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CHEMOMETRIC ANALYSIS OF BIOCHEMICAL LABORATORY DATA OF ONCOLOGY PATIENTS AFTER MORPHINE TREATMENT[1]

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Relationships among serum levels of morphine (M), morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) as well as the results of several standard biochemical tests were investigated in oncology patients, to whom morphine was administered. The level of the serum concentrations of M, M3G and M6G, determined by a new HPLC method, plays an important role in pain resistance; particularly the M3G/M6G ratio is extraordinarily important. The magnitude of this ratio is a complex function of the patient's gender, type of the

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morphine drug and tumor location. Studies of relations between the standard biochemical tests and the M, M3G, and M6G concentrations, and the M3G/M6G ratio, revealed a strong connection between M3G/M6G and the clearance of creatinine.

Introduction

The largest amount of analyses per year is performed in clinical chemistry. Clinical analyses are utilized mainly to specify diagnosis and suggest an appropriate treatment of the patient. Annually, 10 million new cases of cancer are diagnosed worldwide. Cancer is responsible for 6 million deaths yearly and is the second leading cause of mortality in industrialized countries. The World Health Organization recommended morphine as an appropriate analgesic drug for managing moderate and severe pain associated with cancer. After the administration, morphine (M) undergoes extensive metabolism, which primarily occurs in liver. Glucuronidation is the main metabolic pathway producing mostly morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), which play a significant role in dynamic responses of the morphine therapy. M6G exhibits similar affinity for the opioid receptors to that of M; in contrast, M3G has no analgesic effect. Monitoring of morphine and its metabolites concentration in biological fluids may reveal the relationships between the applied doses of the drug, the plasma levels and analgesic effects, as well as to investigate the metabolic rate. Even though the concentration ratios M3G/M and M6G/M are known as approximately 50 and 9, respectively [1,2], they are different for individual patients and enable, together with the concentration ratio M3G/M6G, to characterize the patients who metabolize more M3G or more M6G compared to the average value.

The objective of this work is chemometric elucidation of the relationships among (i) the basic characteristics of the patient (age, weight, height, *BMI* – the body mass index, patient's gender), (ii) morphine treatment (different daily dose, dose/weight ratio - *DW*, two kinds of the administered drug), (iii) serum concentration of morphine and its metabolites (variables *M*, *M3G*, *M6G*) and their ratios (variables *M3G_M*, *M6G_M* and *M3G_M6G*, in which names underline is used instead of slash for practical reasons regarding the used software) (iv) monitored biochemical parameters (*Creat*, *Cl_Creat*, *Urea*, *TP*, *Alb*, *TB*, *GMT*, *ALT*, *AST*, *ALP*, *LDH*), and (v) the corresponding malignant disease (9 tumor locations explained hereafter).

Experimental

Patients' Samples and Further Characteristics

Two sets of patient samples were investigated. The first, smaller set comprises 43 records (21 men, 22 women, patient numbers 1–43), the second larger set contains 103 records (50 men, 53 women, patient numbers 1–103), a subset of which is the first dataset. Only the patients without renal or hepatic dysfunction were included in the first set, however, they were included in the larger second set of data. All patients who participated in the study had cancer and had been on analgesic treatment with morphine longer than one week The morphine doses were not changed during the study and no other opioids were given. Two morphine drugs containing morphinum sulphate were administered: *MST Continus* (Mundifarma, Germany) and *Slovalgin Retard* (Slovakofarma, Slovakia). Despite of regular morphine treatment some patients were not satisfied and felt pain. The samples from five such patients were contained in the smaller data set (numbers 12, 32, 35, 40, 43), and the samples from eleven not satisfied patients were found in the larger data set (numbers 60, 69, 92, 93, 98 and 99 in addition to the cases mentioned before).

Different kind of malignity was diagnosed, which was specified according to nine tumour locations (TL): *Gastrointestinal* (6 in the smaller set – S, 20 in the larger set – L), *Female urogenital* (14 S, 21 L), *Blood tissue* - leukaemia (7 S, 14 L), *Bone* (5 S, 12 L), *Lung* (4 S, 7 L), *Male urogenital* (2 S, 12 L), *Breast* (3 S, 12 L), *Brain* (1 S, 4 L), *Heart* (1 S, 1 L). Given names designate the corresponding TL categorical variables with the value of 1 when the specified diagnosis was true and 0 when it was false (only one of nine TL cases was true for the given patient). The TL variable was used in multivariate data analysis only when the number of patients with TL in the given set was equal to, or larger than 4.

All delivered as well as some newly calculated data were summarized in a table with 103 rows (patients), 21 columns representing three groups of continuous variables: 4 basic characteristics (C), 11 biochemical parameters (D), and 6 morphine variables (E), and with 4 additional columns containing 2 binary variables *Sex* and *Drug*, the nominal variable *Dose* (daily dose of morphinum sulphate) and the ratio *DW* (the dose divided by the weight — considered as a continuous variable).

Standard Solutions

Stock standard solutions were prepared in the following way: morphine hydrochloride (M) $c = 96\ \mu g\ ml^{-1}$) in methanol, morphine-3-glucuronide (M3G) ($c = 82\ \mu g\ ml^{-1}$) and morphine-6-glucuronide (M6G) ($c = 153\ \mu g\ ml^{-1}$) were

prepared in methanol - water mixture (1:1). Morphine hydrochloride standard was obtained from the National Cancer Institute, Slovakia, the M3G standard from the Institute of Forensic Science of the Slovak Police Corps, Bratislava, Slovakia, the M6G standard from Lipomed, Switzerland. Other solutions were prepared diluting the stock solutions with deionised water. Acetonitrile and methanol (HPLC grade) were supplied by Merck, Slovakia. All the other chemicals were Reagent Grade (p.a.) from Lachema, Brno, Czech Republic, except natrium-1-octanesulfonic acid, purchased from Pragolab, Prague, Czech Republic.

Blood Samples Collection and Extraction

Samples (7 mL of venous blood) were obtained using bleeding vacutainer system (Vacutainer 15 027), centrifuged 10 min at 1200 g and the serum was stored at –20 °C until analysis. The columns Chromabond C_{18}-EC were used for the solid-phase extraction. Extraction column was pre-conditioned with a) methanol, b) 10 mmol l^{-1} phosphate buffer in 40 % acetonitrile (pH 2.1), and c) distilled water. A 1 ml serum sample aliquot was loaded onto the extraction column, washed with hydrogen carbonate buffer (pH 9.3) and eluted with 1 ml of methanol. The eluate was evaporated, redissolved in 1 ml H_2O and a 20 µl aliquot was injected onto the HPLC column.

Instrumentation

A Delta Chrom™ DS 030 HPLC pump (Watrex, Slovakia) together with a 20 µl loop Rheodyne 7125 injector (Waters Corp., USA), an autosampler Basic-Marathon (Spark, The Netherlands) and a UV-VIS detector 484 (Waters Corp., U.S.A.) constituted the HPLC system used. A CSW 1 software (Microsoft, USA) was used for the data collection. For the HPLC separations a Symmetry C_{18} (150 × 4.9 mm, 5 µm) reversed-phase column (Waters Corp., U.S.A.) was applied. The UV detection was performed at 210 nm. The mobile phase consisted of 30 mmol l^{-1} phosphate buffer (pH 3) with 1 mmol l^{-1} octanesulfonic acid in 8 % acetonitrile in water. Its flow-rate was 0.8 ml min^{-1} at room temperature.

Applied Chemometric Techniques

Several chemometric techniques were applied: principal component analysis, canonical correlation analysis, cluster analysis (grouping of variables), logistic discrimination, analysis of variance and correlation analysis. Logistic regression enabled to predict the categories of the human samples according to several chosen

criteria. Software packages Stagraphics Plus ver. 5.0 (Manugistics Inc., Rockville, MD, U.S.A.) and Systat 10 (SPSS Inc., Chicago, Ill., U.S.A.) were used for chemometric calculations.

Results and Discussion

A large number of data suitable for chemometric analysis were organized into three tables. Table I brings information on personal characteristics: gender (variable *Sex*), age (*Age*), weight (*Weight*), height (*Height*) of the monitored oncology patients together with their tumour location (categorical variable *TL*), kind of administered morphine drug (categorical variable *Drug*), its dose and frequency (variable *Dose* then represents the total morphine dose per day).

Concentrations of morphine (variable *M*), morphine-3-glucuronide (*M3G*) and morphine-6-glucuronide (*M6G*) in serum of oncology patients as well as their ratios are summarized in Table II. Due to software limitations to express the slash (/) the names of corresponding variables are *M6G_M*, *M3G_M* and *M3G_M6G*. The results of 11 common clinical tests are exhibited in Table III. They characterize the overall clinical state of the monitored oncology patients. The used names of the corresponding variables denote: *Creat* - creatinine, *ClCreat* – clearance of creatinine, *TP* – total protein, *Alb* – albumin, *TB* – total bilirubin, *GMT* – glutamyl transferase, *ALT* – alanine aminotransferase, *AST* – aspartate aminotransferase, *ALP* – alkalic phosphatase, *LDH* – lactate dihydrogenase.

Application of standard as well as robust statistics (mainly the median and the MAD, the median of absolute distances from the median, as the analogs of the mean and the standard deviation) to the data, revealed that:

(1) The concentration of morphine in the blood serum samples of the patients satisfied with analgesic treatment is in the range 14.7 – 70.4 ng ml^{-1}, which is in good agreement with the literature data 17 – 60 ng ml^{-1} [1].

(2) The concentration range for M3G, M6G, M6G/M and M3G/M is very broad, the statistical distribution is considerably asymmetrical (which is typical of clinical results) and, therefore, the only convincing difference was found between the M3G/M6G concentration ratio for the group of patients satisfied with the analgesic treatment and the group of not satisfied patients. For example, for the smaller data set the M3G/M6G mean values and the standard deviations (in brackets) are 15.2 (5.0) and 6.5 (1.9) for the patients with and without pain, respectively. The respective robust values of the median and MAD are 15.0 (6.2) and 6.7 (1.7) for the same two groups. Thus, the increased concentration ratio M3G/M6G can be considered as a measure how successful is the analgesic effect of morphine, which is consistent with the results, described in literature [2].

(3) Among the serum concentrations of M, M3G and M6G and their ratios,

Table I Personal characteristics, tumor location and kind of treatment of the monitored oncology patients

Patient No.	Sex	Age year	Weight kg	Height m	Tumor location	Treatment	Dose mg day^{-1}
1	F	56	70	1.72	Gastrointestinal	MST cont. 30 mg a 12h	60
2	F	76	51	1.56	Female urogenital	MST cont. 30 mg a 12h	60
3	F	30	53	1.64	Female urogenital	Slovalgin 30 mg a 12h	0
4	M	67	83	1.76	Blood tissue	MST cont. 30 mg a 12h	60
5	F	48	67	1.56	Female urogenital	MST cont. 60 mg a 12h	120
6	M	45	70	1.68	Bone	MST cont. 60 mg a 12h	120
7	F	63	71	1.68	Blood tissue	Slovalgin 60 mg a 12h	120
8	F	50	75	1.64	Blood tissue	MST cont. 30 mg a 12h	60
9	F	46	70	1.78	Female urogenital	MST cont. 60 mg a 12h	120
10	F	47	42	1.58	Female urogenital	MST cont. 60 mg a 12h	120
11	M	21	70	1.85	Heart	MST cont. 30 mg a 12h	60
12	M	19	48	1.85	Blood tissue	MST cont. 30 mg a 12h	60
13	M	73	80	1.78	Gastrointestinal	MST cont. 30 mg a 12h	60
14	M	46	83	1.75	Blood tissue	MST cont. 60 mg a 12h	120
15	F	45	65	1.60	Female urogenital	Slovalgin 60 mg a 12h	120
16	M	65	81	1.70	Gastrointestinal	MST cont. 60 mg a 12h	120
17	F	65	77	1.74	Female urogenital	MST cont. 60 mg a 12h	120
18	F	68	75	1.58	Female urogenital	Slovalgin 30 mg a 12h	60
19	F	47	49	1.55	Lung	Slovalgin 30 mg a 12h	60
20	M	40	76	1.72	Blood tissue	MST cont. 60 mg a 12h	120
21	M	61	64	1.68	Male urogenital	MST cont. 30 mg a 8h	90
22	M	53	95	1.78	Gastrointestinal	MST cont. 60 mg a 12h	120
23	M	39	102	1.87	Bone	Slovalgin 60 mg a 12h	120
24	M	52	71	1.77	Lung	Slovalgin 30 mg a 8h	90
25	F	55	41	1.57	Gastrointestinal	MST cont. 30 mg a 8h	90
26	M	66	80	1.73	Gastrointestinal	MST cont. 30 mg a 12h	60
27	F	65	69	1.68	Breast	MST cont. 30 mg a 8h	90
28	F	27	60	1.72	Blood tissue	Slovalgin 60 mg a 12h	120
29	F	44	62	1.73	Female urogenital	Slovalgin 60 mg a 12h	120
30	F	47	52	1.55	Lung	MST cont. 60 mg a 8h	180
31	F	47	62	1.58	Female urogenital	Slovalgin 30 mg a 24h	30
32	M	65	81	1.70	Lung	MST cont. 30 mg a 12h	60
33	F	47	62	1.58	Female urogenital	Slovalgin 30 mg a 12h	60
34	M	45	70	1.68	Bone	Slovalgin 30 mg a 12h	60
35	M	30	70	1.80	Brain	MST cont. 60 mg a 8h	180
36	F	42	80	1.70	Breast	Slovalgin 30 mg a 12h	60
37	F	37	55	1.55	Breast	MST cont. 30 mg a 12h	60
38	F	42	60	1.65	Female urogenital	MST cont. 30 mg a 12h	60
39	F	27	61	1.53	Female urogenital	Slovalgin 60 mg a 12h	120
40	F	52	70	1.68	Female urogenital	Slovalgin 30 mg a 12h	60
41	M	19	70	1.80	Bone	MST cont. 30 mg a 12h	60
42	M	53	85	1.73	Bone	MST cont. 30 mg a 12h	60
43	M	31	78	1.78	Male urogenital	MST cont. 60 mg a 12h	120
44	F	60	56	1.65	Breast	MST cont. 30 mg a 12h	60
45	F	48	62	1.69	Female urogenital	Slovalgin 30 mg a 12h	60
46	M	23	75	1.81	Male urogenital	MST cont. 60 mg a 12h	120
47	M	23	75	1.81	Male urogenital	MST cont. 60 mg a 12h	120
48	M	62	79	1.65	Gastrointestinal	Slovalgin 30 mg a 8h	90
49	M	23	75	1.81	Male urogenital	Slovalgin 10 mg a 8h	30
50	F	51	64	1.68	Female urogenital	MST cont. 120 mg a 8h	360
51	M	51	55	1.68	Blood tissue	MST cont. 30 mg a 12h	60
52	M	51	55	1.68	Blood tissue	MST cont. 60 mg a8h	180

Table I – Continued

Patient No.	Sex	Age year	Weight kg	Height m	Tumor location	Treatment	Dose mg day^{-1}
53	M	62	79	1.65	Gastrointestinal	MST cont. 30 mg a 12h	60
54	M	72	70	1.80	Male urogenital	MST cont. 30 mg a 12h	60
55	M	39	72	1.78	Bone	Slovalgin 30 mg a 12h	60
56	F	69	59	1.64	Breast	Slovalgin 30 mg a 8h	90
57	F	46	58	1.64	Brain	Slovalgin 30 mg a 12h	60
58	F	48	62	1.68	Breast	Slovalgin 30 mg a 12h	60
59	M	41	70	1.84	Gastrointestinal	MST cont. 90 mg a 8h	270
60	F	43	56	1.59	Bone	Slovalgin 30 mg a 12h	60
61	M	62	55	1.68	Male urogenital	MST cont. 90 mg a 8h	270
62	M	62	55	1.68	Male urogenital	MST cont. 90 mg a 8h	270
63	M	62	79	1.65	Gastrointestinal	Slovalgin 10 mg a 4h	60
64	F	43	62	1.67	Female urogenital	MST cont. 30 mg a 12h	60
65	M	23	75	1.81	Male urogenital	Slovalgin 10 mg a 8h	30
66	M	23	75	1.81	Male urogenital	Slovalgin 10 mg a 8h	30
67	F	46	58	1.64	Brain	MST cont. 30 mg a 12h	60
68	F	68	54	1.58	Bone	Slovalgin 30 mg a 8h	90
69	M	41	70	1.84	Gastrointestinal	MST cont. 120 mg a 8h	360
70	F	53	88	1.73	Female urogenital	Slovalgin 10 mg a 12h	20
71	M	62	79	1.65	Gastrointestinal	Slovalgin 20 mg a 8h	60
72	F	64	59	1.65	Breast	Slovalgin 10 mg a 4h	60
73	M	62	63	1.68	Gastrointestinal	MST cont. 30 mg a 12h	60
74	F	67	95	1.62	Breast	MST cont. 60 mg a 8h	180
75	F	67	95	1.62	Breast	MST cont. 60 mg a 12h	120
76	F	67	95	1.62	Breast	MST cont. 60 mg a 12h	120
77	M	77	65	1.68	Gastrointestinal	Slovalgin 30 mg a 8h	90
78	F	60	64	1.58	Bone	Slovalgin 30 mg a 8h	90
79	F	52	39	1.62	Blood tissue	MST cont. 30 mg a 12h	60
80	F	60	64	1.58	Bone	Slovalgin 10 mg a 12h	20
81	F	52	39	1.62	Blood tissue	Slovalgin 10 mg a 4h	60
82	F	60	72	1.62	Blood tissue	MST cont. 30 mg a 12h	60
83	M	77	65	1.68	Gastrointestinal	MST cont. 30 mg a 8h	90
84	M	77	65	1.68	Gastrointestinal	Slovalgin 30 mg a 8h	90
85	F	62	61	1.62	Lung	Slovalgin 30 mg a 8h	90
86	F	62	61	1.62	Lung	Slovalgin 30 mg a 8h	90
87	F	36	61	1.75	Blood tissue	Slovalgin 10 mg a 12h	20
88	M	73	89	1.72	Male urogenital	MST cont. 60 mg a 8h	180
89	M	73	89	1.72	Male urogenital	MST cont. 60 mg a 8h	180
90	F	69	59	1.68	Breast	Slovalgin 10 mg a 6h	40
91	M	28	71	1.79	Blood tissue	Slovalgin 10 mg a 6h	40
92	M	49	94	1.86	Bone	MST cont. 120 mg a 8h	360
93	M	49	93	1.86	Bone	MST cont. 120 mg a 8h	360
94	M	58	63	1.82	Gastrointestinal	Slovalgin 30 mg a 12h	60
95	M	58	63	1.82	Gastrointestinal	Slovalgin 30 mg a 12h	60
96	F	49	67	1.64	Breast	MST cont. 60 mg a 8h	180
97	M	58	62	1.82	Gastrointestinal	MST cont. 60 mg a 8h	180
98	F	80	58	1.64	Brain	MST cont. 60 mg a 8h	180
99	M	60	75	1.70	Lung	Slovalgin 30 mg a 12h	60
100	M	76	82	1.75	Gastrointestinal	Slovalgin 10 mg a 12h	20
101	F	56	66	1.63	Female urogenital	MST cont. 30 mg a 12h	60
102	F	58	62	1.59	Female urogenital	MST cont. 30 mg a 12h	60
103	F	58	62	1.59	Female urogenital	MST cont. 30 mg a 12h	60

Table II Concentration of morphine, morphine-3-glucuronide and morphine-6-glucuronide in serum of oncology patients and their ratios[a)]

Patient No	c(M) ng ml^{-1}	c(M3G) ng ml^{-1}	c(M6G) ng ml^{-1}	c(M6G)/c(M)	c(M3G)/c(M)	c(M3G)/c(M6G)
1	18.8	383	90.8	4.82	20.3	4.21
2	37.4	661	99.7	2.67	17.7	6.63
3	15.6	1312	301.5	19.4	84.4	4.35
4	17.7	835	126.5	7.15	47.3	6.61
5	59.6	1825	594.8	9.98	30.6	3.07
6	44.2	2102	268.6	6.08	47.5	7.82
7	32.8	799	205.7	6.27	24.4	3.88
8	19.5	1015	312.8	16.0	52.1	3.25
9	35.8	4850	734.1	20.5	135.5	6.61
10	34.8	1044	149.5	4.30	10.0	6.98
11	32.9	3000	441.1	13.4	91.1	6.80
12	18.9	7711	405.8	21.5	408.0	19.00
13	43.9	3629	500.6	11.4	82.6	7.25
14	57.2	6214	781.4	13.7	108.6	7.95
15	47.1	2105	605.4	12.9	44.7	3.48
16	58.4	6389	1168	20.0	109.4	5.47
17	64.6	4303	1049	16.3	66.6	4.10
18	14.7	940	290.0	19.7	63.8	3.24
19	18.5	1323	270.4	14.6	71.5	4.89
20	38.4	1254	216.2	5.63	32.7	5.80
21	49.5	3841	346.5	7.00	77.6	11.09
22	22.4	2219	312.8	14.0	99.0	7.09
23	19.5	2481	351.4	18.0	127.3	7.06
24	41.4	1851	216.2	5.22	44.7	8.56
25	37.4	2349	330.8	8.85	62.8	7.10
26	36.0	2179	345.8	9.61	60.5	6.30
27	43.8	1894	258.8	5.91	43.2	7.32
28	23.9	5505	984.5	41.2	230.3	5.59
29	15.9	3141	458.1	28.8	197.6	6.86
30	70.4	18179	2751	39.1	258.2	6.61
31	-	841	116.8	-	-	7.20
32	18.8	3227	150.8	8.01	171.4	21.40
33	37.4	661	99.7	2.67	17.7	6.63
34	17.7	946	126.5	7.15	53.5	7.48
35	82.1	7284	484.5	5.90	88.7	15.03
36	35.8	4850	734.1	20.5	135.5	6.61
37	21.8	2108	262.3	12.0	96.7	8.04
38	15.4	1459	175.1	11.4	94.8	8.33
39	32.4	2242	251.8	7.77	69.2	8.90
40	19.5	3128	312.8	16.0	160.4	10.00
41	38.1	1442	149.5	3.92	37.8	9.64
42	34.8	1452	162.8	4.67	41.7	8.92
43	65.1	2550	234.9	3.61	39.2	10.85
44	21.7	1256	202.6	9.32	57.8	6.20
45	21.6	1200	160.0	7.40	55.5	7.50
46	18.6	1407	178.1	9.57	75.6	7.90
47	15.7	938	146.5	9.30	59.6	6.40
48	33.5	1763	347.6	10.4	52.7	5.07
49	40.9	940	131.3	3.21	23.0	7.16
50	25.1	834	101.7	4.05	33.2	8.20
51	35.7	2082	289.1	8.10	58.4	7.20

Table II – Continued

Patient No	c(M) ng ml^{-1}	c(M3G) ng ml^{-1}	c(M6G) ng ml^{-1}	c(M6G)/c(M)	c(M3G)/c(M)	c(M3G)/c(M6G)
53	37.1	5097	698.2	18.8	137.2	7.30
54	28.4	5054	636.3	22.4	178.0	7.94
55	32.9	1677	178.3	5.42	51.0	9.41
56	29.4	1549	286.9	9.76	52.7	5.40
57	18.9	2175	328.9	17.4	115.1	6.61
58	21.5	1003	145.5	6.77	46.7	6.89
59	39.5	2569	481.7	12.2	65.0	5.33
60	15.7	1597	96.8	6.17	101.8	16.49
61	18.8	481	91.0	4.83	25.6	5.29
62	37.4	750	117.8	3.15	20.1	6.37
63	17.7	684	127.1	7.19	38.7	5.38
64	23.5	1154	147.6	6.29	49.2	7.82
65	51.7	4147	575.9	11.1	80.2	7.20
66	19.6	667	119.2	6.08	34.1	5.60
67	18.7	1234	300.9	16.1	66.0	4.10
68	25.4	1508	205.7	8.10	59.4	7.33
69	13.8	2989	131.1	9.50	216.6	22.80
70	-	577	99.4	-	-	5.80
71	41.6	1258	297.4	7.15	30.2	4.23
72	28.9	1450	232.6	8.05	50.2	6.62
73	31.3	1320	350.1	11.2	42.2	3.77
74	29.5	3196	402.0	13.6	108.4	7.95
75	19.8	1640	227.1	11.5	82.8	7.22
76	51.8	4053	517.0	9.98	78.2	7.84
77	19.3	670	94.5	4.90	34.8	7.09
78	21.4	1919	312.1	14.6	89.9	6.15
79	33.0	5334	623.1	18.9	161.8	8.56
80	37.1	7288	720.1	19.4	196.3	10.12
81	19.5	1440	217.2	11.1	73.9	6.63
82	37.4	1861	353.8	9.47	49.8	5.26
83	45.1	4739	965.1	21.4	105.1	4.91
84	58.1	1010	161.5	2.78	17.4	6.25
85	22.4	1180	151.9	6.78	52.7	7.77
86	36.7	1376	230.1	6.27	37.5	5.98
87	41.8	1659	235.3	5.63	39.7	7.05
88	-	942	105.9	-	-	8.90
89	26.9	2861	311.0	11.6	106.4	9.20
90	36.7	2872	398.9	10.9	78.3	7.20
91	41.6	2844	338.6	8.14	68.4	8.40
92	16.9	6177	293.7	17.4	365.5	21.03
93	18.1	6428	313.6	17.3	355.1	20.50
94	22.1	1092	178.1	8.06	49.4	6.13
95	15.4	888	150.0	9.74	57.7	5.92
96	32.1	1257	169.8	5.29	39.1	7.40
97	29.7	1569	230.8	7.77	52.8	6.80
98	17.9	2006	103.4	5.77	112.1	19.40
99	14.8	2028	102.4	6.91	136.9	19.80
100	-	788	114.2		-	6.90
101	23.9	1417	272.5	11.4	59.3	5.20
102	19.8	1063	174.2	8.80	53.7	6.10
103	18.4	992	171.1	9.30	53.9	5.80

[a]c – concentration, M– morphine, M3G – morphine-3-glucuronide, M6G – morphine-6-glucuronide. The number of digits is relevant to, or by one digit larger than, the corresponding precision

Table III Biochemical data on the monitored oncology patients - results of the performed biochemical tests in serum[a)]

Patient No.	*Creat* μmol l^{-1}	*ClCreal* ml s^{-1}	*Urea* mmol l^{-1}	*TP* g l^{-1}	*Alb* g l^{-1}	*TB* μmol l^{-1}	*GMT* μkat l^{-1}	*ALT* μkat l^{-1}	*AST* μkat l^{-1}	*ALP* μkat l^{-1}	*LDH* μkat l^{-1}
1	60	1.87	3.20	54.0	31.4	11.2	0.28	0.11	0.89	0.79	4.04
2	61	1.02	2.80	69.4	39.4	18.4	0.48	0.25	0.34	0.50	5.42
3	71	1.57	3.10	72.1	41.5	19.1	0.45	0.30	0.29	1.02	4.50
4	109	1.25	7.20	78.8	42.5	8.30	1.13	0.52	0.53	0.98	5.42
5	77	1.53	5.40	70.1	40.2	17.8	0.92	0.42	0.48	1.00	6.71
6	96	1.56	4.30	73.4	39.9	14.2	0.87	0.19	0.27	1.65	5.80
7	89	1.17	3.70	68.8	38.5	11.4	1.04	0.23	0.20	0.90	3.99
8	66	1.95	5.20	68.2	39.4	5.40	1.01	0.27	0.34	0.72	6.75
9	81	1.57	3.90	74.1	38.9	10.8	0.79	0.22	0.56	0.90	4.62
10	92	0.81	6.00	70.0	40.0	13.4	0.56	0.46	0.28	1.02	5.04
11	82	2.28	4.10	74.7	41.4	6.40	1.15	0.35	0.28	0.72	4.39
12	105	1.24	11.7	79.4	39.1	5.80	0.79	0.58	0.31	0.82	5.10
13	78	1.54	4.10	69.4	38.7	6.40	0.53	0.41	0.39	0.85	6.10
14	89	1.97	2.40	92.7	45.7	8.40	0.97	0.35	0.42	1.32	5.43
15	94	1.25	9.20	79.4	42.1	19.1	0.50	0.30	0.26	1.30	7.84
16	108	1.26	6.50	69.4	38.1	18.1	1.02	0.29	0.27	0.94	5.81
17	86	1.28	7.40	73.8	39.1	15.9	0.67	0.27	0.31	1.07	4.57
18	79	1.31	2.60	59.8	32.1	17.9	0.99	0.38	0.26	0.91	6.42
19	86	1.01	11.4	61.4	31.8	15.4	1.12	0.49	0.69	1.72	7.92
20	103	1.66	5.40	72.1	39.8	9.40	0.41	0.59	0.42	0.72	4.18
21	105	1.08	4.90	69.4	34.5	5.60	1.07	0.14	0.58	0.80	6.34
22	104	1.79	3.80	68.3	39.1	17.6	0.56	0.30	0.64	1.06	4.66
23	87	2.66	3.80	77.9	41.5	4.10	0.49	0.54	0.34	1.33	4.94
24	92	1.53	4.10	66.1	39.4	2.10	0.92	0.20	0.28	0.67	4.05
25	61	1.09	6.60	62.9	34.6	11.7	0.84	0.55	0.34	2.82	4.07
26	104	1.28	9.40	66.4	35.1	9.30	0.28	0.13	0.28	2.61	3.56
27	80	1.24	2.80	71.4	36.1	8.10	0.51	0.22	0.28	0.90	4.59
28	81	1.60	5.20	69.1	34.1	9.20	0.31	0.19	0.29	0.63	3.84
29	82	1.39	4.80	81.3	41.4	7.70	0.43	0.92	1.02	1.03	6.32
30	93	0.99	11.2	60.5	25.8	6.50	0.74	0.22	0.27	3.96	3.93
31	88	1.25	6.70	81.1	42.7	8.50	0.31	0.44	0.38	1.23	6.07
32	108	2.43	7.90	69.1	34.5	17.4	0.59	0.62	0.38	0.58	4.12
33	92	1.20	7.50	80.0	40.4	15.4	0.49	0.58	0.34	0.72	7.80
34	96	1.56	5.80	96.0	43.8	19.4	0.65	0.92	0.48	1.02	7.12
35	86	2.01	8.60	49.4	29.4	17.8	0.81	0.22	0.28	1.04	4.92
36	73	2.05	4.90	74.6	26.7	15.1	0.72	0.24	0.48	1.02	6.15
37	55	1.97	4.00	58.8	36.4	23.4	0.31	0.23	0.28	1.30	5.39
38	81	1.39	2.50	69.4	35.8	4.80	0.78	0.49	0.42	1.21	4.79
39	86	1.53	7.60	52.7	30.7	11.8	1.01	0.22	0.39	0.54	6.67
40	87	1.35	8.40	49.9	31.6	8.20	0.91	0.17	0.30	0.89	7.42
41	86	2.13	3.20	75.3	44.3	12.6	0.74	1.16	0.89	1.27	4.94
42	91	1.83	3.10	77.9	39.5	11.6	0.32	0.98	0.55	1.05	5.88
43	85	2.25	4.20	70.2	34.4	8.30	0.36	0.21	0.34	0.74	5.44
44	606	0.14	33.9	69.5	32.5	22.5	5.33	0.27	1.39	3.81	40.6
45	435	0.25	20.3	54.8	29.6	15.0	1.20	0.66	0.65	1.02	6.49
46	279	0.71	19.1	66.9	33.7	3.40	1.15	0.24	0.20	1.63	7.18
47	222	0.89	14.0	58.8	34.5	3.10	0.78	0.36	0.81	1.01	4.47
48	202	0.69	21.5	60.3	32.7	9.00	0.65	0.21	0.22	2.06	7.19
49	280	0.70	21.4	65.4	32.1	5.80	1.08	0.34	0.22	1.59	7.19
50	73	1.49	3.10	69.1	34.5	8.30	0.62	0.21	0.29	0.97	5.14
51	73	1.51	7.20	65.4	33.2	18.9	0.67	0.48	0.24	1.02	6.15
52	75	1.47	6.90	66.4	32.9	17.1	0.72	0.52	0.29	0.98	6.84

Table III – Continued

Patient No.	*Creat* μmol l^{-1}	*ClCreal* ml s^{-1}	*Urea* mmol l^{-1}	*TP* g l^{-1}	*Alb* g l^{-1}	*TB* μmol l^{-1}	*GMT* μkat l^{-1}	*ALT* μkat l^{-1}	*AST* μkat l^{-1}	*ALP* μkat l^{-1}	LDH μkat l^{-1}
53	256	0.54	24.3	64.8	33.5	20.0	2.26	0.21	0.13	1.73	8.15
54	372	0.29	32.1	72.4	38.2	14.4	2.06	0.16	0.23	1.84	6.13
55	64	2.55	6.40	56.0	36.2	15.7	0.30	0.42	0.21	0.95	4.68
56	61	1.31	4.64	50.0	31.2	7.00	0.47	0.13	0.38	0.92	6.77
57	217	0.48	9.64	53.8	28.0	29.5	9.90	2.12	1.21	3.17	10.1
58	58	1.88	5.58	50.9	27.1	29.7	5.33	0.48	0.47	4.80	23.6
59	80	1.95	6.00	69.8	33.1	7.60	2.98	2.64	0.49	2.38	15.7
60	78	1.33	7.60	71.4	34.5	10.2	0.33	0.19	0.23	0.85	6.90
61	125	0.77	8.60	79.3	38.9	9.50	0.64	0.17	0.22	1.83	5.15
62	102	0.95	7.60	66.0	33.4	8.60	0.72	0.15	0.25	1.49	4.95
63	180	0.77	19.4	62.4	31.4	10.5	0.69	0.28	0.23	1.98	6.15
64	84	1.37	4.50	66.1	34.5	15.8	0.75	0.24	0.38	1.02	7.84
65	444	0.44	30.0	78.4	36.0	9.30	1.72	0.18	0.48	3.16	4.78
66	638	0.31	41.8	69.1	38.1	5.60	1.15	0.91	0.58	1.44	3.35
67	198	0.53	8.15	54.1	29.5	25.1	6.15	2.10	1.18	2.90	11.2
68	146	0.51	12.7	55.2	29.4	10.8	0.25	0.08	0.16	0.81	12.7
69	85	1.83	5.90	68.1	32.9	8.50	1.18	2.15	0.52	2.14	13.2
70	95	1.54	5.00	51.0	21.5	9.50	0.75	0.05	0.16	1.46	5.48
71	240	0.58	21.4	62.1	30.5	20.0	2.00	0.22	0.18	1.65	7.80
72	102	0.84	13.6	66.7	33.2	34.1	3.40	1.25	4.31	4.24	32.1
73	84	1.31	7.00	62.0	32.3	17.7	0.92	0.25	0.58	4.61	7.57
74	1283	0.10	24.3	58.3	18.8	18.4	0.27	0.97	1.52	0.85	35.6
75	1403	0.09	23.7	57.4	21.4	21.5	0.26	0.81	1.39	0.92	33.5
76	1326	0.09	22.8	59.1	22.1	20.9	0.28	0.80	1.43	0.92	31.4
77	72	1.28	4.10	55.4	22.4	15.4	0.84	0.28	0.89	1.62	4.91
78	94	1.04	4.50	61.4	32.1	15.1	0.84	0.17	0.38	1.64	66.7
79	218	0.30	10.4	56.5	30.1	13.2	2.65	0.26	0.27	2.43	8.15
80	72	1.34	3.90	59.6	31.8	18.7	0.71	0.15	0.42	2.01	41.9
81	288	0.23	15.9	53.8	31.8	4.80	1.07	0.29	0.34	3.27	6.72
82	64	1.72	8.60	56.7	34.7	4.60	1.66	0.52	0.36	1.17	10.5
83	60	1.53	3.90	52.8	21.4	16.8	0.73	0.21	0.84	1.59	5.78
84	73	1.26	4.80	55.8	22.1	20.7	0.69	0.26	0.82	1.92	5.98
85	197	0.46	16.4	67.1	31.4	6.50	1.58	4.73	6.80	1.72	18.6
86	145	0.63	14.3	64.0	29.4	8.80	1.69	9.82	7.26	2.92	11.6
87	94	1.29	10.8	55.5	35.3	8.60	2.58	0.54	0.16	1.46	11.5
88	210	0.64	11.0	52.3	26.0	11.1	2.21	0.27	0.55	3.27	19.4
89	207	0.65	10.3	51.9	27.4	10.4	2.09	0.37	0.54	3.11	17.1
90	133	0.60	19.8	58.7	28.6	9.50	1.18	0.15	0.34	1.49	7.43
91	121	1.48	14.0	60.7	27.1	9.40	3.09	1.44	0.38	1.29	4.13
92	83	2.32	6.80	69.6	36.0	2.00	1.70	1.47	0.74	2.02	8.11
93	81	2.35	7.90	64.2	35.3	7.40	1.52	0.75	0.28	1.60	9.14
94	593	0.20	60.8	54.6	22.0	188	6.57	0.79	1.06	9.32	17.4
95	536	0.22	52.5	52.1	19.2	213	10.6	1.49	3.02	11.2	16.3
96	170	0.69	3.30	73.3	41.4	5.30	0.68	0.22	0.28	0.95	6.97
97	532	0.22	56.9	58.1	24.8	324	10.0	4.41	6.54	18.9	12.1
98	57	1.67	19.4	37.3	17.9	19.1	0.58	0.43	0.20	1.45	6.81
99	133	1.01	7.25	68.4	39.0	13.2	0.74	0.76	0.27	1.78	8.41
100	52	2.27	2.10	63.8	32.1	16.9	0.28	0.12	0.74	2.22	7.62
101	125	0.85	17.8	60.2	27.9	4.80	2.97	0.24	0.48	3.17	32.1
102	179	0.54	12.4	68.1	33.8	8.80	0.54	0.11	0.43	1.41	22.1
103	126	0.77	9.60	69.4	34.7	9.40	0.34	0.18	0.54	1.02	19.8

*) Creat - creatinine, ClCreat - clearance of creatinine, TP - total protein, Alb – albumin, TB – total bilirubin, GMT - glutamyl transferase, ALT - alanine aminotransferase, AST - aspartate aminotransferase, ALP - alkalic phosphatase, LDH - lactate dihydrogenase. The number of digits is relevant to, or by one digit larger than, the corresponding precision

evaluated separately for men and women, the median values in *c*(M3G) and mainly in the *c*(M6G)/*c*(M) ratio are particularly informative. Pronounced

differences between men and women are documented by the ratios men/women 1.334 for c(M3G) and 0.556 for c(M6G)/c(M). It means that the median levels in men are considerably higher in M3G but significantly lower in M6G/M.

(4) Important concentration ratio M3G/M6G, expressed as the ratio of the male median values divided by the female median values, is 1.184 indicating a higher M3G/M6G ratio in men. However, compared to the corresponding median values (7.82 and 6.61 for men and women, respectively), the men and women MAD values are relatively large (2.12 and 2.33, respectively), therefore, this effect is not very distinct. The results presented in this and previous paragraph are valid for the smaller data set, however, the same evaluation for 103-patient set confirms the same trend even though the differences between men and women are moderately smaller in the cases of c(M6G)/c(M) and c(M3G)/c(M6G).

Concerning the relationships among the variables, the correlation matrix of pair correlations consisting of 99 patient records and 22 variables (*Age*, *Weight*, *Height*, *BMI*, *Dose*, *Creat*, *ClCreat*, *Urea*, *TP*, *Alb*, *TB*, *GMT*, *ALT*, *AST*, *ALP*, *LDH*, *M*, *M3*, *M6*, *M3M_M*, *M6M_M*, and *M3M_M6M*) was build in the first step. As regards the correlations of the ratio M3G/M6G, the highest correlations were found with *M3M_M* ($p < 0.0001$), *Dose* ($p = 0.0002$), *ClCreat* ($p = 0.0016$), *Height* ($p = 0.0019$), and *M3* ($p = 0.0043$). Neither of the other pairs produced a correlation with $p < 0.10$.

Techniques of multidimensional data analysis can uncover hidden relations among data [3,4]. Some basic information can be extracted by the principal component analysis, PCA. Its goal is to make a linear combination of original variables in the way keeping maximum of the variability contained in the data set in a minimum number of principal components. The main advantage of PCA is that a lower number of principal components can satisfactorily substitute a higher number of original variables. Moreover, the newly computed variables are hierarchically ordered, i.e. the first principal component (PC1) is most informative, then follows the PC2, PC3, etc. [3–5].

Figure 1 shows the PC2 vs. PC1 plot composed of four basic personal variables plus *Dose* and the dose/weight ratio (*DW*) (these two variables, which are special personal characteristics, will be sometimes called the "dose variables"), as well as the "morphine" variables *M*, *M3G* and *M6G*, representing respective serum concentrations. It is obvious that the PC1 axis is a combination of the two dose and three morphine variables and the PC2 is mostly composed of *Weight*, *BMI*, *Height* and *Age*, with a small contribution of *M*. The PC1 axis is therefore the "dose" or "morphine" axis, whilst the PC2 represents the basic personal characteristics of the patients. Even though not the same, the PCA biplots for the smaller and larger data sets provide the same conclusions.

Figure 2, depicting the PC2 vs. PC1 plot, is more informative. Instead of the

originally determined morphine variables the ratios M3G/M, M6G/M, M3G/M6G and M6G/M3G are used here. The rays in the biplot represent the respective variables. The smaller the angle between two rays in the biplot, the stronger is the association between them. If the angle is 180° a strong but inversely proportional relationship is indicated, on the contrary, a 90° angle means that the variables are independent. However, these statements are strictly valid only when the contribution of further principal components (PC3, PC4, ...) is insignificant. The opposite positions of the *M3G_M6G* and *M6G_M3G* variables is obvious. In fact, the use of *M6G_M3G* is redundant and substatiated here only for explaining geometry of the biplot representation. More informative is the superposition of the variables *DW* vs. *Age* since their almost opposite location means that the *DW* ratio decreases with the growing age. Relative closeness of *Dose*, *M3G_M* and *Height* to *M3G_M6G* indicates a close relation of the mentioned variables to the M3G/M6G ratio, which is linked to the patient's discomfort with the morphine treatment. The close relation of *Height* to *M3G_M6G* is surprising and has not been described so far, however, it has been confirmed by several independent methods. Due to incompleteness of the data for all monitored oncological patients, only the results for 99 of them were used here.

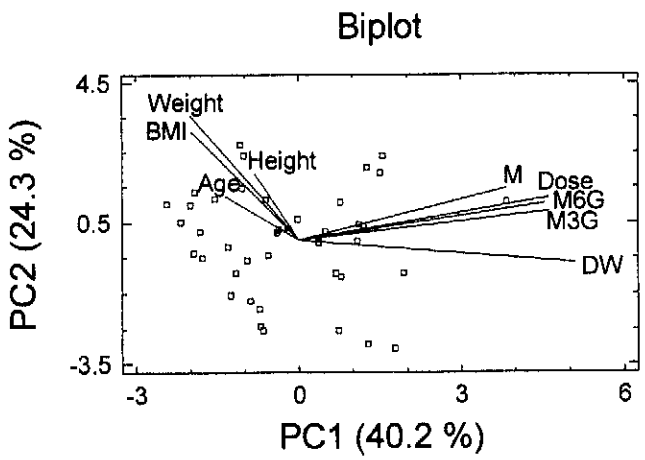

Fig. 1 Principal component analysis biplot PC2 vs. PC1 representing relations between morphine variables and personal characteristics (including morphine daily dose) of 42 oncological patients (the smaller data set, without the data of patient No. 31)

Cluster Analysis, based on clustering variables or objects according to their mutual distances, is one of aforementioned independent methods. The smaller the distance, the more similar are studied variables or objects [3,4]. Figure 3 shows clustering of 12 variables using Ward's method and squared Euclidean distances. It is seen that *M3G_M6G* is closest to *Height*, then *Dose* with *DW* following in the

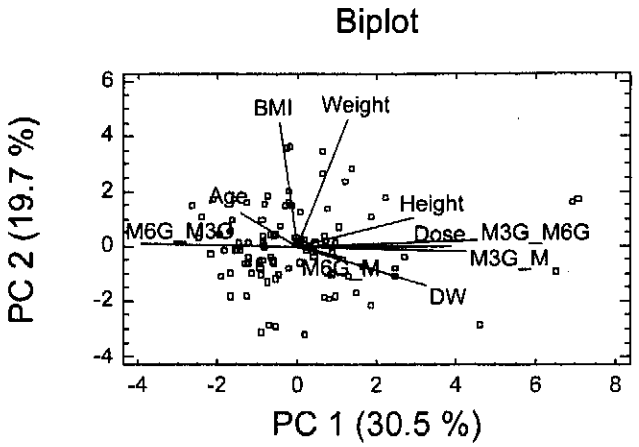

Fig 2 Principal component analysis biplot PC2 vs. PC1 representing relations between the ratio morphine variables and personal characteristics (including morphine daily dose) of 99 oncological patients (the larger data set)

neighbouring cluster. The closest position of *Height* to *M3G_M6G* was found also when this way of clustering was made for the smaller set of oncological patient samples. When 11 monitored biochemical parameters were added to the previous experiment then the clustering of *M3G_M6G* with *ClCreat* and then with *Height* proceeded. The next joint cluster with the aforementioned was that with *Dose* and *DW*. Thus, an important association between *M3G_M6G* and clearance of creatinine has been discovered.

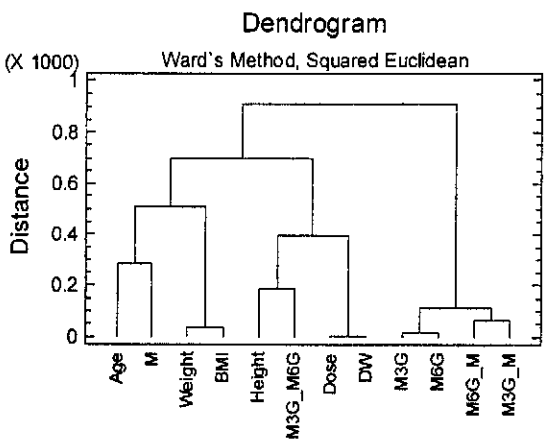

Fig. 3 Dendrogram of Ward's method of hierarchical cluster analysis with squared Euclidean distances showing the distances among 12 selected variables, which represent personal characteristics of 99 oncological patients (the larger data set), morphine daily dose, the morphine and corresponding glucuronides serum concentrations and their ratios

Canonical Correlation Analysis (CCA), performed for 103 patient data in such way that one group of variables included *Height*, *Weight*, *Dose* and *Age* (this time without *BMI* and *DW*), and *M3G_M6G* alone represented another group, also confirmed the importance of *Height* among "personal" variables. The larger the coefficient in the canonical correlation equation (considering its absolute value), the stronger the correlation of the respective variable with the ratio M3G/M6G. The absolute values of coefficients (+1.152, –20.670, –0.141 and –0.129 assuming the order of the variables given above) testify that *Height* is the most important and is related to the discomfort of the patients. When 11 biochemical variables were added to 4 "personal" variables and a similar canonical correlation equation was computed, seven variables mostly correlated to *M3G_M6G* are *ClCreat* (coefficient +0.775), *Urea* (+0.609), *Dose* (+0.530), *Height* (0.470), *ALT* (+0.370), *Weight* (–0.367) and *ALP* (–0.346). The found probability value $p = 0.0037$ indicated highly significant CCA.

Logistic regression (LR) [4,6–8] uses a categorical dependent variable, which in our case is *M3G_M6G* (the M3G/M6G ratio) but used here as a categorical, binary variable. For the given patient, it acquires the value of one if it is higher than the median value of the given data set, and zero if it lower or equal to the median. The independent variables in LR can be continuous as well as categorical, so that LR is ideally suited to explore a possible effect of tumor location, represented by the corresponding categorical variables, in addition to the influence of other variables already described. The following independent variables were used: five or eight categorical variables of the tumour locations for the smaller and larger data set, respectively, the "personal" variables *Age*, *Weight*, *Height* and *Dose*, the morphine serum concentration (variable *M*), and two binary independent variables: the patient's gender (variable *Sex*) and morphine treatment by MST Continus or Slovalgin Retard (variable *Drug*). Categorical tumor location variables are: *BloodTissue*, *Bone*, *FemaleUrogenital*, *Gastrointestinal*, and *Lung* in the smaller data set, plus *Brain*, *Breast*, and *MaleUrogenital* in the larger data set. The tumor location variable was included into the list of variables when at least four patients belonged to the respective category. Then the following coding was used: 1 for the patient samples pertaining to the given tumour location, 0 — pertaining to another tumor location.

The degree of importance of the selected variables for the M3G/M6G ratio follows from comparing the coefficients in the logistic equation given in the LR results output. When examining the smaller data set, the most important regression coefficients with the absolute value larger than 1 were found for *Bone*, *BloodTissue*, *Sex*, *Lung*, *Gastrointestinal* and *FemaleUrogenital* (+10.49, –7.46, –5.84, –5.32, –4.91, and –3.30 in the mentioned order). With regard to the M3G/M6G ratio the most important variable is *Bone*; all five tumour location variables are among the best six.

In the larger data set altogether 26 variables were examined: 4 "personal" variables, 11 biochemical variables, *M* (morphine serum concentration) and 10

binary variables, among them 8 tumour location (TL) variables plus *Sex* and *Drug*. The TL variables exhibited again large logistic regression coefficients. According to the absolute value of logistic regression coefficents the importance of the variables for the M3G/M6G ratio decreased in order *Height*, *Bone*, *Lung*, *Breast*, *FemaleUrogenital*, *Sex*, *Drug*, *Brain*, *BloodTissue*, etc. A special importance of *Height* and patient's gender (*Sex*) was already noted when using other chemometric techniques. Classification success in logistic regression for discriminating 2 classes of patients — with high and low M3G/M6G ratio — was 70.2 %, which indicates a significant discrimination. It also means that using the elaborated logistic model it is possible to predict patient's satisfaction /dissatisfaction with analgesic treatment by morphine drugs.

Conclusion

Two sets of data describing analytical and biochemical test results as well as several personal characteristics of 43 and 103 oncology patients undergoing analgesic treatment with morphine drugs were studied using the multivariate data analysis techniques. Common basic as well as robust statistics were also implemented. Chemometric elucidations of the relations among morphine and its metabolites concentrations in serum, their dependency on the patient treatment (daily dose, kind of morphine drug), personal characteristics and gender, tumor location diagnosis, and customarily monitored biochemical parameters were performed in order to understand and predict pain resistance of the patients.

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