PROCEEDINGS OF SCIENCE

Thermal behavior of blood plasma isolated from children with leukemia. A DSC study.

B. Antonova [1], K. Batchvarov [2], *Yoan Dimitrov [1], B. Tenchov [1], B. Avramova [2], M. Yordanova [2], D. Konstantinov [2]

[1] Medical Faculty, Medical University–Sofia, Zdrave 2 Street, Sofia, Bulgaria

[2] Department of Pediatric Hematology and Oncology, University Hospital "Queen Joanna", Byalo More 8 Street, Sofia, Bulgaria

E-mail: bantonova@medfac.mu-sofia.bg

Differential scanning calorimetry (DSC) is a highly sensitive technique that is frequently used to determine the denaturational heat capacity profiles of protein solutions. In the present study, we applied DSC to characterize for the first time the thermal denaturation profiles of blood plasma (BP) and cerebrospinal fluid (CSF), isolated from children diagnosed with leukemia. Leukemia is the most common type of cancer in children. Our Differential scanning calorimetry measurements revealed significant alterations in the blood plasma and cerebrospinal fluid heat capacity profiles for children with acute lymphoblastic leukemia (ALL) compared to children in continuous remission. These alterations appear to correlate with the severity of the disease (tumor load, blast count). Here we present a selection of blood plasma heat capacity profiles which illustrate our main findings. These results give preliminary indication that body fluids denaturation profile changes due to leukemia could add additional information in the diagnostic and monitoring process of disease.

Key words: differential scanning calorimetry (DSC); protein denaturation; blood plasma (BP); cerebrospinal fluid (CSF), leukemia, non-Hodgkin lymphoma

11th International Conference of the Balkan Physical Union (BPU11),
28 August - 1 September 2022
Belgrade, Serbia

*Speaker

PoS(BPU11)234

Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent type (20%-30%) of all pediatric cancers. It was ultimately lethal in the past, but is nowadays curable in more than 80% of the patients with contemporary combined chemotherapy treatment. The central nervous system (CNS) could be engaged in about 3% of ALL cases and clinically presented with leukemic cells in the cerebrospinal fluid (CSF).

Differential scanning calorimetry (DSC) has recently emerged as a promising and reliable new method in medicine. Its application is based on using the heat capacity profiles of protein denaturation, mostly of blood plasma (BP), but also of other body fluids, for diagnosing and monitoring of diseases [1, 2]. DSC was found to detect specific aberrations in the thermal behavior of blood plasma in diseases, such as rheumatoid arthritis, Lyme disease, diabetes type I, but also various types of tumors, such as brain tumors (glioblastoma multiforme and low-grade astrocytoma [3, 4]), lung cancer [5], cervical cancer [6], multiple myeloma and Waldeström's macroglobulinemia [7, 8], and colorectal cancer [9]. Its potential for disease diagnostics and monitoring based on studying blood plasma samples has been demonstrated in a number of cases. In our previous work on lung cancer [5], we applied DSC to evaluate the efficacy of the chemotherapy treatment and found that the BP thermogram recovers its "healthy" shape with the chemotherapy progress. In the present work, we used DSC for the first time to characterize thermal denaturation profiles of BP and CSF, isolated from pediatric patients with leukemia.

Materials and methods

Blood plasma (BP) and cerebrospinal fluid (CSF) samples were obtained through diagnostic procedures in patients with newly diagnosed or relapsed leukemia and Non-Hodgkin lymphoma admitted to the University Hospital "Queen Joanna", Sofia, Bulgaria. BP was isolated by centrifugation of 3 ml of blood for 15 min at 4000 RCF. The supernatant BP was removed, diluted 10 times in saline (0.9% solution of sodium chloride in water), and used in the DSC experiments. CSF was obtained by lumbar punctures and examined for the presence of cells and proteins. This study was approved by the Ethics Committee of the Medical University - Sofia. The parents of the participating children have signed an informed consent.

The DSC measurements were made using a Nano DSC instrument with 300 μl measuring cell (Thermal Analysis Instruments). Heating and cooling scans were performed at 1 °C /min scan rate in the range 20-110°C. The first heating scans displayed the thermal denaturational profile of the native samples, while subsequent heating scans displayed practically identical profiles without detectable thermal events, typical of denatured samples. As in our previous DSC studies [4, 5, 10], following the method proposed in [11], the second scans were used as baselines and subtracted from the first DSC scans in order to determine the excess heat capacity profiles during the sample denaturation.

Results and discussion

In the present work we investigated using DSC about 120 BP samples from 55 children, aged 1 to 17 years, mostly diagnosed with leukemia. A comprehensive analysis of the recorded DSC thermograms is beyond the scope of this study and will be given in a subsequent publication. The main objective in the current investigation was to identify possible deviations in the

thermogram shape from the norm in pediatric leukemia. As is known, the DSC thermograms of BP are dominated by three major protein fractions. In BP from healthy individuals, these fractions are albumin, the most abundant BP protein, with denaturation peak at about 63°C, a heterogeneous mixture of immunoglobulins melting in the range 70-75°C, and the smallest one, fibrinogen, which melts at ~52°C. The application of DSC in medical diagnostics is based on identifying deviations of the protein melting transitions from the norm, which can be associated with the various illnesses and disorders. While these deviations are quite small or non-existent in many diseases, in our current experiments we observed significant deviations of all three major blood plasma protein fractions in children with leukemia. In the Fig. 1. a selection of BP thermograms is depicted with visible shift of the BP denaturational transitions to lower and higher temperatures.

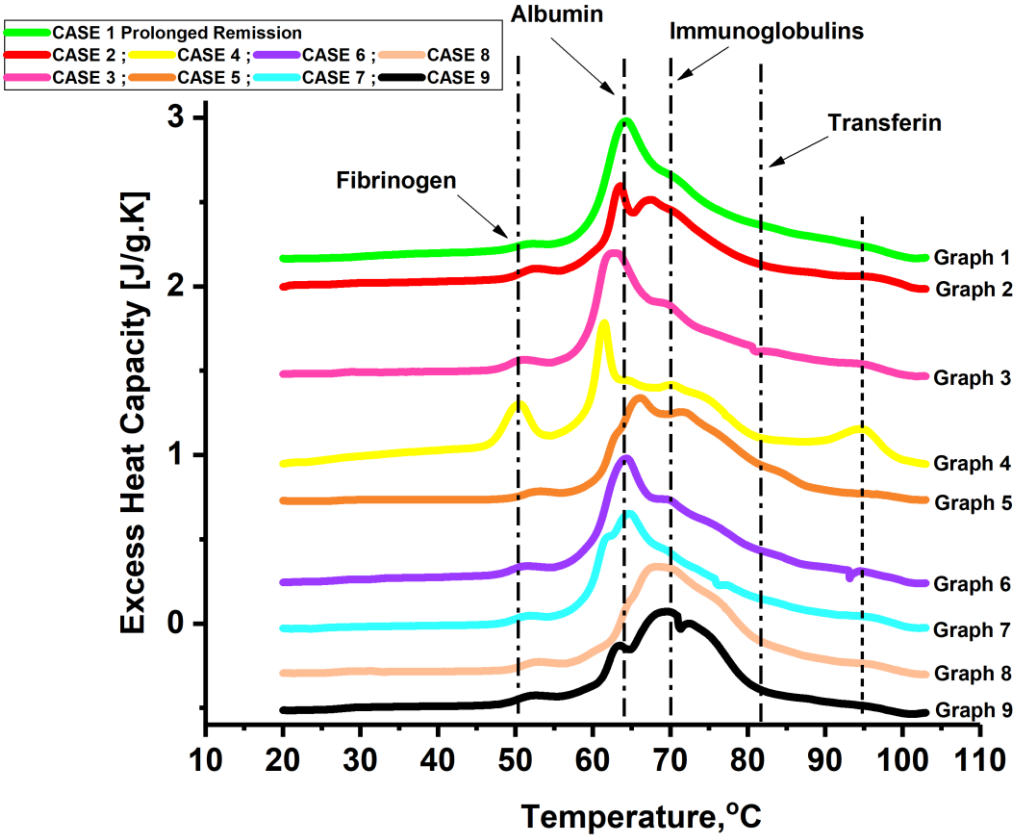

Figure 1. DSC heat capacity profiles of blood plasma isolated from children with leukemia and l non-Hodgkin lymphoma. The major transition peaks display significant shifts along the temperature axis in the various cancers. Graph 1. ALL in continuous remission (cured) with ceased antileukemic treatment few months beforehand (4-year-old boy); Graph 2. ALL in second relapse (15-year-old girl); Graph 3. Extramedullary relapse of ALL (16-year-old boy); Graph 4. Newly diagnosed ALL (3-year-old boy); Graph 5. ALL – newly diagnosed, upward transition shifts, WBC 104,7 G/L (16-year-old boy); Graph 6 ALL with combined CNS and bone marrow relapse (8-year-old boy); Graph 7. Chronic Myeloid Leukemia in a lymphoid blast crisis (67% blast in-

filtration in bone marrow, normal peripheral blood WBC, 17-year-old boy); Graph 8. Newly diagnosed Non-Hodgkin lymphoma with bone marrow infiltration (leukemic distribution to the blood marrow (5-year-old boy); Graph 9. Newly diagnosed Non-Hodgkin lymphoma with bone marrow infiltration, WBC 40.5 G/L (8-year-old boy).

As is evident from Fig. 1, the major denaturational transitions of blood plasma display significant shifts along the temperature axis in the various types of cancer illustrated in Fig. 1. Examination of the DSC patterns in Fig. 1 leads to the following conclusions of interest:

1) Cases of children with ALL, in which there is no marked progression of the neoplastic process, are typified by BP heat capacity profiles resembling those of healthy individuals (cases 1 and 6).

2) Acute lymphoblastic leukemia (ALL) brings about downward shifts of the major transition peaks in the BP heat capacity profiles (cases 2, 3 and 4).

3) Although in lymphoid transformation and without high peripheral blood tumor burden BP heat capacity profile in the CML patient (case 7) differs significantly from the heat capacity profiles typical of ALL (compare case 7 for CML with cases 2, 3 and 4 for ALL).

4) There are visible differences between the BP heat capacity profiles of ALL (cases 2, 3 and 4) and those of non-Hodgkin lymphoma (cases 8 and 9). While the BP denaturational transitions shift downwards in the ALL cases, the BP transitions in non-Hodgkin lymphoma shift upwards.

Although at present we are unable to offer interpretations of the observed effects, it is highly probable that the differences between the DSC heat capacity profiles shown in Fig. 1 mirror different events at molecular level in different lymphoid proliferation conditions (pure ALL, lymphoid blast crisis of CML, leukemisation of non-Hodgkin lymphoma) and at different state of disease control (remission, progression, transformation). These events result in different states of the major blood plasma proteins, respectively, in different shapes of the blood plasma thermograms recorded by DSC.

Another noteworthy observation is illustrated in Fig. 2. It is that the blood plasma heat capacity thermograms are sensitive not only to the type, but also to the severity of the disease.

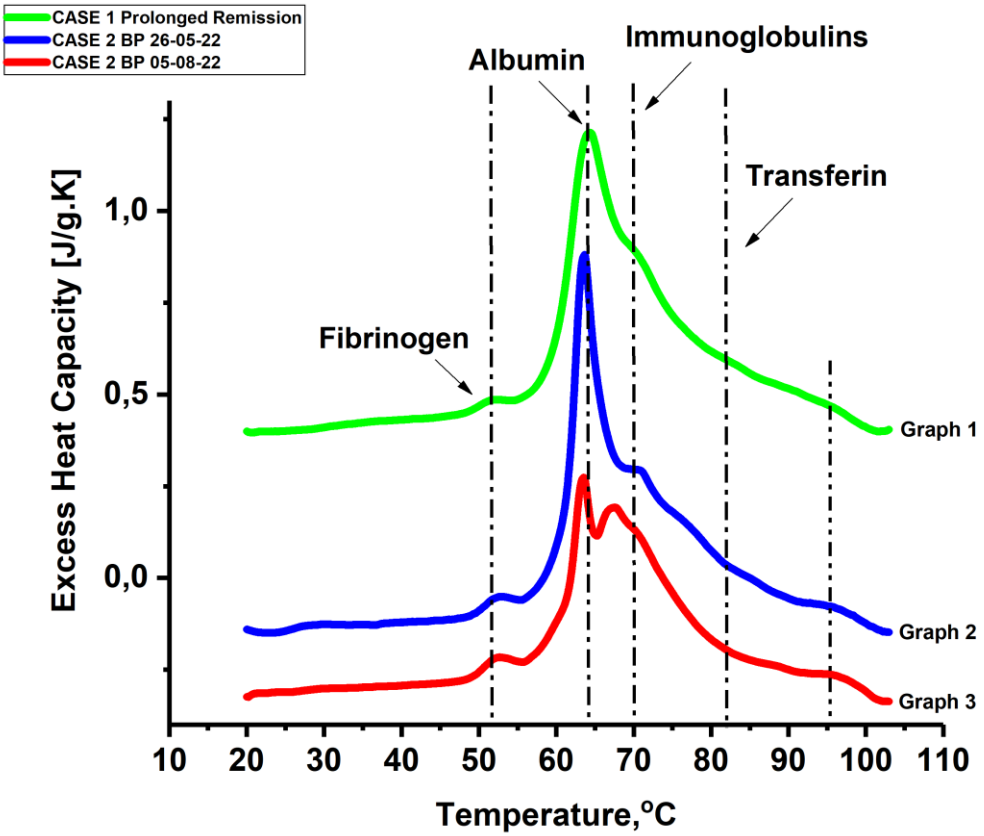

Figure 2. DSC heat capacity profiles of three ALL cases. Graph 1. ALL-continuous remission (age – 4 years); Graph 2 shows the BP thermogram during an ALL after remission (15-year-old girl); Graph 3 shows a subsequent DSC recording of blood plasma taken from the same patient two and a half months later (disease progression).

While case 2, BP 26-05-23 and case 2, BP 05-08-23 in Fig. 2 strongly differ from the remission case 1, the blood plasma thermogram in case 2, BP 05-08-23 worsens and further deviates from the remission thermogram in case 1 (paralleling the ALL progression in the two and a half month interval between BP thermogram measurements shown in graphs 2 and 3).

In the course of the present work we also applied DSC to record the denaturational heat capacity profiles of CSF isolated from children diagnosed and treated for leukemia. Similarly to the results of BP, the obtained CSF thermograms also turned out to be sensitive to the type and severity of the leukemias. However, the results about the CSF thermal behavior need further analysis and verification.

Conclusions

The presented results appear to be the first DSC investigation on blood plasma and cerebrospinal fluid from children with lymphoid neoplasms. While a detailed analysis of the thermograms will be presented in a subsequent publication, here we emphasize the main conclusion of the obtained results – the denaturational heat capacity profiles of blood plasma seem sensitive and display unusually large deviations from the norm in children with various types of

lymphoid neoplasms. These patterns appear to be a sensitive indicator of the disease progression and suggest that DSC may present an additional appropriate diagnostic and monitoring method in pediatric lymphoid neoplasms.

Acknowledgements

Support of the Medical University – Sofia, Bulgaria, Grant D-140/14-06-2022 of the Medical Sciences Council at Medical University - Sofia, is acknowledged.

References

[1] N. C. Garbett, C. S. Mekmaysy, C. W. Helm, A. B. Jenson, J. B. Chaires. Differential scanning calorimetry of blood plasma for clinical diagnosis and monitoring. Exp Mol Pathol. 2009;86(3):186-191.

[2] N. C. Garbett, C. S. Mekmaysy, L. DeLeeuw, J. B. Chaires. Clinical application of plasma thermograms. Utility, practical approaches and considerations. Methods. 2015;76:41-50.

[3] A. A. Chagovetz, C. Quinn, N. Damarse, L. D. Hansen, A. M. Chagovetz, R. L. Jensen. Differential Scanning Calorimetry of Gliomas: A New Tool in Brain Cancer Diagnostics Neurosurgery. 2013;73(2):289-295.

[4] B. Antonova, E. Naydenov, R. Koynova, K. Tumangelova-Yuzeir, B. Tenchov. Exothermic transitions in the heat capacity profiles of human cerebrospinal fluid. Eur Biophys J. 2020;49:231-238.

[5] R. Koynova, B. Antonova, B. Sezanova, B. Tenchov. Beneficial effect of sequential chemotherapy treatments of lung cancer patients revealed by calorimetric monitoring of blood plasma proteome denaturation. Thermochim Acta. 2018;659:1-7.

[6] N. C. Garbett, M. L. Merchant, C. W. Helm, A. B. Jenson, J. B. Klein, J. B. Chaires. Detection of Cervical Cancer Biomarker Patterns in Blood Plasma and Urine by Differential Scanning Calorimetry and Mass Spectrometry. PLoS One. 2014;9(1).

[7] S. Todinova, S. Krumova, A. Danailova, V. Petkova, M. Guenova, G. Mihaylov, Lidia Gartcheva, Stefka Germanova Taneva . Calorimetric markers for monitoring of multiple myeloma and Waldenstrom's macroglobulinemia patients. European Biophysics Journal with Biophysics Letters. 2018;47(5):549-559.

[8] Svetla Todinova, Sashka Krumova, Ralitsa Radoeva, Lidia Gartcheva, Stefka G Taneva. Calorimetric markers of Bence Jones and nonsecretory multiple myeloma serum proteome. Anal. Chem. 2014, 86, 24, 12355–12361 2014 Dec 16;86(24):12355-61. doi: 10.1021/ac503677d. Epub 2014 Dec 5.

[9] S. Todinova, S. Krumova, P. Kurtev, V. Dimitrov, L. Djongov, Z. Dudunkov, et al. Calorimetry-based profiling of blood plasma from colorectal cancer patients. Biochimica Et Biophysica Acta-General Subjects. 2012;1820(12):1879-1885.

[10] S. Abarova, R. Koynova, L. Tancheva, B. Tenchov. A Novel DSC Approach for Evaluating Protectant Drugs Efficacy against Dementia. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2017;1863:2934-2941.

[11] K. Takahashi, J. M. Sturtevant. Thermal-Denaturation of Streptomyces Subtilisin Inhibitor, Subtilisin Bpn', and the Inhibitor-Subtilisin Complex. Biochemistry. 1981;20(21):6185-6190.