

LUNG TUMOR-ASSOCIATED PLEURAL FLUIDS: DIAGNOSTIC AND PROGNOSTIC VALUE OF INFLAMMATORY MARKERS



J Kotyza(1), Karin Buňatová(1), D Havel(2), J Vrzalová(3), M Pešek(2)

(1) Dep. of Biochemistry, Medical Faculty, Charles University in Plzeň, Czech Republic

(2) Dep. of Pneumology, University Hospital in Plzeň, Czech Republic

(3) Laboratory of Immunoanalysis, University Hospital in Plzeň, Czech Republic

ABSTRACT

We analyzed protein levels of 13 markers of inflammation (IMs) in pleural effusions (PEs). IM levels in tumor associated effusions (n=116) were compared with those of parainflammatory PEs (n=30), transudates (n=18), and serum values, and evaluated regarding cancer origin, histology, cytology, pleural involvement, treatment history and survival period.

It appears that most IMs are highly expressed in many tumor-associated PEs reflecting to some extent origin and localization of tumors. Despite lower efficacy of IMs in the differentiation between exudative PEs, some IMs, especially IL-8 and VEGF, may represent novel prognostic markers of malignant processes in the pleural space.

INTRODUCTION

A pleural effusion (PE) is an excessive accumulation of fluid in the pleural space caused by hemodynamic factors (transudates) or, more frequently, by local factors like malignancies and inflammatory processes (exudates).

To relate protein levels of IMs to underlying malignant processes in the pleural space, PEs from lung cancer patients were subjected to a multifactorial analysis covering 13 IMs including C-reactive protein (CRP), urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), soluble vascular adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), myeloperoxidase (MPO) and matrix metalloproteinase-9 (MMP-9). IM levels in tumor associated effusions were compared with those of parainflammatory PEs, transudates and serum values, and evaluated regarding cancer origin, histology, cytology, pleural involvement, treatment history and survival period.

PATIENTS

PE samples were obtained from consecutive 164 patients undergoing thoracentesis or thoracoscopy for therapeutic or diagnostic reasons. All patients gave their informed consent and the protocol was approved by the University Hospital Ethics Committee.

METHODS

IL-6, IL-8, IL-10, MCP-1, sVCAM-1, sICAM-1, MPO, MMP-9, VEGF, and PAI-1 were quantitated by xMAP Technology on a Luminex 100 analyzer (Luminex Corp., USA). CRP was analyzed with the immunoturbidimetric method using the K-Assay set (Kamiya Biomedical Company) and uPA was quantitated by a commercial immunoadsorbent sandwich assay (uPA ELISA Oncogene Science, Bayer HealthCare LLC, Cambridge, USA). Analytical data were evaluated by nonparametric tests.

TABLE I - COMPARISON OF PLEURAL EFFUSION AND SERUM VALUES OF INFLAMMATORY MARKERS

	Pleural effusions Median IR (n=89)	Sera Median IR (n=89)	Wilcoxon two-sample test (p)	Spearman's rank correlation
CRP (mg/L)	13.0 (4.0 25.0)	53.0 (22.0 106)	<0.001	r=0.83 p=0.001
PAI-1 (ng/mL)	37.9 (12.3 117.3)	19.2 (13.8 28.6)	0.001	r=0.09 p=0.51
IL-6 (pg/mL)	8771 (4452 14294)	25.4 (11.2 45.7)	<0.001	r=0.49 p=0.001
IL-8 (pg/mL)	85.3 (27.8 315.5)	9.3 (4.7 18.6)	<0.001	r=0.29 p=0.04
IL-10 (pg/mL)	73.8 (44.4 135.9)	5.8 (2.9 12.7)	<0.001	r=0.11 p=0.21
MCP-1 (pg/mL)	420 (260 535)	165 (99.9 263)	<0.001	r=0.21 p=0.15
TNF-α (pg/mL)	3.9 (1.7 6.1)	2.3 (1.5 3.8)	0.04	r=0.56 p=0.001
VEGF (pg/mL)	170 (21.0 655)	296 (10.7 135)	<0.001	r=0.38 p=0.01
sICAM-1 (ng/mL)	93.8 (64.4 151)	224 (155 324)	<0.001	r=0.34 p=0.02
sVCAM-1 (ng/mL)	363 (275 495)	1124 (888 1476)	<0.001	r=0.24 p=0.09
MPO (ng/mL)	250 (91.5 1017)	210 (144 298)	0.09	r=0.27 p=0.06
MMP-9 (ng/mL)	12.9 (5.9 38.1)	289 (173 503)	<0.001	r=0.04 p=0.77

IR, interquartile range

TABLE II - VALUES OF INFLAMMATORY MARKERS IN PARANEOPLASTIC EFFUSIONS, PARAINFLAMMATORY EFFUSIONS AND TRANSUDATES

	Paraneoplastic exudate (n=116) Median IR	Parainflammatory exudate (n=30) Median IR	Transudate (n=18) Median IR
CRP (mg/L)	19.5 ³ (8.0 37.0)	7.5 (1.0 35.6)	4.1 (1.0 26.8)
uPA (ng/mL)	2.0 ² (1.3 4.1)	3.9 ¹ (1.6 7.7)	1.0 (0.4 1.2)
PAI-1 (ng/mL)	27.7 ^{2,4} (11.0 104)	122 ¹ (38 299)	5.1 (2.0 21.8)
IL-6 (pg/mL)	8.7 ² (4.1 14.3)	17.9 ¹ (5.8 60.9)	0.8 (0.6 1.8)
IL-8 (pg/mL)	106 ¹ (38.2 394)	158 ¹ (48.8 1756)	21.6 (11.4 40.5)
IL-10 (pg/mL)	84.2 ³ (52.7 144)	89.3 (46.7 150)	41.0 (19.9 79.9)
MCP-1 (pg/mL)	442 (277 640)	565 (332 1015)	365 (223 492)
TNF-α (pg/mL)	4.0 (1.9 7.2)	4.7 (2.3 14.6)	3.6 (2.2 4.5)
VEGF (pg/mL)	209 ¹ (34.9 644)	256 ² (18.5 519)	5.5 (3.2 22.8)
sICAM-1 (ng/mL)	96.2 ² (64.4 151)	105 ² (72.1 152.3)	49.6 (31.6 76.9)
sVCAM-1 (ng/mL)	397 ³ (278 526)	515 ¹ (326 829)	201 (163 372)
MPO (ng/mL)	121 ¹ (28.8 551)	214 ¹ (19.8 397)	5.9 (2.3 21.8)
MMP-9 (ng/mL)	12.5 ² (5.4 49.1)	17.5 ¹ (11.4 86.7)	4.3 (1.6 8.4)

IR, interquartile range

¹significant to transudate (Wilcoxon two-sample test) at p<0.001

²significant to transudate at p<0.01

³significant to transudate at p<0.05

⁴significant to parainflammatory exudate at p<0.01

TABLE III - SPEARMAN RANK CORRELATION BETWEEN PLEURAL INFLAMMATORY MARKERS AND SURVIVAL IN LUNG CANCER PATIENTS RELATED TO TUMOR ORIGIN

	Lung tumor effusions not differentiated n=102 r / (p)	Primary tumors n=84 r / (p)	Metastatic tumors n=18 r / (p)
CRP	-0.23 (0.02)	-0.23 (0.04)	-0.19 (n.s.)
uPA	0.14 (n.s.)	0.18 (n.s.)	0.18 (n.s.)
PAI-1	-0.27 (0.01)	-0.26 (0.02)	-0.24 (n.s.)
IL-6	-0.18 (n.s.)	-0.17 (n.s.)	-0.28 (n.s.)
IL-8	-0.36 (0.001)	-0.31 (0.005)	-0.64 (0.01)
IL-10	-0.01 (n.s.)	0.03 (n.s.)	-0.28 (n.s.)
MCP-1	-0.06 (n.s.)	-0.09 (n.s.)	0.12 (n.s.)
TNF-α	0.11 (n.s.)	0.11 (n.s.)	0.19 (n.s.)
VEGF	-0.35 (0.001)	-0.34 (0.002)	-0.34 (n.s.)
sICAM-1	0.22 (0.03)	0.15 (n.s.)	0.55 (0.03)
sVCAM-1	0.27 (0.008)	0.30 (0.01)	0.31 (n.s.)
MPO	-0.20 (0.04)	-0.13 (n.s.)	-0.49 (0.05)
MMP-9	-0.17 (n.s.)	-0.20 (0.07)	-0.11 (n.s.)

RESULTS

IMs were significantly expressed in PEs of paraneoplastic origin when compared to transudates and most serum levels (Table I and Table II). Values in pleura-invading and metastatic tumor associated effusions were typically higher than those of other tumors. Although the highest IM values most frequently accompanied small-cell lung cancer, no significant differences were found between histological subgroups of tumors. Many markers negatively correlated with survival, most prominently IL-8 (r=-0.36, p=0.001) and VEGF (r=-0.35, p=0.001) (Table III).

CONCLUSIONS

The relative autonomy of the pleural space is demonstrated first and foremost by higher pleural compared to serum levels of a variety of IMs, most markedly IL-6, IL-8, and IL-10. On the other hand, a few IMs, such as CRP, are predominant in serum. It appears that most IMs are highly expressed in many tumor-associated PEs reflecting to some extent origin and localization of tumors. Although the differences were not significant, the highest values of PAI-1, TNF-α, sVCAM-1, MPO, MMP-9, IL-1 and IL-10 were found in small-cell carcinoma, the most aggressive of lung tumors. Despite lower efficacy of IMs in the differentiation between exudative PEs, some IMs may represent potential prognostic markers of malignant processes in the pleural space, especially pleural IL-8 and VEGF may be regarded as potential prognostic factors in lung cancer.

References

- Light RW. Disorders of the pleura, mediastinum, diaphragm, and chest wall. In: Kasper DL, Braunwald E, Hauser S, Longo D, Jameson JL, Fauci AS, eds. Harrison's principles of internal medicine, 16th Ed. New York: McGraw-Hill Companies, Inc. 2005; 1565-9.
- Hoheisel G, Izbicki G, Roth M, et al. Proinflammatory cytokine levels in patients with lung cancer and carcinomatous pleurisy. *Respiration* 1998; 65: 183-6.
- Yano S, Sinochara H, Herbst RS, et al. Production of experimental malignant pleural effusion is dependent on invasion of the pleura and expression of vascular endothelial growth factor/vascular permeability factor by human lung cancer cells. *Am J Pathol* 2000; 157: 1893-903.
- Chen YM, Yang WK, Whang-Peng J, Tsai CM, Perng RP. An analysis of cytokine status in the serum and effusions of patients with tuberculosis and lung cancer. *Lung Cancer* 2001; 31: 25-30.
- Terada H, Urano T, Konno H. Association of interleukin-8 and plasminogen activator system in the progression of colorectal cancer. *Eur Surg res* 2005; 37: 166-172.

Acknowledgements

This work was supported by the Ministry of Education, Research Project MSM 0021620819, the Czech Republic.