# CALORIMETRIC TRACKING OF THE PLASMA AND CSF PROTEOME IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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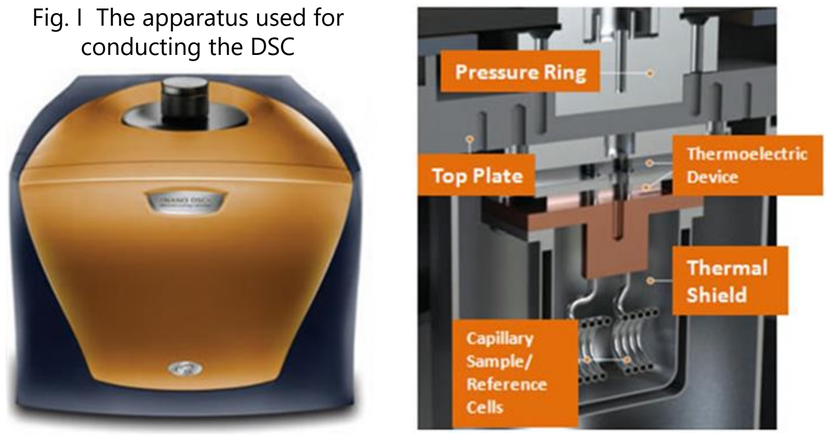
Leukemia is the most common type of cancer found in children. It accounts for around 33% of all malignant diseases in pediatrics.

In this study we have used DSC to compare alterations in the protein thermal denaturation profiles of blood plasma and CSF, taken from children with acute lymphoblastic leukemia (ALL), with the corresponding fluids from other children in continuous remission with healthy clinical and hematological statuses, used as controls. Historically, there have been few studies where this method has been applied to various diseases, in particular in children. However, we believe that it has great potential in medical diagnostics and examinations.

## *Introduction and methods*

Fig. I The apparatus used for conducting the DSC




A new, recently created method of diagnosing and monitoring diseases and receiving information about the mechanisms of their molecular operation is the differential scanning calorimetry (DSC). DSC is a highly sensitive technique that measures temperature-induced conformation changes in proteins. As such, it is useful in measuring the exact values of concentration, denaturation and interaction between proteins and other molecules and allows for observing specific insignificant changes in blood plasma (BP) and CSF (cerebrospinal fluid), related to various pathological processes. This way, plasma and CSF proteins could serve as biomarkers for the diagnosis and monitoring of the disease.

Fig. II Extraction of CSF through lumbar puncture

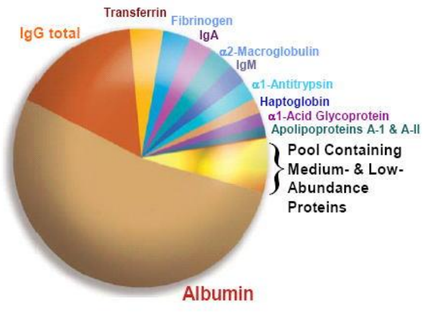



Fig. III Protein components of the blood plasma

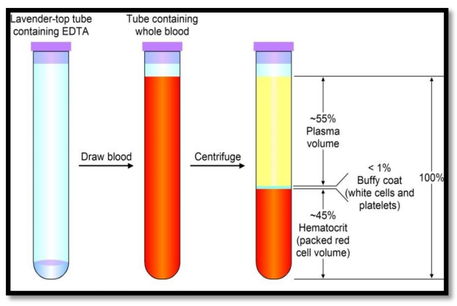



Fig. IV Isolation of blood plasma

ALL causes specific deviations in the DSC thermogram, which increase as the disease progresses. The presence of a smaller neoplastic volume results in deviations that differ from the thermogram of the normal plasma proteome, but in relatively small scale. When the tumor mass is greater, the difference with the control becomes more distinct. The correlation between the calorimetric deviations and the volume of the neoplastic mass is undoubtedly present, however, not in a linear fashion. In some of the patients the thermogram with relatively large leukocyte count does not show significant alteration, while in others there are strong deviations even when the WBC is small.

## *Results and conclusions*

Analysing our examinations regarding DSC studying of CSF in patients with ALL, we can conclude that the neoplastic proteins can be defined with specific endothermic peaks with various shapes around 69-74°C. These changes in the thermogram persist, yet with small alterations in the curves (even when the corresponding intrathecal cytostatic therapy has been applied), for different periods of time after the extinction of the blast cells, proven through conventional examination techniques.

This fact leads us to the conclusion that DSC studying has a vast potential in the near-future as a highly sensitive auxiliary method for diagnosing and tracking the development of the disease in the CSF. While so far we have not come to a distinct resolution as to how specific these changes are for the given malignant proteins, it is crucial to validate the data from the DSC through conventional examination techniques.

The applied thermodynamical method definitely has potential to be introduced in a clinical setting, since it is perfectly suitable for fast detection and screening, requires only a small volume of probe and is not invasive for the patients. We can expect that the systemic registration and analysis of these microcalorimetric thermograms would unveil new horizons for studying and understanding the changes that occur on a molecular scale in the CSF in patients with ALL.

**This study contributes to the validation of the DSC as a powerful non-invasive tool for monitoring malignant diseases and for evaluating the efficacy of the applied chemotherapy.**

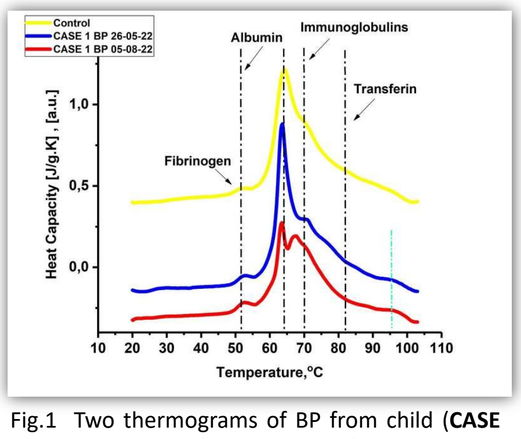



Fig.1 Two thermograms of BP from child (**CASE 1**) with extramedullary relapse of ALL diagnosis from **26.05.2022**, when the neoplastic mass is relatively small, compared to the thermogram from **05.08.2022**, when there is disease progression present and significant increase in the tumor mass, both compared with the BP from a child (**Control**), which is in remission, with ceased antileukemic treatment few months beforehand, having no deviations in the clinical status and the paraclinical examinations.

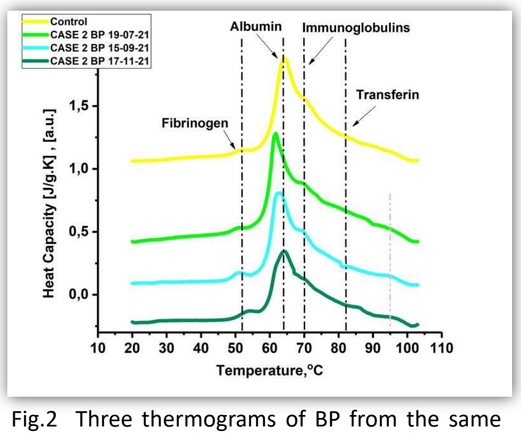



Fig.2 Three thermograms of BP from the same child (**CASE 2**) with three consequent diagnosed relapses: **19.07.2**1 – isolated from the bone marrow; **15.09.21** – extramedullary isolate; **17.11.21** – isolated CNS relapse, all compared with BP from a child (**Control**), which is in remission, with ceased antileukemic treatment few months beforehand, having no deviations in the clinical status and the paraclinical examinations. In all three cases the neoplastic mass is small.

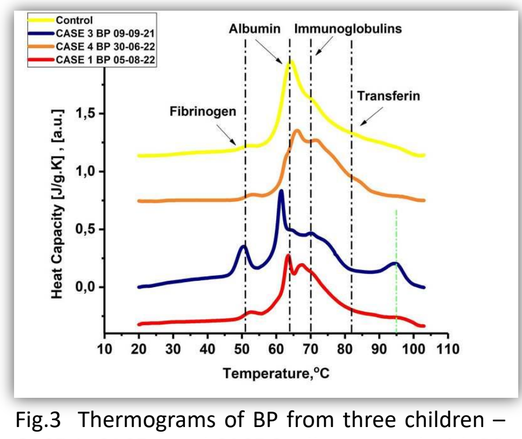



Fig.3 Thermograms of BP from three children – **CASE 1, CASE 4** and **CASE 3,** all compared with BP from a child (Control), which is in remission, with ceased antileukemic treatment few months beforehand, having no deviations in the clinical status and the paraclinical examinations. In **CASE 1** (voluminous tumor masses in the pelvic region) and **CASE 4** (hyperleukocytosis in peripheral blood) a large neoplastic mass is present, and in **CASE 3** the leukocytic invasion is found only in the bone marrow while the WBC in the peripheral blood is small.

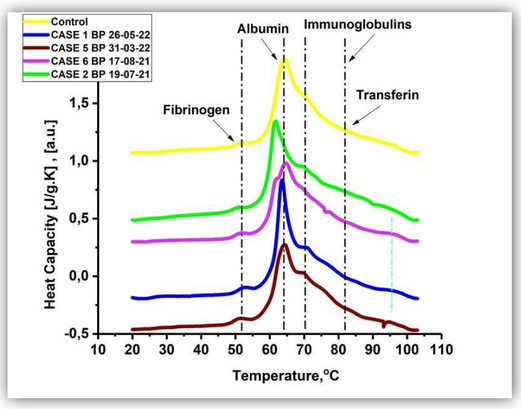



Fig.4 Thermograms of BP from four children – **CASE 1, CASE 5, CASE 6** and **CASE 2** with large neoplastic mass, all compared with BP from a child (**Control**), which is in remission, with ceased antileukemic treatment few months beforehand, having no deviations in the clinical status and the paraclinical examinations. **CASE 1** – newly diagnosed extramedullary relapse of ALL. **CASE 5** – combined CNS and bone marrow relapse of ALL. **CASE 6** – lymphoid blast crisis of a child with chronic myeloid leukemia. **CASE 2** – newly diagnosed isolated bone marrow relapse of ALL.

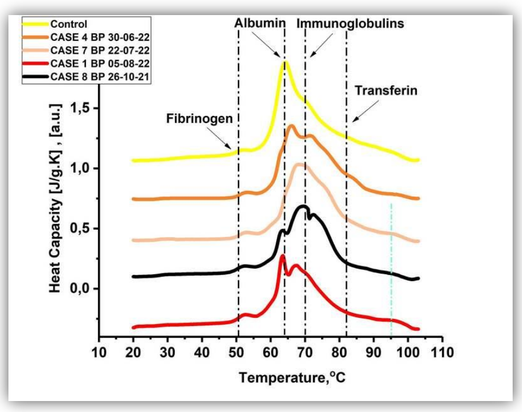



Fig.5 Thermograms of BP from four children – **CASE 4, CASE 7, CASE 1** and **CASE 8** with significant neoplastic mass, all compared with BP from a child (**Control**), which is in remission, with ceased antileukemic treatment few months beforehand, having no deviations in the clinical status and the paraclinical examinations. **CASE 4** – newly diagnosed ALL with hyperleukocytosis in the peripheral blood: WBC = 104,7 g/L. **CASE 7** – newly diagnosed ALL with WBC = 16 g/L, but with voluminous tumor formation in the chest, one of the kidneys is also affected. **CASE 1** – progression of extramedullary relapse of ALL. **CASE 8** – newly diagnosed ALL with WBC = 40,5 g/L, voluminous neoplastic mass affecting the mediastinum, abdomen and testes.

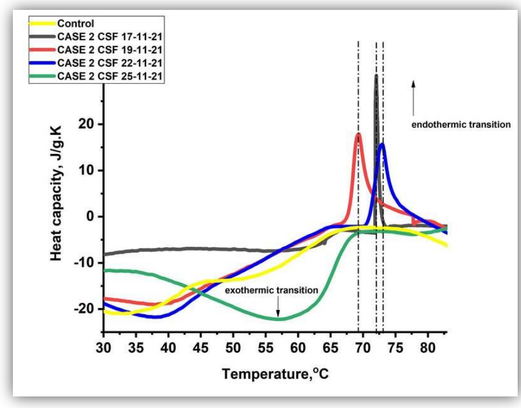



Fig.6 Thermograms of CSF from a 16yo boy – **CASE 2**, with meningeal relapse of ALL, diagnosed on **17.11.2021** with 140 neoplastic cells per microliter. On **19.11.2021** the cells are twice as much – 280 per microliter. After the applied intrathecal drugs, the pleocytosis is negated and upon examination on **22.11.2021** and **25.11.2021** there are no cells in the CSF. The deviations in the denaturation profile are persisting for longer period of time. On **25.11.2021** an exothermic shift is observed, probably caused by the applied therapy. A normal (**control**) profile has been presented, that of a child with ALL, that has never had affected CNS nor currently has deviations in the clinical and haemotological status, continuous remission

Fig.7 Thermograms of CSF from a 8yo boy – **CASE 5**, with combined haemotological and meningeal relapse of ALL, diagnosed on **30.03.2022** with **2500** neoplastic cells per microliter. On **20.04.2022**, after intrathecal cytostatic drug application, the pleocytosis is negated. Following a lumbar puncture on **09.06.2022** a strong endothermic peak is detected, resembling the peak in the **22.11.2021** thermogram of **CASE 2**. On **09.06.2022** another exothermic shift is visualized, due to the applied therapy. A normal (**control**) profile has been presented, that of a child with ALL, that has never had affected CNS nor currently has deviations in the clinical and haemotological status (continuous remission).

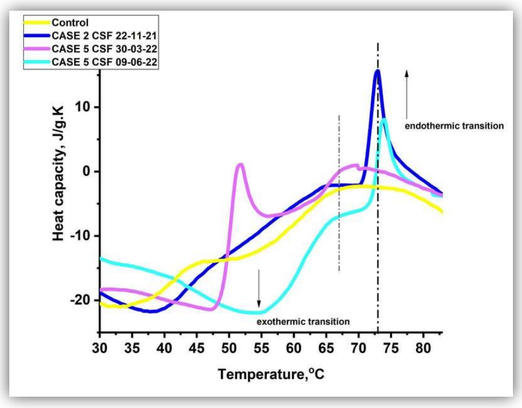
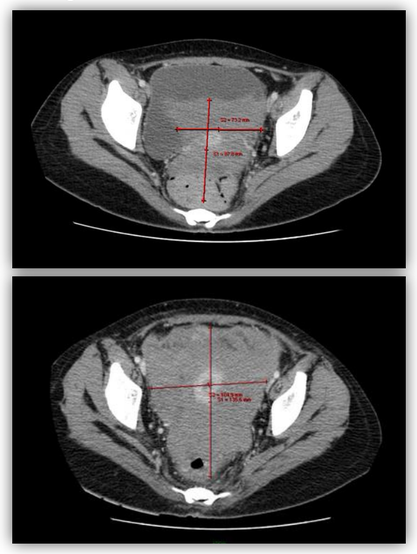



Fig.8, 9 Results from **X-Ray CT** for **CASE 1** from **09.06.22 (top)** and **28.07.22 (bottom)**. The red arrows indicate the size of the tumor formation.



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