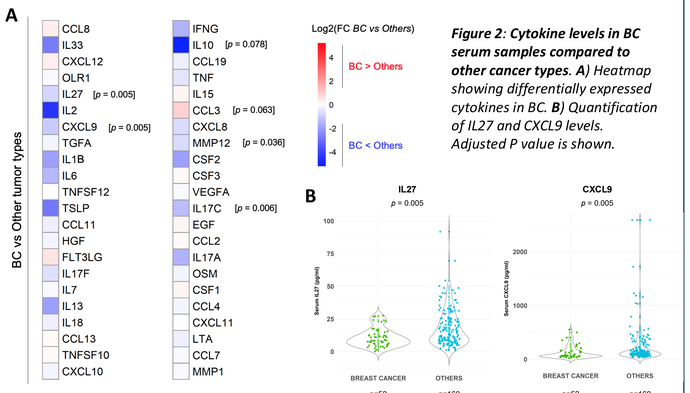


***Figure 2**: Cytokine levels in BC serum samples compared to other cancer types. A) Heatmap showing differentially expressed cytokines in BC. B) Quantification of IL27 and CXCL9 levels. Adjusted P value is shown.*

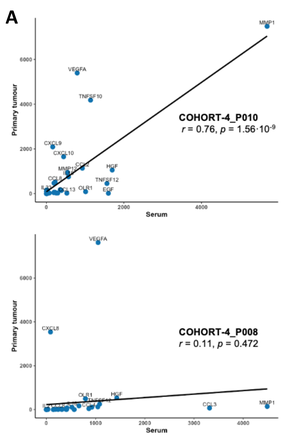
Some cytokines such as IL27, CXCL9 and MMP12 are decreased in serum samples from BC patients compared to patients from other tumor types.

Results 3

Patient ID	Analyzed samples			Path-TR
	Basal (pre-treatment)		Post-treatment	
	Serum	Tumour*	Serum	
COHORT-4_P001	✓	☒	✓	pCR
COHORT-4_P002	✓	✓	✓	pCR
COHORT-4_P008	✓	✓	✓	pCR
COHORT-4_P010	✓	✓	✓	pCR
COHORT-4_P014	✓	✓	✓	pCR
COHORT-4_P029	✓	☒	✓	pCR

* Protein extract from fresh-frozen tumour tissue from the diagnostic biopsy

***Table 2**: Table summarizing the samples analyzed in early TNBC patients treated with neoadjuvant immunotherapy (cohort 4), used in Results 3 and 4 sections.*



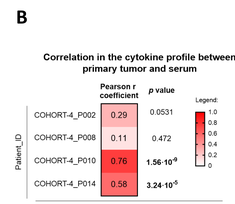


***Figure 3**: Correlation of cytokine levels in serum and fresh tumor samples. A) Independent correlation curves from 2 representative patients. B) Correlation coefficients and P values for all patients analyzed.*

The cytokine profile of serum and tumor samples does not always correlate, supporting the idea that some cytokines may act locally in the tumor microenvironment.

Results 4

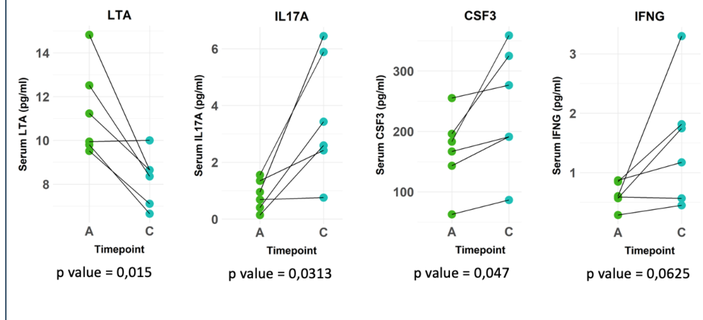


***Figure 4**: Cytokine levels in early TNBC serum samples before (A) and after (C) neoadjuvant chemo-immunotherapy.*

LTA (lymphotoxin alpha), formerly known as TNF-B, decreases after neoadjuvant immunotherapy in serum samples from early TNBC patients, while IL17A, CSF3 (also known as granulocyte colony-stimulating factor, G-CSF) and IFNG increase.

Conclusions

- The circulating cytokine profile of BC patients is not different from other tumor types.
- Some cytokines such as IL27 and CXCL9 are consistently decreased in serum levels from BC patients compared to other cancer patients.
- The serum and tumor cytokine profiles do not always correlate significantly, supporting the idea that some cytokines act locally.
- Neoadjuvant immunotherapy changes the circulating cytokine profile of early TNBC patients.

References

1. Soler MF, Abaurrea A, Azcoaga P, Araujo AM, Caffarel MM. New perspectives in cancer immunotherapy: targeting IL-6 cytokine family. J Immunother Cancer. 2023;11(11):e007530. doi:10.1136/jitc-2023-007530
2. Manzano S, Caffarel MM. Cytokine-centered strategies to boost cancer immunotherapy. Mol Oncol. 2025;19(3):579-583. doi:10.1002/1878-0261.13818
3. Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med. 2020;382(9):810-821. doi:10.1056/NEJMoa1910549

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