Calorimetric Markers for IgD Multiple Myeloma Detection

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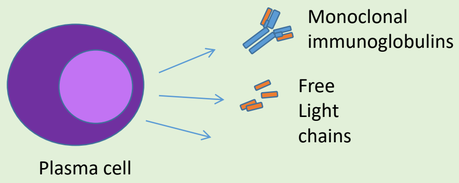
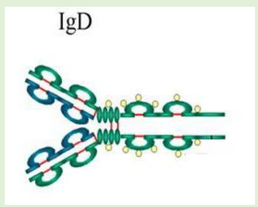
Multiple myeloma (MM) is a hematological disease, that affects B-limphocites in the bone morrow and can provoke monoclonal immunoglobulins (Igs) production (secretory form) or may exists in non-secretory form. In the first case different classes monoclonal immunoglobulins can be detected in the blood sera-IgM, IgG, IgA, IgE or IgD, which determines the isotype of the disease.



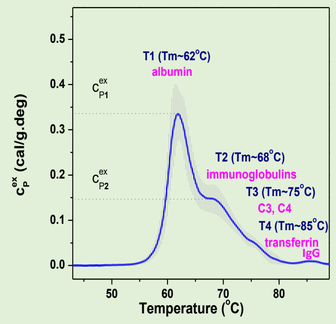


IgD MM is a rare desease, which is characterized by aggressive clinical course, presence of Bence Jones protein, osteolytic lesions, hypercalcaemia, amyloidosis. IgD MM can affect younger population and has lower survival compared to other isotypes. Herein, we applied differential scanning calorimetry (DSC) to characterize the thermodynamic behavior of blood sera derived from patients with IgD MM. The thermodynamic parameters - denaturation temperature (T_m), excess heat capacity (c_p^{ex}) of the successive transitions, Pearson correlation coefficient (r), standardized Euclidean distance (P) and metric similarity (ρ) were determined from the DSC profiles.

AIM: To identify calorimetric markers for IgD MM

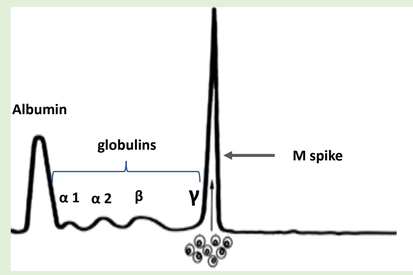
Methods

Differential scanning calorimetry



Typical calorimetric profile of blood serum obtained for healthy controls (mean±SD). The contribution of the major serum proteins to the calorimetric transitions is indicated.

Capillary electrophoresis



Electrophoretic profile typical of patients with multiple myeloma. The contribution of the blood sera proteins is indicated(Capillarys 2, Sebia).

RESULTS

Calorimetric profiles of blood sera from IgD MM patients

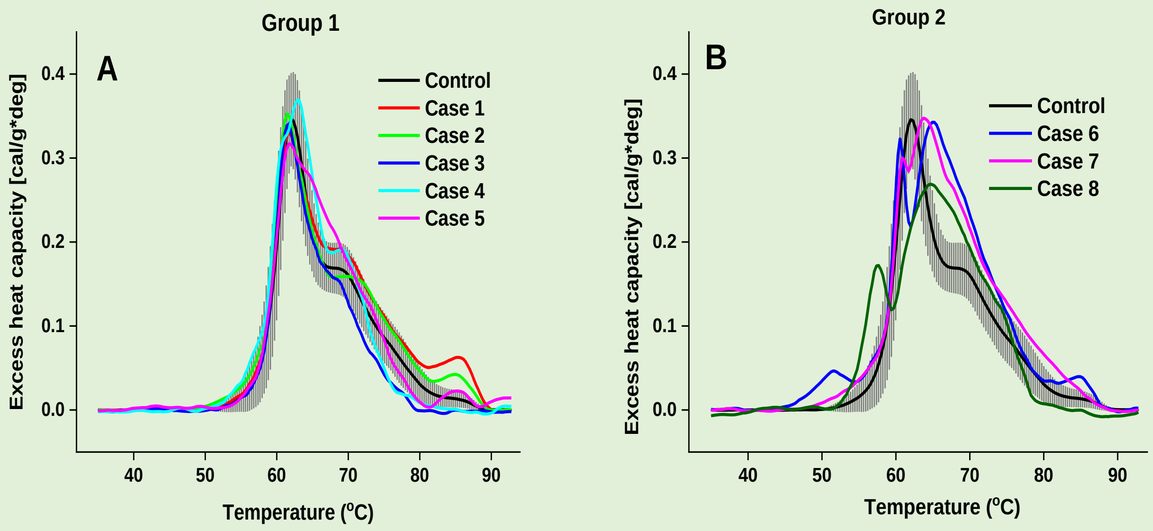


Figure 1. Calorimetric profiles of blood sera obtained from patients with IgD MM. Group 1 – thermograms similar to the controls with main transition centered at 61 – 63 °C (Panel A) and Group 2 with main transition shifted to higher temperatures (64 – 65 °C) (Panel B). For clarity the averaged calorimetric profile of healthy controls is presented at each panel.

Thermodynamic parameters for the different calorimetric groups

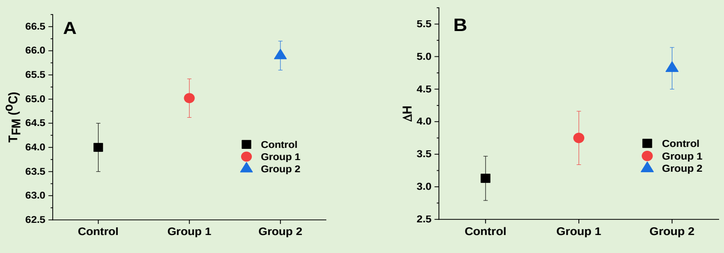


Figure 2. Selected calorimetric parameters (mean ± SD), weighted average center of the thermogram, T_{FM} (Panel A) and change of total enthalpy, ΔH (Panel B), determined from the DSC profiles of blood plasma from for healthy controls, and IgD MM patients (Group 1 and Group 2).

Successive annealing procedure of thermograms for IgD MM sera

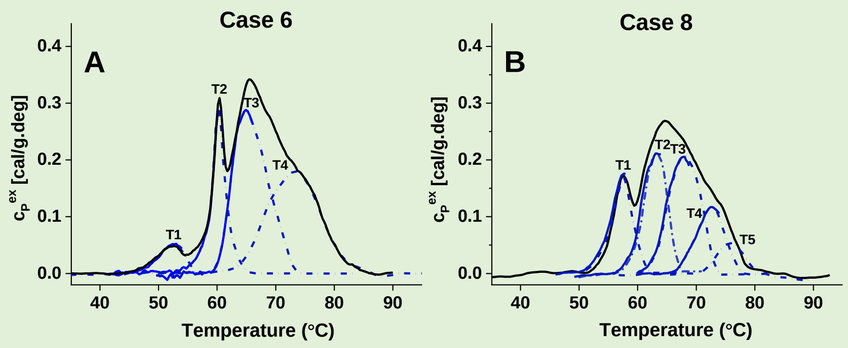


Fig. 3. Successive annealing procedure of thermograms recorded for IgD MM blood sera – case 6 (Panel A) and case 8 (Panel B). The solid black lines represent the initial DSC curve. The calorimetric profiles successively registered after heating (up to temperature slightly above the Tm of the respective transition) and cooling cycles are presented with solid blue line at each panel. The individual components are denoted with dash dot lines.

***Table 1.** Calorimetric parameters transition temperature, T_m, and enthalpy cahnge, ΔH, estimated from the annealing procedure of the thermal denaturation of blood serum from IgD MM patients for each individual Gauss component.*

Case	T_m 1	ΔH 1	T_m 2	ΔH 2	T_m 3	ΔH 3	T_m 4	ΔH 4	T_m 5	ΔH 5
Case 6	52.65	0.26	60.28	0.94	64.9	2.07	73.4	1.99	-	-
Case 8	57.6	3.43	63.25	3.66	67.9	1.39	72.6	0.66	76.1	0.21

***Table 2.** Calorimetric parameters – denaturation temperature, T_m, and excess heat capacity, c_p^{ex} , of albumin and transferrin(Tf)/IgG assigned transition; change of the total calorimetric enthalpy, ΔH, weighted average center of the thermogram, T_{FM}; and similarity parameters (Pearson correlation coefficient, r, standardized Euclidian distance, P, and metric similarity, ρ) estimated from the thermograms of blood sera from healthy individuals and IgD MM patients.*

Groups	T_m^{main} [°C]	c_p^{main} [cal/g*deg]	$T_m^{Tf, IgG}$ [°C]	$c_p^{Tf, IgG}$ [cal/g*deg]	ΔH [cal/g]	T_{FM} [°C]	r	P	ρ
Control	62±0.7	0.37±0.05	81.06±1	0.032±0.05	3.13±0.3	64±1.1	0.98	0.76	0.85
Group 1									
Case 1	61.67	0.3	85.4	0.057	3.99	66.5	0.99	0.72	0.78
Case 2	61.4	0.35	84.9	0.043	4.18	65.6	0.99	0.79	0.84
Case 3	61.5	0.34	–	–	3.16	64.12	0.98	0.52	0.61
Case 4	62.9	0.37	–	–	3.9	63.9	0.98	0.55	0.64
Case 5	61.66	0.31	84.9	0.01	3.5	65	0.98	0.72	0.78
Group 2									
Case 6	64.98	0.34	84.7	0.04	4.93	66.12	0.91	0.7	0.75
Case 7	63.8	0.37	–	–	5.3	66.3	0.95	0.78	0.82
Case 8	64.6	0.27	–	–	4.24	65.3	0.87	0.55	0.62

***Table 3.** Serum levels of the main protein fractions and M protein, determined by capillary electrophoresis, presented as percentage of the total serum content for the cases under study.*

Groups	Albumin (%)	Alpha 1 (%)	Alpha 2 (%)	Beta 1 (%)	Beta 2 (%)	Gamma (%)	M protein (%)
Control	55.8-66.1	2.9-4.9	7.1-11.8	4.7-7.2	3.2-6.5	11.1-18.8	–
Group 1							
Case 1	60.1	3.3	9	6	4.6	17	4.6
Case 2	64.2	4.8	**13.3***	5.6	3.9	**8.2***	3.4
Case 3	62.2	**6.2***	**12.2***	7.1	3.6	**8.7***	0.76
Case 4	**47.6***	4.8	9.2	4.1	3.2	**31.1***	26.4
Case 5	60	**5.3***	**13.2***	5.1	4.7	11.7	9.7
Group 2							
Case 6	**48.6***	**5***	**15.4***	4.4	4.2	**22.4***	17
Case 7	**49.4***	**10.8***	**12.2***	6.2	4.7	16.7	9.7
Case 8	56.63	4.45	8.3	5.73	3.37	**21.53***	13.82

***indicates statistically significant difference from the Controls, p < 0.05**

Conclusions

» Two groups of IgD MM thermograms are defined, based on the thermodynamic parameters.
» The calorimetric parameters: weighted average center of the thermogram and enthalpy change demonstrate higher values for IgD MM patients of both groups compared to those of control ones, more pronounced for Group 2.
» The results show that for 50 % of IgD MM cases the high temperature transition at 86 °C (regarded to be due to transferrin and IgG denaturation) is more pronounced than the controls - an observation that appears to be specific for IgD MM and that might be used as complementary marker for IgD MM detection, however its validation requires further studies.

Acknowledgement. This work is supported by the Bulgarian Ministry of Education and Science under the National Research Program „Young scientists and postdoctoral students”(DCM 168/19.03.2020, A.D.)