Effect of whole-body cryotherapy on morphological, rheological, and biochemical blood indices in individuals with multiple sclerosis

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Abstract

The study aim was to examine the impact of 20 whole-body cryotherapy sessions on biochemical and rheological blood indices in multiple sclerosis individuals. The study group involved 15 women (mean age: 41.53 ± 6.98 years) with diagnosed multiple sclerosis who underwent whole-body cryotherapy sessions. The first control group consisted of 20 women (mean age: 40.45 ± 4.77 years) with multiple sclerosis who received no cryotherapy intervention. The second control group comprised 15 women (mean age: 38.47 ± 6.0 years) without neurological diseases or other chronic conditions who participated in cryotherapy sessions. For blood indices analysis, venous blood was collected twice: on the day of cryotherapy commencement and after the 20 cryotherapy sessions. Blood counts were determined with a hematology analyzer. A laser-optical rotational cell analyzer served to investigate erythrocyte aggregation and deformability. Total serum protein was measured, and proteinogram and fibrinogen values were established. Statistically significant differences were observed in red blood cells, hemoglobin, hematocrit, elongation index, total extent of aggregation, proteins (including fibrinogen). There was no significant effect of the 20 cryotherapy sessions on morphological, rheological, or biochemical blood indices in women with multiple sclerosis. The intervention had a positive impact on the rheological blood properties of healthy women.

Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system, with the key feature of demyelination foci occurrence, especially in the white matter of the brain. MS pathogenesis is not fully understood. The condition is perceived as an inflammatory demyelinating disease in which damage to the axons plays an essential role. There are no current and accurate statistical data assessing the number of people affected by MS. It is estimated that over 2,500,000 people worldwide suffer from MS, including over 630,000 in Europe, and approximately 50,000 in Poland [1, 2]. Most often, the first symptoms appear between 20 and 40 years of age [3].

MS is hypothesized to be associated with a combination of hereditary susceptibility and still undetermined environmental factors [4]. Research suggests that MS may result from a viral infection, although the potential virus has not been identified. Environmental factors that may be indirectly related to the disease onset are also being investigated. The thesis of a significant environmental impact on an increased MS risk is supported by the variable occurrence of the disease in different areas. Studies of the links between MS and the environment have pointed at the influence of, *inter alia*, geoclimatic, seasonal, socioeconomic, racial, and ethnic factors [5, 6].

The beneficial effects of cold on the human body have been known for a long time. In Poland, cold therapy has been successfully applied for over 30 years [7]. The main mechanism that initiates the adaptation process in individuals in a cryochamber is the reduction of skin blood flow, which, in turn, implies an increase in the thermal insulation capacity of the skin [8, 9]. Many studies (among healthy young and middle-aged people) have shown a relationship between whole-body exposure to extremely

low temperatures and changes in the levels of selected enzymes and hormones in body fluids. Morphological and biochemical tests carried out after cryotherapy application indicate an increase in blood serum levels of adrenaline, noradrenaline, adrenocorticotropic hormone, cortisol, testosterone (in men), as well as a decrease in inflammatory response parameters, such as erythrocyte sedimentation rate, Waaler-Rose test, seromucoid [10–14]. After approximately a dozen days of stimulation with cryogenic temperatures, patients with rheumatoid arthritis and physically active individuals present increased hemoglobin (HGB) concentration, platelet counts (PLT), creatinine levels, and glycemia [15, 16]. Some reports show decreased red blood cell (RBC) values in athletes [12, 17–19] and increased white blood cell (WBC) counts [20, 21], while others indicate no RBC or WBC change, most likely because of too few sessions [12, 16, 18, 19, 22]. Blatteis [23] observed a decrease in WBC and RBC in healthy subjects after a series of sessions. In turn, Banfi *et al.* [12] demonstrated a decrease in HGB levels with a simultaneous increase in the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values after the intervention series completion. There is still a lack of studies assessing the effect of whole-body cryotherapy on morphological indices in MS patients.

The aim of the presented study was to investigate and evaluate the impact of a series of 20 whole-body cryotherapy sessions on biochemical and rheological blood indices in individuals with MS.

Methods

Material

The prospective controlled studies were performed at the University of Physical Education in Krakow, Poland, between November 2018 and September 2019. Overall, 80 individuals applied, and 50 women were finally selected to participate in the research program. Before entering the study, each volunteer read the patient information and could ask questions in the case of any doubts. All patients provided their informed consent to participate in the study. The qualification of the subjects is presented in Figure 1.

Ethics statement. The study, registered in the Australian New Zealand Clinical Trials Registry (ACTRN12620001142921, registration date: 2/11/2020), was approved by the Ethics Committee of the Regional Medical Chamber in Krakow (approval No. 87/KBL/OIL/2018 of May, 8/05/ 2018) and followed the tenets of the Declaration of Helsinki.

Key inclusion criteria:

- diagnosed MS – 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10): G35 (the MS groups);
- female sex;
- age: 30–55 years;
- Expanded Disability Status Scale (EDSS) score of 0–6.5 (the MS groups);
- no contraindications to whole-body cryotherapy;

- the subject's written consent to participate in the study.

Key exclusion criteria:

- vitamin D supplementation;
- consuming more than 4 cups of coffee or more than 2 alcoholic beverages per day;
- changing the diet immediately before or during the study;
- participation in other forms of physical activity directly before or during the study.

Participant characteristics. The study group (CRYO-MS) involved 15 women aged 34–55 years (mean age: 41.53 ± 6.98) with diagnosed MS who underwent a series of whole-body cryotherapy sessions.

The first control group (CONTROL-MS) consisted of 20 women aged 32–48 years (mean age: 40.45 ± 4.77) with diagnosed MS who received no intervention of whole-body cryotherapy. Nonprobability sampling was applied. The main criterion for inclusion in the CONTROL-MS group was organizational inability to participate in cryotherapy procedures.

The second control group (CONTROL-CRYO) comprised 15 women aged 30–49 years (mean age: 38.47 ± 6.0) without neurological diseases or other chronic conditions who participated in whole-body cryotherapy sessions.

The participants were included in the research program after obtaining a consent from a neurologist, a rehabilitation physician, and a physiotherapist (assessment of health status, functional status, disease stage, course, and type; determination of the applied treatment: patients with MS were treated mainly with immunomodulating agents and steroids). Body weight was assessed with a Tanita BC 418 MA device (measurement error: 0.1 kg), body height with a tape measure (measurement error: 0.5–1 cm). The Tanita BC 418 MA device also served to establish body composition with the use of the bioelectrical impedance method. The characteristics of the study group and the control groups are presented in Table 1.

Methods

Analysis of blood indices. For the analysis of blood indices, venous blood was collected twice: at baseline (study 1; on the day of whole-body cryotherapy commencement) and after the series of 20 cryotherapy sessions (study 2). In patients who did not undergo the intervention, blood was collected once (study 1). Throughout the project, blood pressure [mm Hg] was monitored in patients receiving cryotherapy: before and after each session. Fasting blood samples were collected in the morning from the basilic, cephalic, or median cubital vein into test tubes:

- with EDTA – for hematological analysis of whole blood; K2 potassium edetate (6 ml) was used as an anticoagulant;
- with clotting activator – for serum testing; the main activator ingredient is SiO_2 (6 ml).

The blood was collected by a qualified laboratory diagnostician, under the supervision of a physician, in accordance with the applicable standards of the Blood Physiology Laboratory of the University of Physical Education in Krakow. The assessment of blood indices was performed in the Blood and Skin Physiology Laboratory of the University of Physical Education in Krakow and in the Department of Clinical Biochemistry of the Krakow Branch of Maria Skłodowska-Curie National Research Institute of Oncology.

The following hematological blood parameters were assessed with the ABX Micros 60 hematology analyzer (USA):

- WBC [10^9/l];
- RBC [10^{12}/l];
- HGB [g/dl];
- hematocrit (HCT) [%];
- PLT [10^9/l];
- MCH [pg];
- mean corpuscular volume (MCV) [fl];
- MCHC [g/dl].

Assessment of elongation and aggregation indices. A laser-optical rotational cell analyzer (LORCA) (RR Mechatronics, the Netherlands) was used to study erythrocyte aggregation and deformability; the results were presented as elongation and aggregation indices. The tests in the above-mentioned device were performed within 30 minutes of blood collection, at 37°C, in accordance with a standard protocol [24–26].

The blood for determining the elongation index (EI) was taken from the test tubes in the amount of 25 µl and added to 5 ml of 0.14 mM polyvinylpyrrolidone (PVP), dissolved in phosphate-buffered saline (PBS). The test sample was placed in the measuring chamber between two concentric cylinders set in rotation. The laser light, passing through the thin layer of red blood cells suspended in the PBS solution, was deflected, giving a diffraction image on the projection screen. The EI results are provided in the range of 0.30–60.30 shear stress measured in pascals. EI is the measure of deformation of red blood cells as they move through the measurement chamber [24–26].

Prior to the actual aggregation test, the blood sample was oxygenated by incubation and mixing with carbogen for 15 minutes. The blood, in the amount of 1.5 ml, was introduced into the measuring chamber of the LORCA analyzer. Within 120 seconds, the cylinder was set in rotation with a shear rate of > 400 • s^{-1}. After 10 seconds, the centrifugation was stopped abruptly, and aggregation of red blood cells began. The result of the computer analysis is a curve of time dependence of the scattered light intensity (for a specific shear rate), i.e., a sylectogram [24–26].

Parameters determining the kinetics of erythrocyte aggregation were investigated:

- aggregation index (AI) [%];
- total extent of aggregation (AMP, amplitude) [a.u.];
- half time kinetics of aggregation (T1/2) [s].

Assessment of protein level. Total serum protein was measured by using a Cobas 6000 analyzer (Roche) (biuret method). The following reaction was applied:

$$\text{protein} + \text{Cu}^{2+} \xrightarrow{\text{alkaline environment}} \text{Cu-protein complex}$$

In alkaline environment, peptide bonds of proteins form a characteristic violet-colored complex with copper ions contained in the biuret reagent. The following reagents were used: R1 – sodium hydroxide 400 mmol/l, potassium sodium tartrate 89 mmol/l; R2 – sodium hydroxide 400 mmol/l, potassium sodium tartrate 89 mmol/l, potassium iodide 61 mmol/l, zinc sulfate 24.3 mmol/l. Serum was used in the samples. The concentrations were automatically calculated by the system. The proteinogram was provided by a Minicap (Sebia) analyzer and the capillary method was applied. The following indices were determined:

- total protein [g/l];
- albumin [g/l];
- alfa-1 globulins [g/l];
- alfa-2 globulins [g/l];
- beta-1 globulins [g/l];
- beta-2 globulins [g/l];
- gamma globulins [g/l];
- the albumin-globulin (A/G) ratio.

Determination of fibrinogen concentration. For the determination of fibrinogen concentration, 50 µl of plasma was added to 100 µl of thromboplastin with calcium chloride. The result is based on the measurement of the time from reagent addition to clot formation; then, the result is converted to g/l. The assessments were performed with a Bio-Ksel Chrom-7 camera (spectrophotometric method).

Description of the intervention. The whole-body cryotherapy sessions were carried out in the Malopolska Cryotherapy Rehabilitation Center in Krakow. The applied cryochamber temperatures were as follows:

- vestibule temperature: –60°C;
- chamber temperature: –120°C.

The time of a single whole-body cryotherapy session in the groups who underwent the intervention in the study was as follows:

- 1.5 minutes (the first session);
- 2 minutes (the second session);
- 3 minutes (the third session on).

One session per day was performed, 5 times a week (every day in the same time period of 3:00–5:00 p.m.). Overall, there were 20 sessions.

Women entering the cryochamber were dressed in swimsuits, high woolen socks, and shoes with a high wooden sole. The parts of the body most exposed to low temperature were covered (the subjects wore gloves, a head covering, and a face mask). After each cryotherapy session, the patients warmed up on a Kettler Corsa cycle ergometer without any resistance for 15 minutes. The sessions were directly supervised by a trained physiotherapist. A physician was present at the Rehabilitation Center throughout the entire duration of each session.

During the procedure, visual contact with the patients was maintained through thermal windows; additionally, the interventions were monitored with a camera and the patients were given information on the current session time (every 30 seconds). The cryochamber is equipped with a visible alarm button, as well as electronic systems monitoring the oxygen level, humidity, and temperature of both the vestibule and the proper chamber.

Statistical analysis. Data are presented as mean values and standard deviations. The normality of distributions was verified with the Shapiro-Wilk test. The differences between the study group and the control groups were assessed by using the one-way analysis of variance (ANOVA) or, if its assumptions were not met, the Kruskal-Wallis test. For post-hoc evaluation, Tukey's test for unequal sample sizes was applied, or, respectively, the multiple comparison test of mean ranks for all Dunn's trials. The dependent variables were compared with Student's *t*-test for related variables or, if its assumptions were not met, with the Wilcoxon test. Independent variables were compared with the Mann-Whitney U test. The significance level of $\alpha = 0.05$ was adopted in the analyses. The applied tests verified two-sided hypotheses. The analyses were performed with the use of the Statistica 13 package (Tibco Software Inc., USA).

Results

A statistically significant difference was observed in the baseline RBC levels; these were lower in MS patients ($4.23 \pm 0.61 \cdot 10^{12}$/l and $4.30 \pm 0.67 \cdot 10^{12}$/l in the CRYO-MS and CONTROL-MS group, respectively) than in healthy women ($4.80 \pm 0.27 \cdot 10^{12}$/l in the CONTROL-CRYO group). Moreover, healthy women were characterized by a statistically significantly ($P = 0.007$) lower RBC level after whole-body cryotherapy application ($4.57 \pm 0.28 \cdot 10^{12}$/l).

There was also a significantly higher mean baseline HGB level in the group of healthy women (CONTROL-CRYO) compared with patients with MS (CRYO-MS) before whole-body cryotherapy ($P = 0.045$). After the intervention, a statistically significantly ($P = 0.014$) lower mean HGB level (3.81%) was noted in the group of healthy women.

When analyzing the HCT level, a lower baseline level was indicated in MS patients receiving cryotherapy (CRYO-MS) (35.97 ± 5.65%) and in the CONTROL-MS group (35.90 ± 5.88%) than among healthy women (CONTROL-CRYO) (40.54 ± 2.50%). Furthermore, healthy women presented a statistically significantly ($P = 0.003$) lower HCT level after the application of whole-body cryotherapy (38.35 ± 2.20%).

As for the EI, a significant difference was observed in the baseline values at the shear stress of 4.24–60.30 Pa: EI was lower in healthy women (CONTROL-CRYO) as compared with MS women who received (CRYO-MS) and who did not receive whole-body cryotherapy (CONTROL-MS) ($P \leq 0.016$). After the intervention, an increase in the EI value was noted in healthy women at the shear stress of 2.19–60.30 Pa. The average level of the index variability was 28.07%.

A statistically significantly higher baseline AMP was determined in healthy women (21.62 ± 4.06 a.u.) than in MS controls who received no cryotherapy (18.16 ± 4.57 a.u.). There were no statistically significant changes in AMP after the intervention.

When analyzing protein levels, lower baseline values of total protein were observed in the groups of MS patients (70.17 ± 3.98 g/l and 70.29 ± 2.79 g/l in the CRYO-MS and CONTROL-MS group, respectively) compared with healthy women (73.56 ± 3.68 g/l). There was also a statistically significant reduction (by 2.49%) in the total protein level in healthy women after whole-body cryotherapy application (71.73 ± 2.53 g/l). Mean albumin levels slightly decreased in healthy women after the intervention (CONTROL-CRYO) ($P = 0.009$). There were no statistically significant changes in albumin levels after the use of whole-body cryotherapy. MS patients presented lower baseline levels of gamma globulins (9.41 ± 2.22 g/l and 9.47 ± 2.29 g/l in the CRYO-MS and CONTROL-MS group, respectively) than healthy patients (11.53 ± 2.37 g/l); the average difference equaled 18.13%. There were no statistically significant changes after whole-body cryotherapy application.

With reference to the mean levels of fibrinogen, a statistically significant increase was observed in healthy women after the cryotherapy intervention (34.13% in the CONTROL-CRYO group), as well as a statistically insignificant increase, owing to a large discrepancy, in patients with MS who received whole-body cryotherapy (44.24% in the CRYO-MS group). There were no statistically significant between-group differences in baseline fibrinogen levels.

Detailed results are presented in Figures 2 and 3 and Tables 2–5.

Discussion

Because of the lack of an unambiguous answer as for MS etiopathogenesis and, consequently, the limited options of causal treatment, no effective therapy has been developed to combat the disease [4]. The currently available interventions allow to reduce the disease activity, inhibit its progression, modify the autoimmune process, influence the intensity of relapses, and alleviate or treat the concomitant clinical symptoms. MS therapy is a difficult process, which is related, *inter alia*, to the multitude of symptoms and their overlapping [27, 28]. Despite the increasingly detailed knowledge about the disease pathomechanism, the pharmacological treatment options that might be widely applied are quite limited. Therefore, symptomatic treatment, including physical activity and physiotherapy, plays an important role besides pharmacotherapy [29, 30].

Rheology investigates blood flow through blood vessels. It concerns whole blood, as well as plasma and morphotic elements, especially red blood cells [31]. The basic factors that influence blood flow involve RBC, red blood cell deformability and aggregation, plasma viscosity, and HCT [31–33].

The phenomenon of erythrocyte deformability plays an important role in the flow of blood cells through capillaries with a diameter even two times smaller than the cells [34]. Normal erythrocytes deform under stress mainly because they do not have cell nuclei, their cytoplasm has a relatively low viscosity, the cell membrane presents favorable viscoelastic properties, and the appropriate shape ensures a high ratio of free surface to volume [26]. Changes in the blood cell shape depend on the quality of the spectrin-actin network in connection with calcium and ATP ions, and the reasons for this capacity decrease mainly involve the cell age, mechanical damage, and disease factors [10, 35].

According to Maeda [36], appropriate erythrocyte deformability plays a key role in the blood flow in the vascular system. In turn, the appropriate erythrocyte shape, intracellular viscosity, and the cell membrane wall stiffness depend on the HGB level, which significantly influences the deformation capacity. Red blood cell deformability is crucial as it allows the cells to pass through capillaries. The lower the deformability, the higher the blood viscosity and the worse the blood flow in the microcirculation [36].

Spontaneous erythrocyte aggregation in whole blood, i.e., the formation of three-dimensional erythrocyte structures, is a reversible physiological phenomenon that plays a key role in blood flow at low shear rates and significantly increases blood viscosity [26]. Erythrocyte aggregation is a complex dynamic phenomenon, activated by various factors, especially plasma-related ones, and pathological processes. Physiological shear stresses are to prevent aggregate formation, and the absence of shear stresses may be among the many causes of cardiovascular disease [37].

The degree of red blood cell aggregation depends on the flow conditions, properties of cell membranes, and physicochemical properties of cells [33]. Small blood vessels, where the shear rates are usually low, are particularly susceptible to the formation of erythrocyte aggregates. This ultimately causes a decrease in blood flow velocity, or even its inhibition, and, as a consequence, under-oxygenation of cells and tissues [38].

The hematological symptoms in MS patients are unspecific. A homogeneous mechanism of their occurrence is difficult to determine and very likely not to exist at all. It has been suggested that hematological changes in MS may be related to such factors as complex humoral and cellular response, biochemical disorders, changes related to the attachment and transport of vitamin B12, the applied pharmacological treatment, and the phase of the disease (relapse or remission) [39–41].

Erythrocyte elasticity is significantly influenced, *inter alia*, by intracellular viscosity, affected by HGB. Appropriate MCH and MCHC values determine the potential for erythrocyte deformability even in cases of hyper- or hypotonic changes in the blood environment [31]. Najim al-Din *et al.* [42] did not find significant HGB level differences between MS patients and those with other neurological diseases; they also confirmed the thesis by Reynolds and Linnell [41] about the tendency for macrocytosis without concomitant anemia in MS patients. In the presented study, a statistically significantly higher mean baseline level of HGB was observed in the group of healthy women compared with the mean level in MS patients before whole-body cryotherapy ($P = 0.045$). After the intervention, a statistically significantly lower mean HGB level was noted in healthy women ($P = 0.014$). The analysis showed no statistically significant differences in MCH after whole-body cryotherapy application or at baseline. However, there was a statistically significant minimal increase in MCHC in healthy women after whole-body cryotherapy application ($P = 0.013$).

Grasso *et al.* [43] found no pathological HGB or RBC values in MS patients. They performed blood tests in MS patients (EDSS score: 1–9) and observed no difference in MCV as compared with the control group. At the same time, as the comparative group consisted of individuals who were not completely healthy, the results were also referred to the assumed physiological norms. Notably, the MCV results exceeded the assumed norms in one person from the study group and one from the control group only. No correlation was established between the patient's functional status expressed in the EDSS score and the MCV value.

In subsequent studies, Kocer *et al.* [44] also failed to confirm macrocytosis in MS patients examined during a relapse. In their research, MCV remained within the normal range in over 77% of the participants, while microcytosis was observed in the others. De Freitas *et al.* [45] also found no differences in MCV between MS patients undergoing steroid therapy and those who received no pharmacological treatment.

In the present study, there were also no statistically significant WBC changes in MS patients compared with the control group. However, a statistically significant difference was observed in the baseline RBC levels, which were lower in patients with MS (CRYO-MS and CONTROL-MS groups) than in healthy women (CONTROL-CRYO group). Baseline MCV did not differ between groups, with a slight MCV reduction observed in healthy women after whole-body cryotherapy application ($P = 0.028$).

The red blood cell distribution width (RDW), a measure of erythrocyte size and volume, affects deformability. A study found an increased RDW in patients with relapsing-remitting MS compared with healthy controls, which may underlie the altered red blood cell deformability in MS. Furthermore, increased RDW was observed to be positively associated with EDSS scores. A study that involved 109 MS patients and 130 healthy individuals failed to control other potential confounding factors affecting RDW

and lacked adequate pathological controls. More research is needed on the use of RDW as a biomarker [46]. Morphological changes in erythrocytes (macrocytes and echinocytes) are positively correlated with MS severity and may impair red blood cell deformability [47].

Platelets also play an important role in the coagulation cascade; they are abundant in MS [48]. Platelets themselves do not directly affect erythrocyte aggregation, but influence thrombotic processes [49, 50], which supports the idea of ischemic tissue damage [51]. In the present study, no statistically significant changes in PLT were established after whole-body cryotherapy application or at baseline.

Brunetti *et al.* [39] observed increased values of whole blood viscosity indices, with coexistent normal levels of plasma viscosity and HCT. They suggested that the raised blood viscosity was due to a decrease in erythrocyte deformability. Later studies by Pollock *et al.* [40] did not confirm this hypothesis, showing no visible differences in erythrocyte deformability between MS patients and healthy controls. Research related to the morphology of erythrocytes was also carried out by Simpson *et al.* [52], who reported that MS patients presented a lower level of sphingomyelin in the erythrocyte cell membrane as compared with the control group. They demonstrated impaired erythrocyte deformability, which can increase blood viscosity in MS patients. The authors observed an increase in MS patients' blood viscosity that varied depending on sex. In women, the values of blood viscosity were significantly higher than in the control group at the shear rates of $1 \bullet s^{-1}$, $10 \bullet s^{-1}$, and $100 \bullet s^{-1}$, while in men, blood viscosity was statistically significantly higher only at one shear rate ($1 \bullet s^{-1}$). Ernst [53] noted, however, that changes in viscosity indices did not always correlate with the level of erythrocyte deformability, as they were also influenced by the administered steroid therapy. This was confirmed by de Freitas *et al.* [45], who investigated the effect of steroid therapy on red blood cell membrane stability. They found that erythrocyte membranes in MS patients were less resistant to damaging factors than erythrocyte membranes in healthy individuals, while steroid therapy improved erythrocyte membrane resistance and brought it closer to that observed in healthy people.

When analyzing the level of HCT in the conducted study, a statistically significant difference was observed in the baseline values: these were lower in MS patients receiving the intervention (35.97 ± 5.65%) and in the MS control group (35.90 ± 5.88%) compared with healthy women (40.54 ± 2.50%). Moreover, healthy women were characterized by a statistically significantly lower HCT level after whole-body cryotherapy application ($P = 0.003$). In turn, with reference to EI, a statistically significant difference was demonstrated in baseline values at the shear stress of 4.24–60.30 Pa: the result was unexpectedly lower in healthy women compared with those with MS who received or who did not receive cryotherapy ($P \leq 0.016$). In healthy women, a favorable increase in EI was noted at the shear stress of 2.19–60.30 Pa after the intervention. The average level of EI variability equaled 28.07%. The application of whole-body cryotherapy significantly increased erythrocyte deformability and decreased HCT values in healthy women, which positively influences their rheological blood properties.

Kowal and Marcinkowska-Gapińska [54] published the preliminary results of their research on the comparison of hemorheological properties in MS patients and individuals with acute cerebral ischemia.

They observed a statistically significantly higher HCT value and a lower measure of red blood cell aggregation capacity in patients with MS compared with those after an acute cerebral ischemia incident. The differences in erythrocyte stiffness and the tendency to aggregation were not statistically significant. In the study, however, MS patients constituted a very small group, and the research was not continued among a wider MS cohort. The mean AI values in the study group and in the control group were not significantly different before and after whole-body cryotherapy. There were also no statistically significant changes in baseline AI or T1/2 values.

Fibrinogen is a 340-kDa glycoprotein synthesized in the liver, with a plasma concentration of approximately 150–400 mg/dl. The protein is involved in blood clotting and hemostasis, as well as in inflammation and tissue repair. Fibrinogen facilitates platelet aggregation by glycoprotein IIb/IIIa receptor binding and forming a fibrin monomer that rapidly polymerizes to create a clot [55, 56]. With inflammatory response, plasma fibrinogen levels rise 2–3-fold, leading to cell aggregation and an increase in blood viscosity [57]. Studies have shown that fibrinogen can modulate inflammatory response by leukocyte activation and synthesis of pro-inflammatory mediators (cytokines and chemokines) [55, 58]. Fibrinogen is not a clear indicator of the blood-brain barrier disruption, but it does activate glial cells, which results in blood-brain barrier dysfunction in MS patients [59].

Acuña *et al.* [60] demonstrated that high plasma fibrinogen levels (> 417 mg/dl) were associated with active changes in magnetic resonance imaging in MS patients during relapses. In an earlier study on fibrinogen in MS patients, Ehling *et al.* [61] showed no elevation of fibrinogen in the cerebrospinal fluid or plasma, probably because they compared fibrinogen levels between MS patients and those with central nervous system infections; in addition, less than a third of MS patients experienced an acute relapse at the time of fibrinogen analysis, with the rest presenting an inactive disease or a chronic progressive course.

Despite the inflammatory events, it seems that patients with relapsing-remitting MS do not have elevated levels of fibrinogen in remission [62]. However, D-dimer levels are elevated [63] and low fibrinogen levels during remission (MS patients vs. controls) do not rule out fibrinogen increase during relapse, especially if one considers the role of fibrinogen in MS pathology, where fibrin is involved in cytokine release and microglia activation in the central nervous system [64, 65]. When analyzing the mean levels of fibrinogen in the present study, no increased values were observed in women with MS, while after the application of a series of 20 whole-body cryotherapy sessions, there was a statistically significant rise in healthy women (by 34.13%) and a statistically insignificant rise (owing to the large discrepancy) in MS patients (by as much as 44.24%).

In clinical practice, plasma fibrinogen may be a valuable and easy biomarker of activity during relapses in MS patients, but prospective studies in larger groups are needed to confirm these results.

It has been found that although all MS patients show an increase in oxidative damage biomarkers, there seems to be no correlation between the degree of the increase and the disease severity. It is now suggested that oxidative changes are not accompanied by inflammatory activity in these patients as

measured by changes in WBC and C-reactive protein levels. This would mean that oxidative stress precedes the inflammatory response, which would indirectly support the hypothesis that oxidative stress alters blood-brain barrier permeability and stimulates monocyte adhesion to vascular endothelium [66]. Erythrocytes may contribute to the pathophysiological mechanisms of MS through impaired antioxidant capacity and altered hemorheology, leading to an increase in oxidative stress and to potential ischemic tissue damage, respectively [67].

Studies on the effect of whole-body cryotherapy in MS patients were also conducted by Bryczkowska *et al.* [68]. In healthy individuals, the first changes in the lipid profile were observed after 20 daily whole-body cryotherapy sessions [20], while in MS patients, after a series of 30 whole-body cryotherapy sessions, researchers did not report significant changes in total protein, albumin, uric acid, and glucose concentrations or in the lipid profile [68]. In the present study, lower baseline total protein levels were demonstrated in the groups of MS patients (70.17 ± 3.98 g/l and 70.29 ± 2.79 g/l) compared with healthy women (73.56 ± 3.68 g/l); there was also a statistically significant reduction of the parameter among healthy women after whole-body cryotherapy application (2.49%). No significant differences were indicated in the other proteinogram indices, i.e., albumin, alpha-1 globulins, alpha-2 globulins, beta-1 globulins, beta-2 globulins, except for lower baseline levels of gamma globulins in the groups of MS patients compared with healthy women (by 18.13% on average).

Studying the role of erythrocytes in MS may reveal further specific differences that could be used as the disease biomarkers, as well as broaden our understanding of the pathological mechanisms of this complex and heterogeneous disease. This, in turn, could lead to the discovery of new and innovative therapeutic targets, thus significantly improving patients' quality of life.

Summing up, in the light of the available literature and the results of own research, it can be concluded that whole-body cryotherapy is an effective method to combat or inhibit the progress of many diseases and their negative consequences, thus contributing to maintaining the best possible body fitness. The presented study is probably the first one to assess the influence of whole-body cryotherapy on rheological blood properties, including EI and AI, in MS women. No pathological or harmful changes were observed after whole-body cryotherapy application. In healthy participants, the intervention resulted in improved erythrocyte flexibility, which adds to better cell oxygenation.

Conclusions

1. There was no significant effect of a series of 20 whole-body cryotherapy sessions on the morphological, rheological, or biochemical blood indices among MS women.
2. The application of whole-body cryotherapy significantly increased erythrocyte deformability and reduced the HCT value (within physiological norms) in healthy women, which positively influenced the rheological properties of blood.
3. Whole-body cryotherapy is an effective and safe form of therapy in MS patients.

Study Limitation

The study is not without limitations. An important aspect that could have affected the trial results is a relatively small number of individuals in the study groups. The obtained results are important for determining the safety of whole-body cryotherapy in the field of clinical trials, and the research should be continued (with randomization) in larger and more diverse groups of patients.

Declarations

Author contributions

B.P., A.T., J.G. designed the study. B.P., A.T., S.P. performed the trial and obtained the samples. B.P., S.P., J.G. qualified and cared for patients. B.P., A.T., J.A., S.P. analyzed the data and interpreted the results. B.P., A.T., J.A. wrote the manuscript. J.M. performed the statistical analysis. All authors read, edited, and approved the final version of the manuscript.

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Competing interests

The authors declare no competing interests.

Data availability

All data generated or analyzed during this study are included in this published article.

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Tables

Table 1. Characteristics of the study group and the control groups. *MS* multiple sclerosis, *EDSS* Expanded Disability Status Scale.

Characteristics		Study group (CRYO-MS)	Control group 1 (CONTROL-MS)	Control group 2 (CONTROL-CRYO)
Age [years]		41.53 ± 6.98	40.45 ± 4.77	38.47 ± 6.00
Body height [cm]		165.93 ± 6.53	167.25 ± 5.85	169.4 ± 5.79
Body weight [kg]		66.75 ± 16.78	68.78 ± 16.54	72.35 ± 13.85
Body mass index [kg/m^2]		24.18 ± 5.68	24.60 ± 6.04	25.22 ± 4.81
Fat [%]		33.26 ± 7.45	34.29 ± 8.23	30.47 ± 6.65
Fat [kg]		23.31 ± 11.40	24.81 ± 11.47	22.82 ± 8.99
Fat-free mass [kg]		43.45 ± 5.68	43.98 ± 5.44	49.55 ± 5.90
Total body water [kg]		31.83 ± 4.21	32.23 ± 4.02	36.28 ± 4.32
EDSS score		3.03 ± 1.67	3.08 ± 1.54	–
Disease duration [years]		11.00 ± 6.49	13.10 ± 5.45	–
Place of residence [%]	City (100,000–199,000 inhabitants)	20.00	15.00	6.67
	City (200,000 inhabitants or more)	73.33	65.00	86.67
	Countryside	6.67	20.00	6.67
Education [%]	Secondary	26.67	5.00	13.33
	Higher	73.33	95.00	86.67
Employment status [%]	Currently employed	86.67	90.00	93.33
	Unemployed	13.33	10.00	6.67
Disease course [%]	Primary progressive	13.33	–	–
	Relapsing-remitting	86.67	100.00	–
Occurrence of relapses [%]	Several times a year	6.67	5.00	–
	Once a year	20.00	25.00	–
	Every few years	60.00	65.00	–
	No relapse, MS progresses	13.33	5.00	–
Occurring disorders [%]	Spasticity	40.00	40.00	–
	Tremor	6.67	10.00	–
	Excessive fatigue	80.00	90.00	–
	Blurred vision	20.00	35.00	–

	Paresthesia	46.67	30.00	–
	Balance disorders	46.67	55.00	–
	Mood disorders	53.33	35.00	–
	Bladder dysfunction	33.33	35.00	–
Pharmacological treatment [%]	Immunomodulating agents	67.67	85.00	–
	Steroid agents	33.33	20.00	–
	None	6.67	5.00	–
	Low-dose naltrexone	6.67	–	–
Psychoactive substances [%]	Coffee	60.00	75.00	67.67
	Cigarettes	6.67	–	–
	Alcohol	–	15.00	6.67
Orthopedic aids [%]	Orthopedic crutches	–	15.00	–
	Nordic walking sticks	13.33	15.00	–

Table 2. ANOVA results: baseline parameters. *MS* multiple sclerosis, *WBC* white blood cell, *RBC* red blood cell, *HGB* hemoglobin, *HCT* hematocrit, *PLT* platelet, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *EI* elongation index, *AI* aggregation index, *AMP* amplitude (total extent of aggregation), *T1/2* half time kinetics of aggregation.

Parameter	CRYO-MS N = 15	CONTROL-MS N = 20	CONTROL-CRYO N = 15	*P* (ANOVA)	*P* (CRYO-MS/ CONTROL-MS)	*P* (CRYO-MS/ CONTROL-CRYO)	*P* (CONTROL-MS/ CONTROL-CRYO)
WBC [10^9/l]	5.01 ± 1.16	4.96 ± 1.27	5.25 ± 1.40	0.786			
RBC [10^{12}/l]	4.23 ± 0.61	4.30 ± 0.67	4.80 ± 0.27	0.013	0.939	0.021	0.048
HGB [g/dl]	12.31 ± 1.07	12.50 ± 1.51	13.39 ± 0.80	0.036	0.899	0.045	0.116
HCT [%]	35.97 ± 5.65	35.90 ± 5.88	40.54 ± 2.50	0.007	0.999	0.043	0.039
PLT [10^9/l]	240.60 ± 80.97	252.70 ± 78.39	275.67 ± 35.49	0.377			
MCV [fl]	85.00 ± 5.37	83.65 ± 5.58	84.40 ± 4.03	0.738			
MCH [pg]	29.54 ± 4.03	29.42 ± 3.87	27.94 ± 1.61	0.355			
MCHC [g/dl]	34.19 ± 5.24	35.14 ± 3.37	33.05 ± 0.65	0.245			
EI 0.30	0.03 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.017	0.941	0.063	0.029
EI 0.58	0.05 ± 0.01	0.05 ± 0.01	0.15 ± 0.20	0.017	0.983	0.051	0.034
EI 1.13	0.11 ± 0.02	0.11 ± 0.02	0.15 ± 0.03	< 0.001	0.797	< 0.001	< 0.001
EI 2.19	0.20 ± 0.03	0.20 ± 0.02	0.21 ± 0.05	0.557			
EI 4.24	0.30 ± 0.03	0.30 ± 0.03	0.26 ± 0.07	0.016	0.999	0.038	0.039
EI 8.24	0.38 ± 0.04	0.38 ± 0.03	0.30 ± 0.09	< 0.001	0.996	< 0.001	< 0.001
EI 15.98	0.45 ± 0.04	0.45 ± 0.04	0.36 ± 0.09	< 0.001	0.996	< 0.001	< 0.001
EI 31.03	0.50 ± 0.04	0.50 ± 0.04	0.40 ± 0.10	< 0.001	0.999	< 0.001	< 0.001
EI 60.30	0.53 ± 0.03	0.52 ± 0.05	0.42 ± 0.09	< 0.001	0.953	< 0.001	< 0.001
AI [%]	62.25 ± 5.65	64.27 ± 9.10	58.28 ± 8.04	0.093			
AMP [a.u.]	19.89 ± 2.26	18.16 ± 4.57	21.62 ± 4.06	0.039	0.442	0.443	0.046
T1/2 [s]	2.26 ±	2.17 ± 0.75	2.81 ± 1.03	0.059			

	0.62						
Total protein [g/l]	70.17 ± 3.98	70.29 ± 2.79	73.56 ± 3.68	0.012	0.995	0.026	0.033
Albumin [g/l]	44.75 ± 2.77	44.46 ± 2.60	45.85 ± 3.49	0.373			
Alfa-1 globulins [g/l]	2.60 ± 0.38	2.61 ± 0.50	2.67 ± 0.33	0.871			
Alfa-2 globulins [g/l]	6.29 ± 0.91	6.39 ± 0.81	5.92 ± 0.57	0.212			
Beta-1 globulins [g/l]	4.03 ± 0.60	4.19 ± 0.45	4.43 ± 0.60	0.140			
Beta-2 globulins [g/l]	3.12 ± 0.61	3.21 ± 0.67	3.19 ± 0.34	0.892			
Gamma globulins [g/l]	9.41 ± 2.22	9.47 ± 2.29	11.53 ± 2.37	0.019	0.997	0.039	0.046
Albumin-globulin ratio	1.80 ± 0.29	1.76 ± 0.33	1.67 ± 0.22	0.460			
Fibrinogen [g/l]	2.78 ± 1.30	3.18 ± 1.28	2.52 ± 0.59	0.231			

Table 3. ANOVA results: post-intervention and CONTROL-MS parameters. *MS* multiple sclerosis, *WBC* white blood cell, *RBC* red blood cell, *HGB* hemoglobin, *HCT* hematocrit, *PLT* platelet, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *EI* elongation index, *AI* aggregation index, *AMP* amplitude (total extent of aggregation), *T1/2* half time kinetics of aggregation.

Parameter	CRYO-MS N = 15	CONTROL-MS N = 20	CONTROL-CRYO N = 15	*P* (ANOVA)	*P* (CRYO-MS/ CONTROL-MS)	*P* (CRYO-MS/ CONTROL-CRYO)	*P* (CONTROL-MS/ CONTROL-CRYO)
WBC [10^9/l]	4.71 ± 1.01	4.96 ± 1.27	5.31 ± 1.35	0.410			
RBC [10^{12}/l]	4.11 ± 0.51	4.30 ± 0.67	4.57 ± 0.28	0.112			
HGB [g/dl]	12.19 ± 1.20	12.50 ± 1.51	12.88 ± 0.73	0.310			
HCT [%]	34.70 ± 4.84	35.90 ± 5.88	38.35 ± 2.20	0.068			
PLT [10^9/l]	238.13 ± 79.14	252.70 ± 78.39	271.73 ± 48.65	0.437			
MCV [fl]	84.27 ± 5.39	83.65 ± 5.58	84.00 ± 4.16	0.939			
MCH [pg]	30.04 ± 4.43	29.42 ± 3.87	28.25 ± 1.60	0.377			
MCHC [g/dl]	35.58 ± 4.46	35.14 ± 3.37	33.60 ± 0.42	0.832			
EI 0.30	0.04 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.045	0.470	0.035	0.354
EI 0.58	0.06 ± 0.02	0.05 ± 0.01	0.07 ± 0.01	< 0.001	0.193	0.029	< 0.001
EI 1.13	0.11 ± 0.02	0.11 ± 0.02	0.15 ± 0.02	< 0.001	0.445	< 0.001	< 0.001
EI 2.19	0.20 ± 0.02	0.20 ± 0.02	0.24 ± 0.02	< 0.001	0.638	< 0.001	< 0.001
EI 4.24	0.30 ± 0.02	0.30 ± 0.03	0.33 ± 0.02	0.001	0.958	0.009	0.004
EI 8.24	0.38 ± 0.03	0.38 ± 0.03	0.40 ± 0.03	0.211			
EI 15.98	0.45 ± 0.03	0.45 ± 0.04	0.47 ± 0.02	0.124			
EI 31.03	0.50 ± 0.03	0.50 ± 0.04	0.52 ± 0.02	0.089			
EI 60.30	0.54 ± 0.03	0.52 ± 0.05	0.56 ± 0.02	0.017	0.482	0.171	0.012
AI [%]	60.67 ± 6.33	64.27 ± 9.10	58.96 ± 7.51	0.238			
AMP [a.u.]	21.05 ± 3.07	18.16 ± 4.57	21.91 ± 2.07	0.007	0.075	0.783	0.015
T1/2 [s]	2.50 ±	2.17 ± 0.75	2.75 ± 1.11	0.147			

	0.75						
Total protein [g/l]	69.64 ± 3.13	70.29 ± 2.79	71.73 ± 2.53	0.125			
Albumin [g/l]	44.70 ± 2.39	44.46 ± 2.60	44.19 ± 3.13	0.878			
Alfa-1 globulins [g/l]	2.53 ± 0.43	2.61 ± 0.50	2.65 ± 0.40	0.739			
Alfa-2 globulins [g/l]	6.11 ± 0.65	6.39 ± 0.81	5.98 ± 0.76	0.270			
Beta-1 globulins [g/l]	4.01 ± 0.58	4.19 ± 0.45	4.55 ± 0.67	0.032	0.646	0.028	0.189
Beta-2 globulins [g/l]	2.99 ± 0.55	3.21 ± 0.67	3.20 ± 0.35	0.447			
Gamma globulins [g/l]	9.35 ± 1.92	9.47 ± 2.29	11.19 ± 2.46	0.046	0.989	0.043	0.099
Albumin-globulin ratio	1.82 ± 0.26	1.76 ± 0.33	1.63 ± 0.25	0.170			
Fibrinogen [g/l]	4.01 ± 1.70	3.18 ± 1.28	3.38 ± 1.17	0.212			

Table 4. Assessment of therapeutic intervention in the CRYO-MS group. *MS* multiple sclerosis, *WBC* white blood cell, *RBC* red blood cell, *HGB* hemoglobin, *HCT* hematocrit, *PLT* platelet, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *EI* elongation index, *AI* aggregation index, *AMP* amplitude (total extent of aggregation), *T1/2* half time kinetics of aggregation.

Parameter	CRYO-MS N = 15		*P* (*t*-Student/Wilcoxon)
	Before	After	
WBC [10^9/l]	5.01 ± 1.16	4.71 ± 1.01	0.330
RBC [10^{12}/l]	4.23 ± 0.61	4.11 ± 0.51	0.547
HGB [g/dl]	12.31 ± 1.07	12.19 ± 1.20	0.506
HCT [%]	35.97 ± 5.65	34.70 ± 4.84	0.482
PLT [10^9/l]	240.60 ± 80.97	238.13 ± 79.14	0.649
MCV [fl]	85.00 ± 5.37	84.27 ± 5.39	0.308
MCH [pg]	29.54 ± 4.03	30.04 ± 4.43	0.532
MCHC [g/dl]	34.19 ± 5.24	35.58 ± 4.46	0.504
EI 0.30	0.03 ± 0.02	0.04 ± 0.02	0.444
EI 0.58	0.05 ± 0.01	0.06 ± 0.02	0.615
EI 1.13	0.11 ± 0.02	0.11 ± 0.02	0.677
EI 2.19	0.20 ± 0.03	0.20 ± 0.02	0.659
EI 4.24	0.30 ± 0.03	0.30 ± 0.02	0.812
EI 8.24	0.38 ± 0.04	0.38 ± 0.03	0.820
EI 15.98	0.45 ± 0.04	0.45 ± 0.03	0.670
EI 31.03	0.50 ± 0.04	0.50 ± 0.03	0.683
EI 60.30	0.53 ± 0.03	0.54 ± 0.03	0.504
AI [%]	62.25 ± 5.65	60.67 ± 6.33	0.386
AMP [a.u.]	19.89 ± 2.26	21.05 ± 3.07	0.092
T1/2 [s]	2.26 ± 0.62	2.50 ± 0.75	0.875
Total protein [g/l]	70.17 ± 3.98	69.64 ± 3.13	0.509
Albumin [g/l]	44.75 ± 2.77	44.70 ± 2.39	0.930
Alfa-1 globulins [g/l]	2.60 ± 0.38	2.53 ± 0.43	0.394
Alfa-2 globulins [g/l]	6.29 ± 0.91	6.11 ± 0.65	0.232
Beta-1 globulins [g/l]	4.03 ± 0.60	4.01 ± 0.58	0.849
Beta-2 globulins [g/l]	3.12 ± 0.61	2.99 ± 0.55	0.126
Gamma globulins [g/l]	9.41 ± 2.22	9.35 ± 1.92	0.699
Albumin-globulin ratio	1.80 ± 0.29	1.82 ± 0.26	0.500
Fibrinogen [g/l]	2.78 ± 1.30	4.01 ± 1.70	0.053

Table 5. Assessment of therapeutic intervention in the CONTROL-CRYO group. *MS* multiple sclerosis, *WBC* white blood cell, *RBC* red blood cell, *HGB* hemoglobin, *HCT* hematocrit, *PLT* platelet, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *EI* elongation index, *AI* aggregation index, *AMP* amplitude (total extent of aggregation), *T1/2* half time kinetics of aggregation.

Parameter	CONTROL-CRYO N = 15		*P* (*t*-Student/Wilcoxon)
	Before	After	
WBC [10^9/l]	5.25 ± 1.40	5.31 ± 1.35	0.859
RBC [10^{12}/l]	4.80 ± 0.27	4.57 ± 0.28	0.007
HGB [g/dl]	13.39 ± 0.80	12.88 ± 0.73	0.014
HCT [%]	40.54 ± 2.50	38.35 ± 2.20	0.003
PLT [10^9/l]	275.67 ± 35.49	271.73 ± 48.65	0.699
MCV [fl]	84.40 ± 4.03	84.00 ± 4.16	0.028
MCH [pg]	27.94 ± 1.61	28.25 ± 1.60	0.090
MCHC [g/dl]	33.05 ± 0.65	33.60 ± 0.42	0.013
EI 0.30	0.05 ± 0.02	0.02 ± 0.01	0.001
EI 0.58	0.15 ± 0.20	0.07 ± 0.01	0.161
EI 1.13	0.15 ± 0.03	0.15 ± 0.02	0.611
EI 2.19	0.21 ± 0.05	0.24 ± 0.02	0.011
EI 4.24	0.26 ± 0.07	0.33 ± 0.02	< 0.001
EI 8.24	0.30 ± 0.09	0.40 ± 0.03	< 0.001
EI 15.98	0.36 ± 0.09	0.47 ± 0.02	< 0.001
EI 31.03	0.40 ± 0.10	0.52 ± 0.02	< 0.001
EI 60.30	0.42 ± 0.09	0.56 ± 0.02	< 0.0001
AI [%]	58.28 ± 8.04	58.96 ± 7.51	0.688
AMP [a.u.]	21.62 ± 4.06	21.91 ± 2.07	0.742
T1/2 [s]	2.81 ± 1.03	2.75 ± 1.11	0.799
Total protein [g/l]	73.56 ± 3.68	71.73 ± 2.53	0.039
Albumin [g/l]	45.85 ± 3.49	44.19 ± 3.13	0.009
Alfa-1 globulins [g/l]	2.67 ± 0.33	2.65 ± 0.40	0.762
Alfa-2 globulins [g/l]	5.92 ± 0.57	5.98 ± 0.76	0.708
Beta-1 globulins [g/l]	4.43 ± 0.60	4.55 ± 0.67	0.136
Beta-2 globulins [g/l]	3.19 ± 0.34	3.20 ± 0.35	0.938
Gamma globulins [g/l]	11.53 ± 2.37	11.19 ± 2.46	0.373
Albumin-globulin ratio	1.67 ± 0.22	1.63 ± 0.25	0.254
Fibrinogen [g/l]	2.52 ± 0.59	3.38 ± 1.17	0.012

Figures

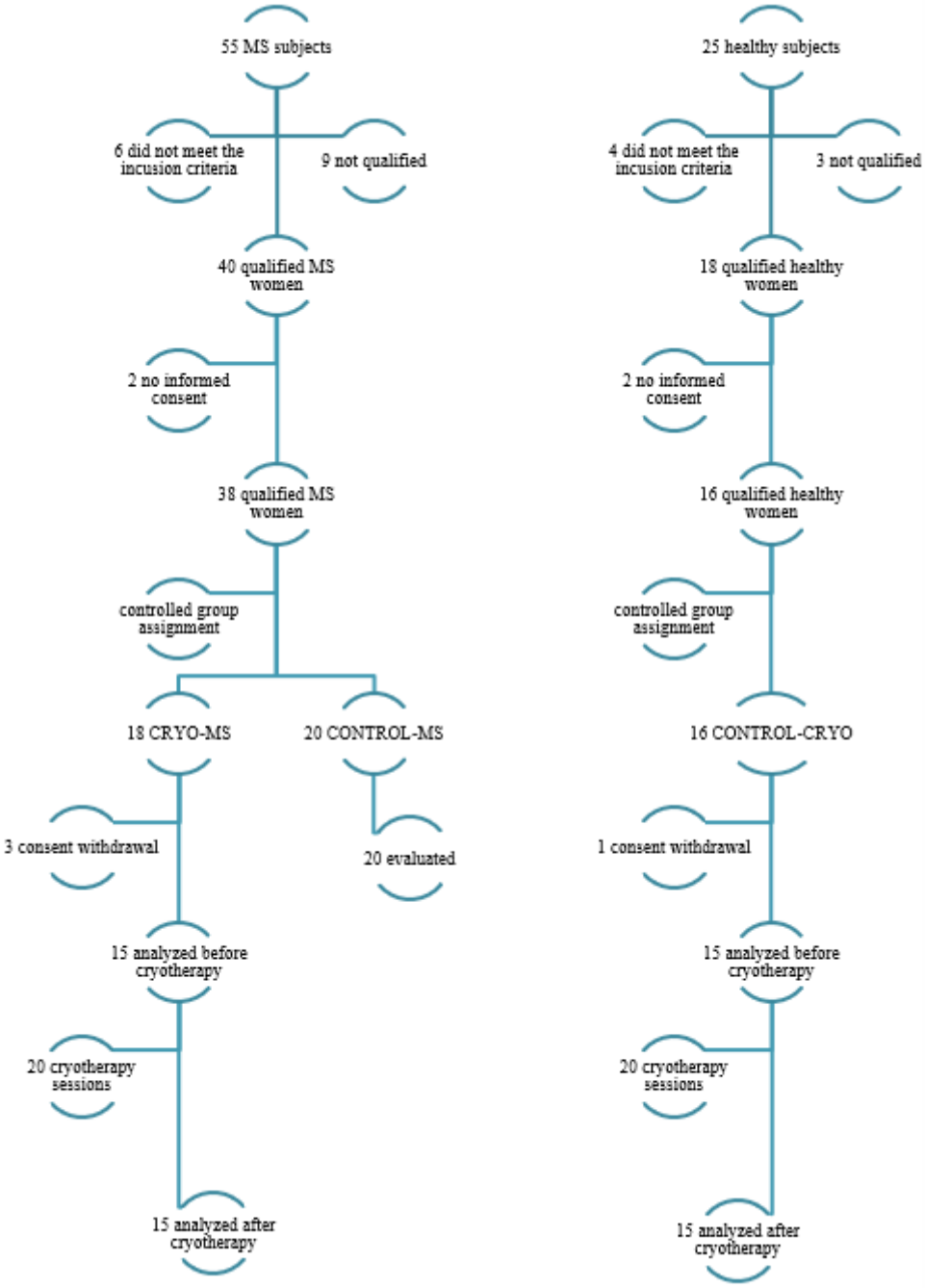

Figure 1

Qualification of the respondents. MS multiple sclerosis

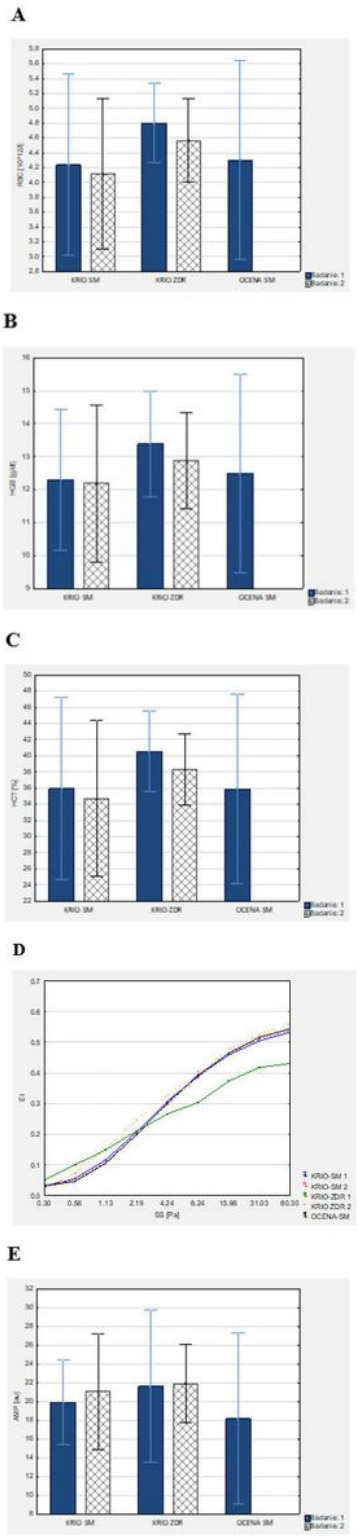

Figure 2

Morphological and rheological blood indices in the study group and the control groups (study 1 and study 2): (A) mean red blood cell (RBC) count [1012/l]; (B) mean hemoglobin (HGB) level [g/dl]; (C) mean hematocrit (HCT) level [%]; (D) mean elongation index (EI) value; (E) mean total extent of aggregation (AMP, amplitude) [a.u.] value.

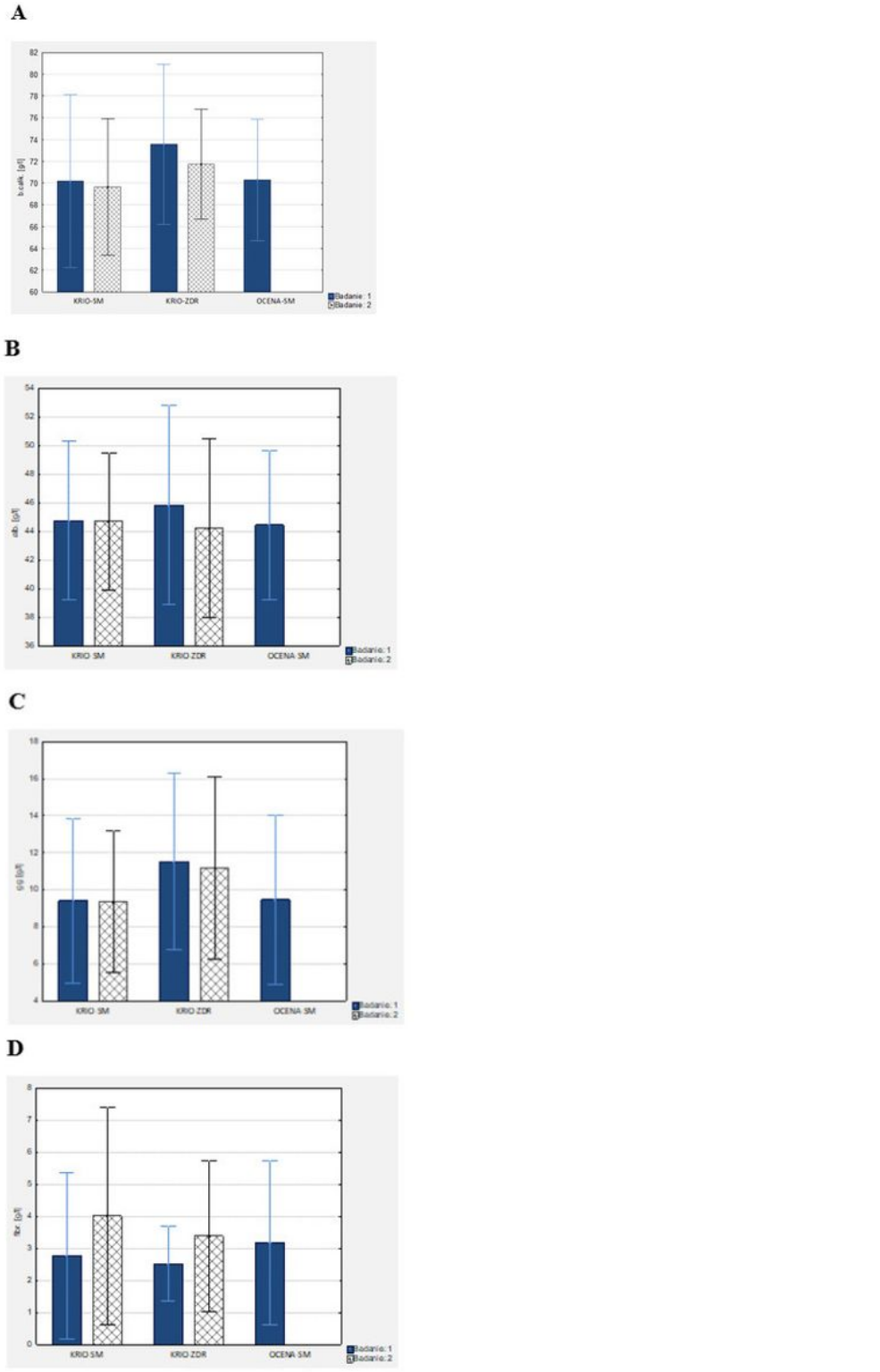

Figure 3

Rheological blood indices in the study group and the control groups (study 1 and study 2): (A) mean level of total protein [g/l]; (B) mean level of albumin [g/l]; (C) mean level of gamma globulins [g/l]; (D) mean level of fibrinogen [g/l].