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Research Article

The Potential of the Quick Detection of Selectins Using Raman Spectroscopy to Discriminate Lung Cancer Patients from Healthy Subjects

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This study aimed at determining the concentration of P-selectins in lung cancer patients in different stages and healthy subjects. Then, the ability of the methodology developed to discriminate the existence of lung cancer was also evaluated. Serum spectra were obtained using Raman spectroscopy (RS). Blood samples were taken from subjects divided into two groups: group 1—comparing data from 22 patients clinically diagnosed with cancer before versus after medical intervention; group 2—comparing data from 10 palliative patients versus 17 healthy volunteers. The RS analysis of the samples revealed the presence of five very similar peaks in both groups 1 and 2. This leads to the conclusion that a medical intervention in cancer cases gives results comparable to those obtained from healthy subjects. The study indicates that the use of Raman spectroscopy can produce a better classification of cancer patients. However, diagnostically the results have not been statistically significant, probably due to the limited number of samples gathered. A larger number of samples would be required for future verification.

1. Introduction

P-selectin is a molecule which belongs to the selectin family together with P-platelets, E-endothelials, and L-leukocytes. It is stored in the granules and the endothelial cell—the Weibel–Palade bodies on platelets. It is a cell adhesion molecule present on the surface of an activated vascular endothelial cell, which is responsible for the interaction of the inner layer of blood vessels with activated thrombocytes [1–4]. The physiological functions of selectins in the processes of inflammation, immune response, wound repair, and homeostasis have been described before [5]. P-selectin, in particular, is an important disease marker as it plays an essential role in many inflammatory processes including cancer, coronary artery disease, stroke, and diabetes [6, 7]. This molecule is stored on the cell surface of the endothelial cell and platelets, and after rapid endothelium stimulation (e.g., by thrombin, histamine, and reactive oxidized substances), P-selectin is translocated on the surface of the endothelial cell. It was recognized that P-selectin may be a candidate involved in the metastatic process [8–10]. There are studies reporting that P-selectin plays a functional role in metastasis formation in breast, colon [11, 12], and lung cancers [10, 13]. There was a study which explained a deeper role of P-selectin. The study showed that after the removal of the cell surface mucin from tumor cells in the lungs of mice (in the absence of P-selectin), a reduction of metastasis can be observed [8]. In several laboratory studies, P-selectin is described as a potential candidate for biomarkers whose higher concentration is presented and involved in the

metastatic process [8, 11, 14–16]. Raman spectroscopy can provide information about the conformation of macromolecules such as lipids, proteins, and nucleic acids. This method has been used to assess certain factors in several diseases such as diabetes, Alzheimer's, and also cancer. The differences in the involved molecules (lipids, proteins, and nuclear acids) occur in the tertiary and quaternary structures, so it is not possible to determine precisely which molecule caused differences in the spectra. Raman spectroscopy and statistical analysis can be used to discriminate between a serum sample from a lung cancer patient and a healthy sample. In this study, the intensity of P-selectin and its correlation with different clinical stages of lung cancer patients were described. P-selectin plays a crucial role in the recruitment of leukocytes into the environment of the tumor and contributes to the formation of the tumor cell or/and its migration. It was found that some band ratios were significant and corresponded to the concentration of P-selectin in serum samples. Those specific bands might be helpful in screening for lung cancer using Raman spectroscopy of serum samples.

2. Materials and Methods

This prospective study was recorded in http://clinicaltrials.gov Identifier: NCT02758678 and was conducted in the Regional Clinical Hospital in Zielona Góra between November 2015 and April 2016. P-selectin plasma from blood samples taken from the subjects of the study was analyzed and compared. This study was approved by the Ethics Committee at the Medical Council in The Regional Medical Chamber, Zielona Góra, Poland (no. 2/57/2015). The subjects participating in the study provided both oral and written informed consent.

2.1. Subject Characteristics. The subjects of the study were divided into 2 groups. Group 1: 36 patients who had undergone surgical staging procedures of whom 22 patients had a confirmed lung cancer and the remaining 12 had a nonmalignant tumor. Blood samples were taken just before the medical procedure (surgery) and were next compared to the blood samples taken after 3 months , in the follow-up. Group 2: blood samples from ten patients with disseminated disease (blood taken before palliative radiotherapy) were compared with the blood from 17 healthy volunteers. Prior to the medical procedure (surgery and radiotherapy), all patients in both groups had their condition histologically and/or cytologically confirmed. Only patients with disease and without any previous chemo/radiotherapy history were included in the study. The histopathological data were defined according to the *Union for International Cancer Control TNM Classification* (UICC, 7th edition) [17]. The patients' characteristics are shown in Table 1. The blood was processed immediately after it was drawn. Next, the blood samples were analyzed with Raman spectroscopy methods.

2.2. Serum Separation and Raman Spectroscopy Analysis. Blood samples were obtained from a total of 49 patients. A set of P-selectin was purchased from Randox Laboratories Limited (UK). A single 2 ml heparinised peripheral blood sample was centrifuged to get serum specimens. The Raman spectra (RS) of the solid residues from the serum samples were measured by placing 10 μl of serum from the aliquots onto an aluminium substrate and allowed to dry for at least 30 min. A Renishaw inVia confocal Raman spectrometer with a Leica microscope, 50x objective (numerical aperture of 0.75), and WIRE 2.0 software were used. The Raman system was calibrated with a silicon standard using the Raman peak at 520 cm^{-1}. Multiple scans were conducted on the solid residue by moving the substrate on an X-Y stage. The wavelength of excitation was 785 nm. The radius of the beam was 3.0 μm, and the laser power irradiation over the sample was 5mW. Each spectrum was taken with an exposure of 30 s, 10 accumulation and collected in the region from 450 to 1500 cm^{-1}.

Table 1: Patients and clinical tumor characteristics.

Characteristics of patients	Group 1	Group 2	Control group
Age, years			
Median (range)	63 (53–77)	68 (58–80)	59 (47–70)
Gender			
Male	17	3	7
Female	5	7	10
Histology			
Non-small cell carcinoma	3	3	
Adenocarcinoma	13	5	
Squamous	6	2	
Surgery	22	0	
Non surgery	0	10	
TNM factor			
T1a/T1b/T2a/T2b/T3/T4	4/6/6/3/2/1	0/1/1/0/4/4	
N0	15	1	
N1N2/N3	6/1/0	1/7/1	
M0	22	1	
M1	0	9	

T, tumor; N, nodes; M, metastases.

2.3. Statistical Analysis. The statistical analysis of the registered Raman spectra was conducted [18]. It was originally constructed for the so-called matrix-assisted laser desorption/ionization (MALDI) spectrometry. This approach uses a binary discriminate analysis and works by data-adaptive thresholding of the expression values and peak selection and subsequent ranking of the dichotomized features using a relative entropy measure (as a generalization of the "peak probability contrast"). The methodology of the analysis, i.e., R scripts reproducing all described computational procedures, is available under the GNU General Public License from CRAN "MALDIquant" package [19].

3. Results

The MALDI computational procedures used on the spectra from the 2 groups revealed five probable peaks differentiating the analyzed two sets of samples (Table 2).

Table 2: Identified peaks.

Peak ID	1	2	3	4	5
Before intervention vs after intervention	853	1005	1208	1320	1449
t-test p value (dependent samples)	0.6140	0.06846	0.0424	0.8632	0.6825
Palliative vs healthy	855	1007	1210	1320	1449
t-test p value (independent samples)	0.6049	0.1781	0.1089	0.02756	0.2215

Based on these peaks, very similar in both groups, a comparable effect can be established between the results from patients after intervention in cancer cases as well as from palliatives and the results from healthy subjects. Then, using real spectrometric data, the differences between the groups of patients were calculated using standard Student's t-tests for dependent and independent samples. Only two peaks, 1208 (before intervention vs after intervention) and 1320 (palliative vs healthy), were assessed statistically significant which means higher P-selectin intensity for before-intervention subjects and for palliative subjects; the difference for 1005 peak (before intervention vs after intervention) was on the border of the statistical significance (see p values in Table 2). Some illustrative plots of particular spectra for a palliative patient and a healthy person are presented combined in Figure 1.

However, the estimated diagnostic power is not statistically satisfactory (Table 3).

It can be seen from Table 3 that a better classification of patients can be made using the spectra analysis of the samples from group 2 (palliative vs healthy), but probably due to the limited number of samples gathered, the reported results are not statistically significant so far and a larger number of samples are required in the future to verify these findings.

4. Discussion

Due to the progress of the neoplastic process, the morphology and biochemistry of the tissue changes. Hence Raman spectroscopy as a fast and nondestructive technique to analyze it seems to be useful. Biochemical markers of cancer cells, such as selectins, can be analyzed by Raman spectroscopy by comparing healthy cells and diseased tissue [20].

The main goal of the study was to detect the P-selectin intensity in patients with lung cancer before and after surgical procedures and in palliative patients compared to healthy volunteers. A simple approach to differential expression and classification for Raman spectra was presented. In this small sample study, the differences in the relative intensity of five probable peaks were obtained in two sets of samples. A P-selectin increase was observed in the lung cancer patients before the surgical procedure and the palliative patients. A similar result was presented in the study with a more advanced Raman spectroscopy [21] (extensive spectral database and neural network analysis). It was shown

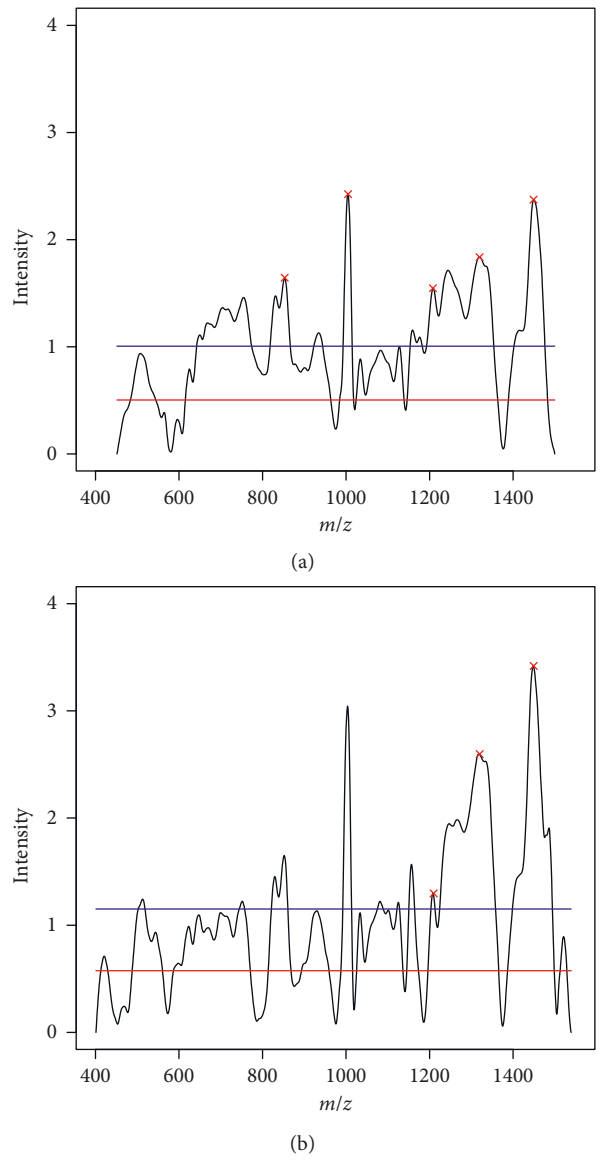

Figure 1: Raman spectra (with identified peaks and noise lines): (a) palliative patient (b) healthy person.

Table 3: Diagnostics.

Diagnostic measures	Accuracy	Sensitivity	Specificity	ppv	npv
Before intervention vs after intervention	0.50	0.57	0.44	0.50	0.51
Palliative vs healthy	0.66	0.30	0.88	0.58	0.68

*ppv = positive predictive value; npv = negative predictive value.

that the use of these tools could provide fast and reliable diagnosis of different types of cancers in clinical settings. Another study [13] obtained similar results, where increased P-selectin value differed significantly from the levels in healthy individuals. The carcinoma mucin induces microthrombi formation and triggers platelet activation and

P-selectin expression [22, 23]. Another clinical study [24–26] also reported changes of inflammatory and coagulatory biomarkers which led to a reduction in parameters which might be a contribution to the progression of cancer. The use of the P-selectin expression as a marker with its simple and quick diagnosis could help to better determine the stage of cancer and in turn lead to a more appropriate choice of treatment methods.

5. Conclusions

Raman spectroscopy can be characterized as a noninvasive procedure which makes it a very good candidate for a prediagnostic tool. We plan to extend our study to a larger population and repeat the statistical analysis in the future.

Data Availability

Statistical analysis and data used to support the findings of this study are available from the corresponding author upon request (andrzej.tukiendorf@gmail.com).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References

[1] G. S Kansas, "Selectins and their ligands: current concepts and controversis," *Blood*, vol. 88, no. 9, pp. 3259–3287, 1996.

[2] H. Läubli and L. Borsig, "Selectins promote tumor metastasis," *Seminars in Cancer Biology*, vol. 20, no. 9, pp. 169–177, 2010.

[3] L. Erpenbeck and M. P. Schön, "Deadly allies: the fatal interplay between platelets and metastasizing cancer cells," *Blood*, vol. 115, no. 117, pp. 3427–3436, 2010.

[4] H. Amano, Y. Ito, F. Ogawa et al., "Angiotensin II type 1A receptor signalling facilitates tumour metastasis formation through P-selectin–mediated interaction of tumour cells with platelets and endothelial cells," *American Journal of Pathology*, vol. 182, no. 2, pp. 553–564, 2013.

[5] K. Ley, M. Laudanna, I. Cybulsky, and S. Nourshargh, "Getting to the site of inflammation: the leukocyte adhesion cascade updated," *Nature Reviews Immunology*, vol. 7, no. 9, pp. 678–689, 2007.

[6] D. E. Lorant, M. K. Topham, R. E. Whatley et al., "Inflammatory roles of P-selectin," *Journal of Clinical Investigation*, vol. 92, no. 2, pp. 559–570, 1993.

[7] T. J. M. Molenaar, J. Twisk, S. A. M. de Haas et al., "P-selectin as a candidate target in atherosclerosis," *Biochemical Pharmacology*, vol. 66, no. 5, pp. 859–866, 2003.

[8] L. Borsig, R. Wong, J. Feramisco, D. R. Nadeau, N. M. Varki, and A. Varki, "Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis," *Proceedings of the National Academy of Sciences*, vol. 98, no. 6, pp. 3352–3357, 2001.

[9] K. V. Honn, D. G. Tang, and J. D. Crissman, "Platelets and cancer metastasis: a causal relationship?," *Cancer and Metastasis Reviews*, vol. 11, no. 3-4, pp. 325–351, 1992.

[10] F. Heidemann, A. Schildt, K. Schmid et al., "Selectins mediate small cell lung cancer systemic metastasis," *PLoS One*, vol. 9, no. 4, Article ID e92327, 2014.

[11] S. Köhler, S. Ullrich, U. Richter, and U. Schumacher, "E-/P-selectins and colon carcinoma metastasis: first in vivo evidence for their crucial role in a clinically relevant model of spontaneous metastasis formation in the lung," *British Journal of Cancer*, vol. 102, no. 3, pp. 602–609, 2010.

[12] K. Stübke, D. Wicklein, L. Herich, U. Schumacher, and N. Nehmann, "Selectin-deficiency reduces the number of spontaneous metastases in a xenograft model of human breast cancer," *Cancer Letters*, vol. 321, pp. 89–99, 2012.

[13] Y. V. Dumanskiy, O. Y. Stoliarova, O. V. Syniachenko, and E. D. Iegudina, "Endothelial dysfunction of vessels at lung cancer," *Experimental Oncology*, vol. 37, no. 4, pp. 277–280, 2015.

[14] L. Borsig, R. Wong, R. O. Hynes, N. M. Varki, and A. Varki, "Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis," *Proceedings of the National Academy of Sciences*, vol. 99, no. 4, pp. 2193–2198, 2002.

[15] Y. J. Kim, L. Borsig, N. M. Varki, and A. Varki, "P-selectin deficiency attenuates tumor growth and metastasis," *Proceedings of the National Academy of Sciences*, vol. 95, no. 16, pp. 9325–9330, 1998.

[16] H. L¨aubli and L. Borsig, "Selectins as mediators of lung metastasis," *Cancer Microenvironment*, vol. 3, no. 1, pp. 97–105, 2010.

[17] L. H. Sobin, M. K. Gospodarowicz, and C. Wittekind, *TNM Classification of Malignant Tumours*pp. 208–215, UICC, Geneva, Switzerland, 7th edition, 2010.

[18] S. Gibb and K. Strimmer, "Differential protein expression and peak selection in mass spectrometry data by binary discriminant analysis," *Bioinformatics*, vol. 31, no. 19, pp. 3156–3162, 2015.

[19] S. Gibb and K. Strimmer, "MALDIquant: a versatile R package for the analysis of mass spectrometry data," *Bioinformatics*, vol. 28, no. 17, pp. 2270-2271, 2012.

[20] R. R. Alfano, C. H. Liu, W. L. Sha et al., "Human breast tissue studied by IR Fourier transform Raman spectroscopy," *Lasers in Life Science*, vol. 4, pp. 23–28, 1991.

[21] A. Pavićević, S. Glumac, J. Sopta, A. Popović-Bijelić, M. Mojović, and G. Bačić, "Raman microspectroscopy as a biomarking tool for in vitro diagnosis of cancer: a feasibility study," *Croatian Medical Journal*, vol. 53, no. 6, pp. 551–557, 2012.

[22] G. Bendas and L. Borsig, "Cancer cell adhesion and metastasis: selectins, integrins, and the inhibitory potential of heparins," *International Journal of Cell Biology*, vol. 2012, Article ID 676731, 10 pages, 2012.

[23] A. J. Tafur, E. Wolny, R. McBane et al., "Three-month cumulative incidence of thromboembolism and bleeding after periprocedural anticoagulation management of patients with active cancer," *Blood*, vol. 114, no. 22, p. 491, 2009.

[24] E. Wolny-Rokicka, K. Brzezniakiewicz-Janus, J. Wydmański, A. Tukiendorf, and A. Zembroń-Łacny, "Analysis of haemostasis biomarkers in patients with advanced stage lung cancer during hypofractionated radiotherapy treatment," *Journal of International Medical Research*, vol. 46, no. 5, pp. 1876–1883, 2018.

[25] E. Wolny-Rokicka, J. Wydmański, A. Tukiendorf et al., "The correlation of blood parameters with size in cases of neoplastic tumor," *Asian Pacific Journal of Cancer Prevention*, pp. 1710–2484, 2018.

[26] E. Wolny-Rokicka, J. Wydmański, A. Tukiendorf, P. Mróz, and A. Zembroń-Łacny, "Study of appraisal of basic-haemostatic markers in lung cancer patients in follow up after radiotherapy," *Medical Science Monitor*, vol. 24, 2018.