Supplementary Information

Exploratory infrared spectroscopic study of erythrocytes in cancer chemotherapy

Mihai Gutu^{*a*}, Mihaela Avadanei^b*, Mihai Marinca,^c Cipriana Stefanescu,^{*b*}

^a Biophysics and Medical Physics Department, Medicine Faculty, "Gr. T. Popa" University of Medicine and Pharmacy, 6, Universitatii Str., Iasi Romania,

^b"Petru Poni" Institute of Macromolecular Chemistry, 41A Aleea Grigore Ghica Voda, Iasi, Romania.

^CRegional Oncology Institute, 2-4 Gen. Henri Mathias Berthelot St., 700483 Iasi, Romania,

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Fig. S2. The C-H stretching (a) and fingerprint (b) regions of averaged control and cancerous erythrocytes, as a function of number of chemotherapy cycles (CTH), and their corresponding second derivative spectra (a') and (b'). The shaded areas in (b') highlight the spectral domains were differences between the average spectra were observed: change in proportion of some secondary structures in proteic part, and changes at the CH₂ groups level, observed in the CH₂ scissoring bands, respectively.

Fig. S3. Difference spectra between the averaged data of digestive cancer cases receiving chemotherapeutics and untreated subjects, in the regions assigned to OH/NH/CH stretching (left) and fingerprint vibrations (right). The shaded areas highlight the significant differences at the CH_2/CH_3 and amide group level, respectively. The spectra noted "0 CTH" are referring to the differences between patients receiving no chemotherapy and those from the control group.

Fig. S4. (a) Normalized FTIR spectra of erythrocytes in the CH bending/scissoring vibration region for the control samples as compared to those from the cancer cases. (b) Plot of PC-1 versus PC-2 for the normal against the pathological cases: black trace – normal samples, red trace – abnormal samples. The ellipses delimitate the samples clustering. (c) Loading plots of PC-1 (A) and PC-2 (B)

Fig. S5. (a) The normalized FTIR spectra of erythrocytes in the region of phosphate group stretching vibrations for the control samples as compared to those from the cancerous cases. (b) Plot of PC-1 versus PC-2 for the normal against the pathological cases: black trace – normal samples, red trace – abnormal samples. The ellipses delimitate the samples clustering. (c) Loadings plots of the first two principal components: PC-1 (A) and PC-2 (B)

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pathological cases: black trace – normal samples, red trace – abnormal samples. The ellipses delimitate the samples clustering. (c) Loadings plots of the first two principal components: PC-1 (A) and PC-2 (B) **Physiological parameters and correlation with the spectral parameters**

| | | v(=CH)/v(CH ₃) | v(=CH)/ v(CH _{tot}) | $\widetilde{v}_{(v_{sym}(CH_2))}$ | v as (CH2)/v as(CH3) | vas(CH2)/v(=CH) | δ _{sciss} (CH ₂)/ δ _{as} (CH ₃) | Amide I/II |
|-----------------------|---|----------------------------|---|-----------------------------------|---|---|---|------------|
| Age | r | -0.108 | -0.134 | -0.061 | 0.068 | 0.015 | 0.362 | -0.039 |
| | p | 0.714 | 0.649 | 0.837 | 0.819 | 0.960 | 0.203 | 0.894 |
| Cth | r | 0.522 | 0.255 | 0.022 | 0.570* | -0.313 | -0.137 | -0.539* |
| | p | 0.083 | 0.379 | 0.942 | 0.045* | 0.275 | 0.642 | 0.047* |
| RBC | r | 0.423 | 0.306 | 0.134 | -0.270 | 0.364 | 0.559* | -0.183 |
| НСТ | p | 0.132 | 0.288 | 0.648 | 0.350 | 0.200 | 0.038* | 0.532 |
| НСТ | r | 0.253 | 0.109 | -0.141 | -0.350 | $v_{as}(CH_2)/v(=CH)$ $\delta_{sciss}(CH_2)/sinctify (CH_3)$ 0.0150.3620.9600.203-0.313-0.1370.2750.6420.3640.559*0.2000.038*0.0900.5220.7610.0560.0200.4700.9470.0900.440-0.2150.1150.461-0.0350.0260.9060.9310.4390.1280.1160.663-0.1460.2170.6170.4560.181-0.0670.5350.820-0.038-0.2550.8970.378-0.455-0.0100.1020.974 | -0.045 | |
| | p | 0.383 | 0.711 | 0.630 | 0.819 0.960 0.200 0.570^* -0.313 -0.13 0.045^* 0.275 0.642 -0.270 0.364 0.559 0.350 0.200 0.038 -0.350 0.200 0.038 -0.350 0.090 0.522 0.220 0.761 0.050 -0.264 0.020 0.470 0.362 0.947 0.090 -0.175 0.440 -0.21 0.550 0.115 0.46 0.031 -0.035 0.020 0.916 0.906 0.93 -0.318 0.439 0.122 0.268 0.116 0.666 0.456 -0.146 0.217 0.101 0.617 0.455 -0.298 0.181 -0.06 0.302 0.535 0.821 | 0.056 | 0.879 | |
| Hb | r | 0.257 | 0.120 | -0.132 | -0.264 | 0.020 | 0.470 | -0.097 |
| | p | 0.375 | 0.684 | 0.653 | 0.362 | 0.947 | 0.090 | 0.742 |
| PL | r | -0.259 | -0.022 | 0.277 | -0.175 | 0.440 | -0.215 | 0.010 |
| | p | 0.372 | 0.942 | 0.337 | 0.550 | 0.115 | 0.461 | 0.974 |
| Cholester ol total | r | -0.448 | -0.453 | -0.408 | 0.031 | -0.035 | 0.026 | 0.312 |
| | p | 0.108 | 0.103 | 0.147 | 0.916 | 0.906 | 0.931 | 0.278 |
| Triglyceri | r | -0.388 | -0.497 | -0.013 | -0.318 | 0.439 | $\delta_{ns}(CH_3)$ A 15 0.362 60 0.203 13 -0.137 75 0.642 64 0.559* 00 0.038* 90 0.522 61 0.056 20 0.470 47 0.090 40 -0.215 15 0.461 35 0.026 06 0.931 39 0.128 16 0.663 46 0.217 17 0.456 81 -0.067 35 0.820 38 -0.255 97 0.378 75 -0.056 86 0.849 55 -0.010 02 0.974 | 0.070 |
| ues | p | 0.170 | 0.942 0.337 0.550 3 -0.453 -0.408 0.031 3 0.103 0.147 0.916 3 -0.497 -0.013 -0.318 0 0.071 0.964 0.268 0 0.149 -0.110 0.456 | 0.116 | 0.663 | 0.812 | | |
| Blood | r | 0.189 | 0.149 | -0.110 | 0.456 | -0.146 | 0.217 | -0.160 |
| glucose | p | 0.518 | 0.611 | 0.709 | 0.101 | 0.617 | 0.456 | 0.586 |
| Leucocyt | r | -0.395 | -0.213 | -0.088 | -0.298 | $v_{as}(CH_2)/v(=CH)$ $o_{sciss}(C)$ 0.015 0.36 0.960 0.20 -0.313 -0.13 0.275 0.64 0.364 0.559 0.200 0.033 0.200 0.033 0.090 0.52 0.761 0.05 0.020 0.47 0.947 0.09 0.440 -0.21 0.115 0.46 -0.035 0.02 0.906 0.93 0.439 0.12 0.116 0.66 -0.146 0.21 0.617 0.45 0.181 -0.02 0.535 0.82 -0.038 -0.23 0.897 0.37 -0.475 -0.03 0.086 0.84 -0.455 -0.01 | -0.067 | 0.001 |
| 05 | p | 0.162 | 0.466 | 0.766 | 0.302 | 0.535 | 0.820 | 0.997 |
| Lymphoc | r | -0.308 | -0.275 | -0.103 | -0.369 | -0.038 | -0.255 | 0.126 |
| yus | p | 0.284 | 0.342 | 0.725 | 0.195 | 0.897 | 0.378 | 0.668 |
| VEM | r | -0.254 | -0.291 | -0.429 | -0.149 | -0.475 | -0.056 | 0.155 |
| | p | 0.382 | 0.313 | 0.126 | 0.611 | 0.086 | 0.849 | 0.596 |
| HEM | r | -0.126 | -0.181 | -0.344 | -0.069 | -0.455 | -0.010 | 0.034 |
| | p | 0.669 | 0.536 | 0.228 | 0.814 | 0.102 | 0.974 | 0.908 |

 Table S1. Hematological, biological and FTIR erythrocytes parameters correlations for digestive cancer

*Statistic significant

Cth- chemotherapy cycles number, RBC- red blood cell number, HCT- hematocrit, Hb- haemoglobin level, PL- platelets

| Levene's Test for Equality of Variances | | | | t-test for Equality of Means | | | |
|---|-----------------------------|--------|-------|------------------------------|--------|---------------------|-----------------|
| | | f | Sig. | t | df | Sig. (2- tailed) | Mean Difference |
| RBC | Equal variances assumed | 3.210 | 0.082 | -4.910 | 33.000 | 0.000 | -0.704 |
| | Equal variances not assumed | | | -4.567 | 21.251 | 0.000 | -0.704 |
| НТС | Equal variances assumed | 10.086 | 0.003 | -5.923 | 33.000 | 0.000 | -6.868 |
| | Equal variances not assumed | | | -5.246 | 17.598 | 0.000 | -6.868 |
| Hb | Equal variances assumed | 12.618 | 0.001 | -5.960 | 33.000 | 0.000 | -2.555 |
| | Equal variances not assumed | | | -5.205 | 16.706 | 0.000 | -2.555 |
| PL | Equal variances assumed | 3.853 | 0.058 | 2.261 | 33.000 | 0.030 | 57.095 |
| | Equal variances not assumed | | | 2.017 | 18.086 | 0.059 | 57.095 |
| Total Cholesterol | Equal variances assumed | 1.410 | 0.243 | -2.795 | 33.000 | 0.009 | -28.452 |
| | Equal variances not assumed | | | -2.617 | 21.830 | 0.016 | -28.452 |
| Tryglicerides | Equal variances assumed | 0.349 | 0.559 | 0.676 | 33.000 | 0.504 | 9.000 |
| | Equal variances not assumed | | | | 32.326 | 0.480 | 9.000 |
| Leukocytes | Equal variances assumed | 0.007 | 0.935 | -3.155 | 33.000 | 0.003 | -1.613 |
| | Equal variances not assumed | | | -3.226 | 30.067 | 0.003 | -1.613 |
| Limfocytes | Equal variances assumed | 0.108 | 0.745 | -3.061 | 33.000 | 0.004 | -0.691 |
| | Equal variances not assumed | | | -2.941 | 24.146 | 0.007 | -0.691 |
| VEM | Equal variances assumed | 0.601 | 0.444 | -0.712 | 33.000 | 0.481 | -1.320 |
| | Equal variances not assumed | | | -0.677 | 23.177 | 0.505 | -1.320 |
| HEM | Equal variances assumed | 1.404 | 0.245 | -1.597 | 33.000 | 0.120 | -1.152 |
| | Equal variances not assumed | | | -1.452 | 19.435 | 0.162 | -1.152 |
| V(=CH)/V(CH _{3as}) | Equal variances assumed | 7.514 | 0.010 | -6.718 | 33.000 | 0.000 | -0.001 |
| | Equal variances not assumed | | | -7.794 | 28.392 | 0.000 | -0.001 |

Table S2. Independent Samples Test for digestive cancer vs. control group (healthy men group)

| V(CH _{2as})/V(CH _{3as}) | Equal variances assumed | 13.483 | 0.001 | -3.196 | 33.000 | 0.003 | -0.080 |
|---|-------------------------|--------|--------|--------|--------|-------|--------|
| | Equal variances n | -3.655 | 30.089 | 0.001 | -0.080 | | |
| V(=CH)/V(CH _{tot}) | Equal variances assumed | 8.280 | 0.007 | -3.912 | 33.000 | 0.000 | 0.000 |
| | Equal variances n | -4.433 | 30.979 | 0.000 | 0.000 | | |
| ΔV1/2(CH2sciss) | Equal variances assumed | 13.642 | 0.001 | 3.163 | 33.000 | 0.003 | 0.907 |
| | Equal variances n | 3.765 | 24.861 | 0.001 | 0.907 | | |
| V _{as} (P-O) | Equal variances assumed | 7.540 | 0.010 | -0.923 | 33.000 | 0.363 | -0.035 |
| | Equal variances n | -1.075 | 27.870 | 0.292 | -0.035 | | |
| | Equal variances n | -2.358 | 29.429 | 0.025 | -7.364 | | |
| Amide I/II | Equal variances assumed | 3.160 | 0.085 | -0.139 | 33.000 | 0.890 | -0.006 |
| Equal variances not assumed | | | | -0.119 | 15.366 | 0.907 | -0.006 |
| * | | | | | | | |