Overexpression of syndecan-1, MUC-1, and putative stem cell markers for diagnosis and treatment monitoring of breast cancer leptomeningeal metastasis by cerebrospinal fluid flow cytometry.

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Background

Cancer is a mosaic of tumor cell subpopulations, where only a minority is responsible for disease recurrence and cancer invasiveness. We focused on one of the most aggressive circulating tumor cells (CTCs) which, from the primitive tumor, spreads to the central nervous system (CNS), evaluating the expression of prognostic and putative cancer stem cell markers in breast cancer (BC) leptomeningeal metastasis (LM). Moreover, the role of flow-CSF was evaluated in one patient for disease treatment monitoring.

Methods

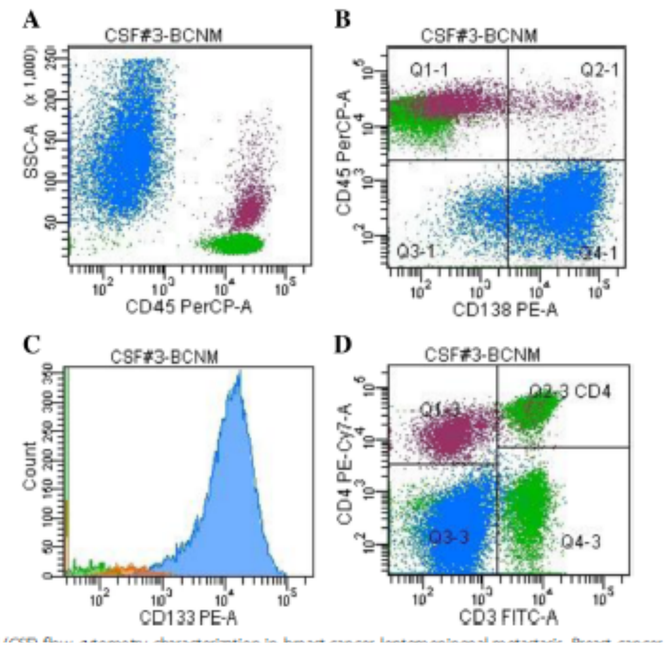
Flow cytometry immunophenotypic analysis of cerebrospinal fluid (CSF) samples (4 ml) was performed in 14 consecutive cases of BCLM. Syndecan-1 (CD138), MUC-1 (CD227) CD45, CD34, and the putative cancer stem cell markers CD15, CD24, CD44, and CD133 surface expression were evaluated on CSF floating tumor cells. The tumor-associated leukocyte population was also characterized.

Results

Despite a low absolute cell number (9 cell/µl, range 1–86), the flow cytometry characterization was successfully conducted in all the samples. Syndecan-1 and MUC-1 over-expression was documented on BC cells in all the samples analyzed; CD44, CD24, CD15, and CD133 in 77%, 75%, 70%, and 45% of cases, respectively. A strong syndecan-1 and MUC-1 expression was also documented by immunohistochemistry on primary breast cancer tissues, performed in five patients. The CSF tumor population was flanked by T lymphocytes, with a different immunophenotype between the CSF and peripheral blood samples ($P \leq 0.02$). In one case flow-CSF monitoring was performed after lumbar puncture treatment, documenting a decrease percentage of tumor cells as well as an increase of CSF T-lymphocytes.

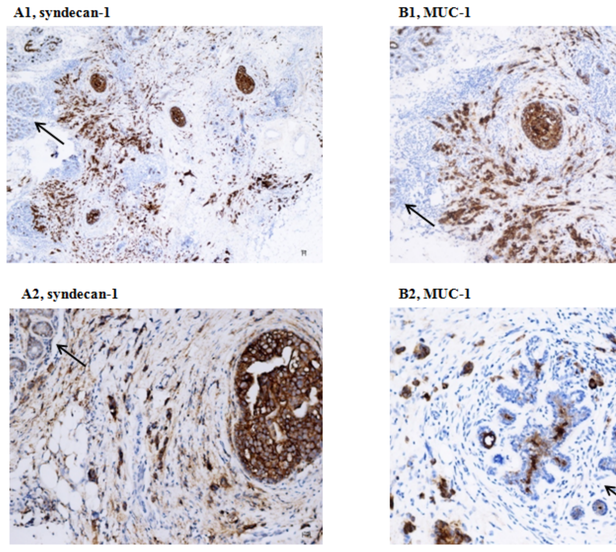
Histological and immunohistochemical staining of the primary breast cancer tissue from 13 patients with leptomeningeal metastasis

Case n°	Histology	pTNM	ER	PgR	*HER2*
1	na	na	na	na	na
2	Infiltrating ductal carcinoma	na	pos	pos	neg
3	Infiltrating ductal carcinoma	pTc1, N0, stage 1	neg	neg	neg
4	Infiltrating lobular carcinoma	na	neg	pos	pos
5	na	na	na	na	na
6	Infiltrating ductal carcinoma	pT4b, N3a, M1 (UICC 2002)	40%	70%	neg
7	Infiltrating lobular carcinoma	pT2, pN3a, M0	75%	100%	pos
8	na	na	na	na	na
9	Infiltrating lobular carcinoma	pT1c, N1bi, Mx (UICC 1997)	30%	30%	neg
10	Infiltrating ductal carcinoma	na	pos	pos	neg
11	Infiltrating lobular multifocal	pT1c (m); pN1biv; Mx (UICC 1997)	20%	20%	pos
12	Infiltrating ductal carcinoma	na	neg	neg	neg
13	Infiltrating ductal carcinoma	pT1c(m), N2, Mx (UICC 2002)	neg	40%	pos



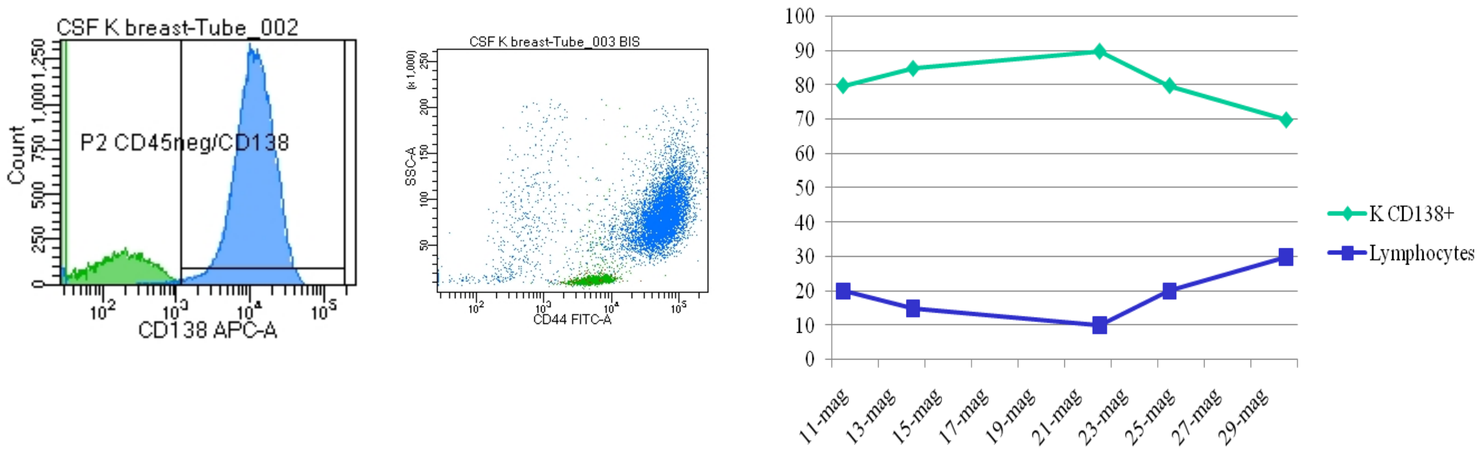
CSF flow cytometry characterization in breast cancer neoplastic meningitis

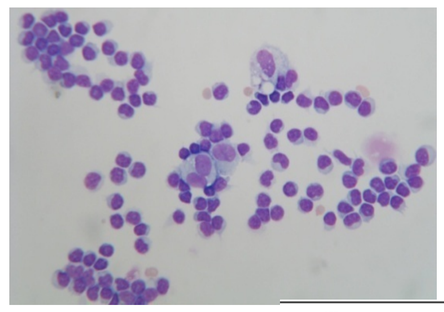
Brest cancer cells (blue) are CD45neg CD138+ and CD133+ sided by CD45 positive lymphocyte and monocytes



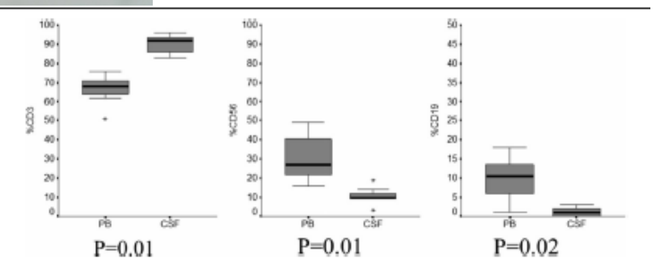
Primary breast cancer immunohistochemical staining of patients with leptomeningeal metastasis.
Brest cancer cells are syndecan-1 (CD138) and MUC-1 strongly positive.
Non-neoplastic breast epithelium (arrow) shows glandular architecture with weak staining for both CD138 and CD227

CSF flow cytometrymonitoring in one patients with BCNM





CSF Tumor –associated leukocytes



Conclusions

Flow cytometry can be successfully employed for solid tumor LM characterization even in CSF samples with low cell count. This in vivo study documents that CSF floating BC cells overexpress prognostic and putative cancer stem cell biomarkers related to tumor invasiveness, potentially representing a molecular target for circulating tumor cell detection and LM treatment monitoring, as well as a primary target for innovative treatment strategies. The T lymphocyte infiltration, documented in all CSF samples, suggests a possible involvement of the CNS lymphatic system in both lymphoid and cancer cell migration into and out of the meninges, supporting the extension of a new form of cellular immunotherapy to LM.