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Rapid Cytoreduction by Plateletapheresis in the Treatment of Thrombocythemia

Bela Balint, Mirjana Pavlovic and Milena Todorovic

Abstract

The objective of this chapter is to provide a systematic overview of current knowledge regarding therapeutic apheresis—primarily therapeutic plateletapheresis (TP)—and to summarize evidence-based practical approaches related to cytoapheresis treatment of “hyperthrombocytosis” or “extreme thrombocytosis” (ETC). Our results of platelet (Plt) quantitative/qualitative analyses and evaluation of efficacy of apheresis systems/devices—on the basis of Plt removal and in vivo Plt depletion—will be presented. Our preclinical researches confirmed that in Plt concentrates, the initial ratio of discoid shapes was 70%, spherical 20%, and less valuable (dendritic/balloonized) shapes 10%—with morphological score of platelets (MSP = 300–400). After storage, the ratio of discoid and spherical shapes was decreased, while the less valuable ones progressively increased (MSP = 200). Electron microscopy has shown discoid shapes with typical ultrastructural properties. Spherical shapes with reduced electron density and peripheral location of granules/organelles were detected. Also, dendritic shapes with cytoskeletal “rearrangement,” membrane system integrity damages, and pseudopodia formations were documented. Our clinical study demonstrated that TP was useful in ETC treatment and should help prevention of “thrombo-hemorrhagic” events—until chemotherapy, antiplatelet drugs, and other medication take effect. During TP treatment, Plt count and morphology/ultrastructure were examined. Plt functions by multiplate analyzer were evaluated. We concluded that intensive TP was an effective, safe, and rapid cytoreductive treatment for ET.

Keywords: platelet, morphology-ultrastructure, thrombocythemia, apheresis, plateletapheresis

1. Introduction

Besides supportive treatment using whole blood, red blood cell (RBC), platelet (Plt), and plasma transfusions, hemotherapy, in an extensive viewpoint, involves also performing different apheresis procedures to remove or replace altered or overproduced and accumulated blood constituents (cell and plasma contents, pathogens, metabolic products, etc.). Hemotherapy also involves collection and ex vivo manipulation of stem cells (SCs) with a goal of reconstitution of hematopoiesis in performing conventional transplantations and for use in the fields of regenerative medicine.

Thus, the basic aim of hemotherapy is reconstitution and maintenance of a “hemobiological” (hematological, immunological, hemorheological) equilibrium or homeostasis by the recovery of lacking blood components, as well as through the use of apheresis and other “hemo-modifying” procedures. By doing this, the circulating blood volume is reestablished, but also the “blood oxiform function” (capacity for oxygen binding/transport), hemostasis, and activity of “immune response mediators” are restored. The rationalized and well-timed hemotherapy is contributing to increased survival of patients with some “life-threatening” conditions.

During supportive hemotherapy of patients with potential beneficial clinical response, it is essential to perform the most rational and “well-timed” intervention in the most appropriate manner—that is determining the most efficient and approachable therapeutic procedures and techniques. Also, it is imperative to optimize specific indications of blood component therapy with rationalized thresholds.

Also, the most sensitive point during planning and performing therapeutic apheresis (TAph), especially cytapapheresis, is determination of the most optimal approach to the treatment or, even better, the “time-frequency-intensity continuum” of the procedure. The introduction of cytapapheresis treatment requires determination of total blood cell quantity within the organism, sometimes even determination of the ratio between cells in intravascular versus extravascular space. The evaluation of hematopoietic potential and the reserve in bone marrow, as well as calculation of the intensity of cell production (“cytogenesis-kinetics”) in the peri-apheresis period, is important too.

Before evaluation of results of TP in the treatment of our patients, several principles and standards of the apheresis treatment will be presented. Thus, this chapter will treat briefly general information related to apheresis treatment [without therapeutic plasma exchange (TPE)] with specific emphasis upon cytoreductive potential or thrombocytodepletion using cytapapheresis treatment and lastly with presentation of our own results using TP procedures by comparison of the efficiency of different generations and types of blood cell separators.

While practically all details on the ultrastructure of Plts are already identified and explained, some morphological and functional properties of these blood elements will be very concisely recapitulated. Therefore, prior to presenting of our own data of Plt fundamental researches and in clinical study setting, some elementary morphological/ultrastructural and functional characteristics of Plts will be given.

2. Apheresis/cytapheresis: a systematic overview

Apheresis or hemapheresis is a “hemomodulatory” procedure in which blood components (cells or plasma) are collected from donors (the so-called apheresis donation) or specific quantitatively or qualitatively altered constituents/factors are removed from the bloodstream, while the remainder (seldom combined with normal blood cells) is returned to the patient’s circulation (named as TAph) [1–4].

The basic goal of TAph procedures is to reduce the “patient’s load” of specific substances, responsible for the development of disease, to the levels that will allow clinical improvement [4–6]. TAph treatment should be done regularly or always combined with “immunomodulatory” (e.g., immunochemotherapy) and other palliative (drug) therapies. Based upon the criterion of what is the removed blood constituent—with specific “pathogenic factors or substrate”—it is possible to classify the TAph procedures into TPE and therapeutic cytapapheresis (TC) [5–15].

TPE is a replacement of the patient’s plasma with an adequate substitution fluid (albumin in saline) or with normal (allogeneic) or modified (immunoabsorbed

autologous) plasma. The use of “selective” TPE results in reduced risk of side effects (immunomodulation or virus transmission) since “purified” autologous plasma is used as a replacement fluid [11, 12].

TC is an effective “disease-modifying” therapeutic approach in which elevated or altered cells are removed from the blood, while the residue is returned into the circulation. The basic goal of the evidence-based TC procedure is a reduction of the “patient’s load” with overproduced cell quantity or functionally altered cells, responsible for disease development [16–18]. TC procedures combined with immunotherapy and other medications result in “hemobiological” and overall clinical recovery. Thus, the main objective of TC procedures (symptomatic or prophylactic) is to reduce the patient’s excessive cellular count—ex vivo Plt removal with in vivo Plt depletion (Plt removal/depletion)—and to alleviate symptoms created by these cells [1, 2, 18].

The goal of novel TC procedures—such as photopheresis, granulocyte adsorptive apheresis, and RBC exchange (RBCX)—is the substitution of abnormal or “disease-responsible” cells, as well as ex vivo modification of “immunocompetent cells” prior to reinfusion, getting frequently long-term beneficial clinical effects. RBCX procedures are indicated for treatment of patients with irregular RBCs and consecutive hemorheological abnormality with anemia and other hemobiological disturbances [6, 7, 16].

Using standard TC procedures, the excessive cellular quantity is removed from the patient’s bloodstream with intention to reduce total blood cell count in organism and to prevent the symptoms and signs caused by various “cytemias.” According to blood cells removed, TCs can be categorized into therapeutic leukapheresis (TL), erythrocytapheresis (TE), and TP [6, 16–18].

The aim of TLs is white blood cell (WBC) count reduction and reverse of the “hyperleukocytosis-leukostasis” syndrome, regression of organomegaly and lymphadenomegaly, and improvement in general clinical status [2, 15–17]. TLs represent a useful “cytoreductive” therapy during “leukostasis crisis” when the number of circulating WBC $\geq 150 \times 10^9/L$. In the treatment of patients with excessive number ($500 \times 10^9/L$ or more) of leukemic cells, their removal by TL would have been adequate for “partial excision” of tumor mass even 1000–1500 g of “total cell mass” in removed cell suspension—that is in the apheresis product (AphP) [7, 17].

The objective of TE treatment is to obtain a hemorheological advance. Besides removal of the excess of RBCs from patient’s circulation, with TE it is possible to achieve hematological and hemorheological recovery (cellular hyperviscosity reduction) with consecutive improvement of tissue perfusion and oxygenation [2, 17].

Thrombocytapheresis or TP is useful in the treatment of patients with thrombocytopenia and symptomatic thrombocytosis until chemotherapy, antiplatelet drugs, and other medications takes effect. The purpose of TP might be determined as a method for rapid reestablishment of Plt overproduction, following prevention of “thrombo-hemorrhagic” events. Thromboembolism is more frequent than bleeding crisis, and the arterial thromboses (followed by cerebrovascular, coronary, or other occlusive episodes) happen more often than venous events [17–22].

The use of TP treatment is indicated when the Plt number $\geq 1500 \times 10^9/L$ (“hyperthrombocytosis” or “extreme thrombocytosis”), although the degree of thrombocytosis and the complexity of clinical status are not always causally associated. However, Plt quantification is undoubtedly helpful in predicting patient’s hemostatic risk [22–29]. The details of TP treatment will be presented and discussed further below.

Concisely, the goal of our original and innovative “multi-manner” TAph is to offer the possibility of rapid returning from a “life-threatening” (irreversible) emergency—altering simultaneously more than one blood constituent(s)—to the

“district” of unbalanced, but reversible clinical condition, with a possibility for recovery of patient’s hematological and overall clinical homeostasis [13–15].

It is important to highlight and emphasize that TApH is an aggressive method, by which there is a direct intervention in patient’s circulation; therefore it might be followed by side effects and complications. In order to minimize them, different criteria for selection of blood component donors and patients have to be applied. The analysis of laboratory parameters (blood cell count and biochemistry examination, screening testing for coagulation, etc.) is required too, along with clinical follow-up of the patients for which the TApH treatment is predicted.

The use of TApH procedures is not possible without (1) updated personal knowledge in hematology, immunology, biochemistry, and hemorheology; (2) rationalized education for treatment of acute “life-threatening” conditions; and (3) clinical experience related to working with extracorporeal circulation and cardiopulmonary reanimation. For that reason, TApH is applied only in medical centers with tertiary level of health protection and in countries with developed medical service.

3. Platelet morphology, ultrastructure, and functionality

Following contact with subendothelial structures at the sites of vascular injury, the “connecting” of Plts (adhesion) will occur in the presence of the von Willebrand factor (vWF). Then Plts are activated—a process which involves a number of “cell surface receptors” for agonists (ADP, thrombin, thromboxane A₂ (TXA₂), collagen, etc.), as well as specific signal transduction pathways. Activation process increases the intracellular concentration of calcium ions—by releasing from specific cytoskeleton structures and calcium influx across cell membrane. Elevated calcium concentration results in ultrastructural (morphological) and functional Plt alterations [17, 30–33].

After activation, Plts change their morphology—from discoid into spherical (seldom to dendritic) shapes—as a consequence of reorganization of the membrane-system and cytoskeleton network (“shape change reaction”). Specific granules are centralized intracellularly, and their contents are discharged into the open canalicular system and then to the outside of the cell (“releasing reaction”). The alteration in shape is the beginning of a series of reactions during Plt response to the action of agonists. Thus, discoid Plts transform into spherical or dendritic shapes, with pseudopod formation. These changes are practically prompt and do not require the presence of large amounts of extracellular calcium ions and fibrinogen. This “early” stage of Plt response is conditioned by polymerization of actin and by disconnecting of some marginal microtubule bonds. Elevated intracellular calcium concentration stimulates the phospholipase A₂ (which releases arachidonic acid from membrane phospholipids). Arachidonic acid is then metabolized lastly into TXA₂—a potent Plt activator [6, 31–37].

The “shape change reaction” allows the Plts to interact with one another (“clumping”) in the blood to form aggregates. Therefore, the phenomenon of the Plt aggregation involves sequences of “platelet-to-platelet” interactions. This process requires the presence of extracellular calcium ions, as well as fibrinogen, with very close Plt surface contact [18, 30–32].

In a few words, Plt ultrastructure consists of three structural/functional zones: “peripheral zone,” “sol-gel zone,” and “organelle zone,” as well as the “membrane-system.”

The “peripheral zone” is responsible for Plt integrity and adhesion/aggregation—mediated by receptors and ligands (agonists). This zone consists of glyco-calyx (external region), cytoskeleton or submembrane area, and cell membrane. Also, it incorporates various absorbed coagulation factors and receptors for

thrombin, ADP, collagen, vWF, thrombospondin, TXA2, prostacyclin, fibrinogen, fibronectin, etc. [18, 31–33].

The “sol-gel zone” is a viscous cytoskeleton matrix, which contains an open canalicular system or microtubules, microfilaments, some vesicles, and secretory organelles. Microtubules support the membrane contractile cytoskeleton and maintain a discoid Plt shape. In the microfilaments actin, myosin, actomyosin, and other substances were detected. Actin microfilaments in the “sol-gel zone” form actin cytoskeleton (matrix for organelles), while actomyosin is responsible for Plt contraction and may promote granule secretion [6, 32–35].

The “organelle zone” includes alpha-granules, dense granules, and lysosomal granules, glycogen particles, mitochondria, and others. Alpha-granules (50–80 per Plt) consist of vWF, fibronectin, platelet factor 4, beta-thromboglobulin, thrombospondin, platelet-derived growth factor (PDGF), and fibrinogen. The dense granules (3–8 per Plt) include adenine nucleotides, serotonin, pyrophosphate, calcium, and magnesium ions. The lysosomal granules (up to 3 per Plt) contain acid hydrolases, cathepsin D and E, lysosomal-associated membrane protein (LAMP)-2 and some lipolytic enzymes, and CD63. Finally, the mitochondria are also in the “organelle zone” and provide Plt energy requirements. They are also a deposit and an important discharger of calcium ions [17, 30–35].

The open canalicular system of “membrane-system” can be used for transport of some plasma components (e.g., fibrinogen) to alpha-granules and for release of granular contents following Plt activation. The dense tubular system operates as a depot of calcium ions, and it is active during Plt contractions. Prostaglandin synthesis is discovered in these systems also after Plt activation [6, 30–32].

Generally, investigation of Plt functions is important for diagnosis of pathological conditions with qualitative Plt disorders—e.g., hemorrhage tendency or risk of interventional bleeding, especially in patients treated by antiplatelet drugs—also for determination of “storage lesions” (liquid-state conserved or cryopreserved cells), as well as for the establishment of the “critical threshold” for Plt supportive therapy [31–38].

Testing by Multiplate analyzer—a rapid assay of whole-blood impedance aggregometry—can be used to detect Plt dysfunction and for prediction of bleedings and/or some thrombotic events, also to resolve a personalized antiplatelet therapy (including monitoring of the treatment efficacy too), as well as to reduce of Plt transfusion needs. However, this testing does not give information concerning the Plt “shape change reaction” and the reversibility of aggregation and consequently cannot be used for diagnosis of some specific Plt function disorders [18]. The results of our own Plt functional examinations will be shown afterward.

In our initial cryobiological research studies [34–38], cells were investigated in the “buffy coat”-derived Plt concentrates (BC-PCs) intended for prophylactic or therapeutic transfusions. Recently, in our clinical study [18], Plts were evaluated before and following TP treatment in the peripheral blood of ET patients, as well as in the removed AphP.

In these studies, cells were quantified in the donors’ blood samples and also in BC-PC units, as well as in the peripheral blood of patient before vs. after TP procedures and in the AphP using flow cytometry by Advia 2120 counter (Bayer, Germany). Morphological properties of Plts were examined by phase-contrast microscope (Polyvar, Reichert-Jung, Austria). The value of morphological score of platelets (MSP) was determined according to the percentage of different platelet shapes. Numerical valuation used for Plt shapes is as follows: ballooned = 0; dendritic = 1; spherical = 2; and discoid = 4. Ultrastructure of platelets was examined with electron microscope (Philips 201 C, Philips, The Netherlands). The Plt function by Multiplate analyzer (Dynabyte GmbH, Germany) was evaluated. Surface

antigens were analyzed by flow cytometer Epics XL (Coulter, USA) using specific monoclonal antibodies [34–38].

Our preclinical studies [34–38] verified that in BC-PC units immediately after preparation (“unmanipulated” or unfrozen cells), the ratio of discoid shapes was about 60–80%, spherical approximately 15–25%, and functionally less valuable (dendritic or ballooned) shapes around 5–5%; then the value of MSP was between 300 and 400. During storage, the incidence of discoid and spherical shapes progressively decreased, while the less valuable ones increased. As a result, values of the MSP also reduced gradually. In BC-PC units stored in liquid state for up to 5 days, the MSP was approximately 200 [36].

The objective of our early cryobiological researches [34–42] was to determine an optimized cryopreservation protocol with minimized “thermal damages” (cryo-injury) of frozen/thawed cells—retaining maximum quantitative (cell count) and qualitative (cell functionality, morphology, and ultrastructure) recovery. Different cryopreservation protocols with controlled-rate (“microprocessor-controlled”) or uncontrolled-rate (“dump-freezing” with no programmed cooling rate) freezing procedures, in combination with dimethyl sulfoxide (DMSO; final concentrations = 5, 6, and 10%), were compared [34–38].

Preclinical researches demonstrated that the frequency of discoid shapes in all Plt cryopreservation settings was lower (range: 39–58%) than in “unmanipulated”

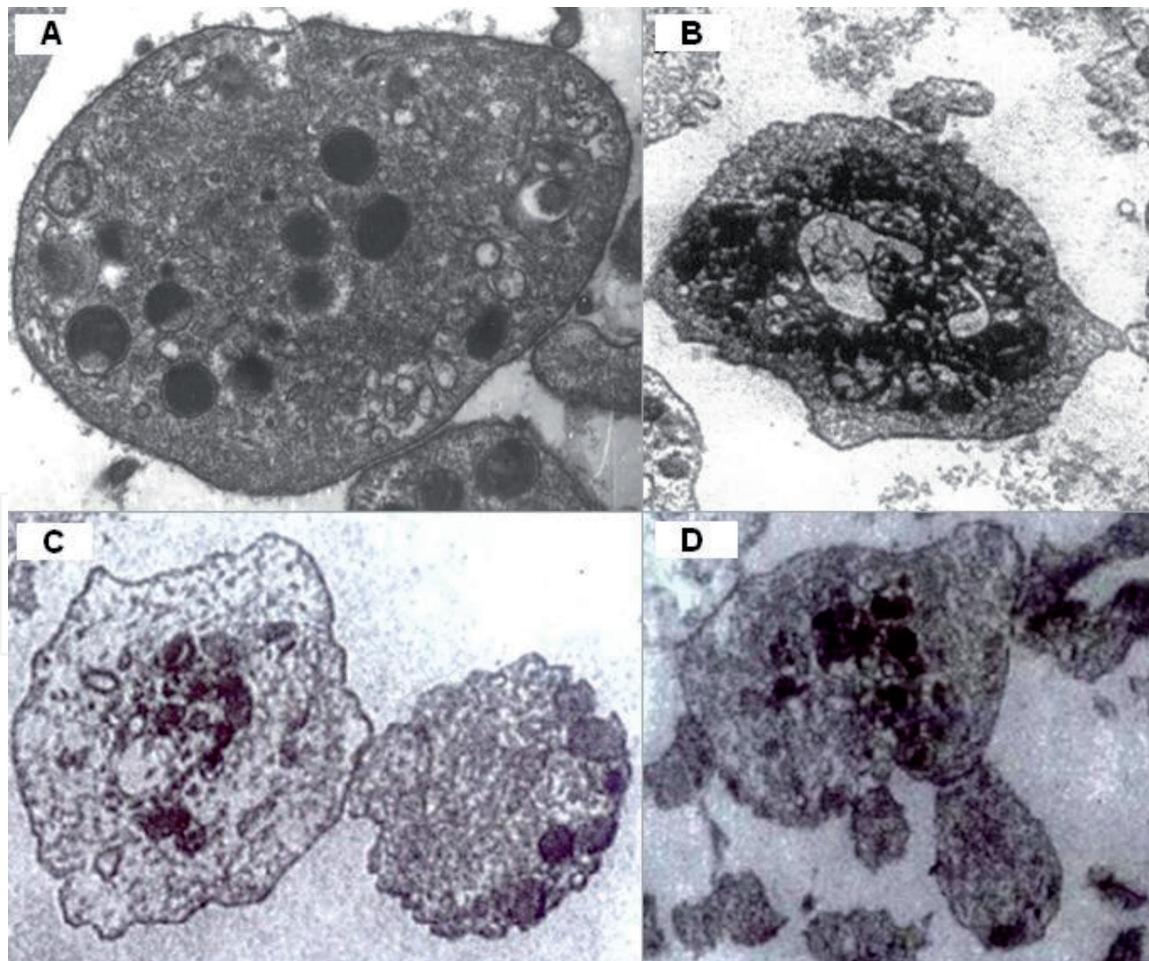


Figure 1.

Ultrastructure of Plts in cryobiological setting (our original data). Several discoid shapes had an unchanged “ultrastructural arrangement,” with minor membrane injuries (A). Spherical shapes showed an inferior “electron density” of the cytoskeleton, somewhat inferior cell structure compared to discoid shapes—which was manifested by marginal location of alpha-granules, dense granules, lysosomal granules, and other organelles in the cytoskeleton (B and C). Several dendritic shapes with reduced quantity of alpha granules, numerous pseudopodia, and unclear cell edges were also confirmed (D). Sporadic ballooned shapes with critical membrane damages and multiple ruptures, as well as fragments of the destroyed Plts, were also detected.

(unfrozen) BC-PC group [34]. It is also confirmed that controlled-rate freezing provides higher percentage of discoid and spherical shapes—with resultant superior MSP value. In the opposite, a higher proportion of functionally less valuable shapes (dendritic or ballooned) was observed in uncontrolled-rate cryopreservation setting [35].

Electron microscopy in our Plt cryopreservation researches [35–38] had shown the occurrence of different Plt types—mainly discoid and spherical, as well as sporadic dendritic or ballooned shapes (as presented in **Figure 1**).

Finally, in our recent clinical study [18], the “shape ratio” and ultrastructure of Plts in peripheral blood of ET patients with ETC (treated by TP) were also investigated (Plt shapes are visualized in **Figure 2**).

A reduction of Plt function in patients with some disorders, also during liquid-state storage or following cryopreservation, is usually caused by stated ultrastructure “rearrangement” or “reorganization.” The Plt membrane-system damage or destruction—with increased membrane permeability—can happen during the “phase change of the lipid.” Finally, the “disarrangement” of receptors on the Plt membrane results in interruption of signal transduction pathways during activation process.

Our clinical study [18] also confirmed that the baseline aggregability of Plts in the patient’s venous blood sample was 918 AU*min (normal = 923–1509 AU × min) by TRAP test (AU = aggregation unit; 10 AU × min is equal to one AUC [area under the curve] value; TRAP = thrombin receptor activating peptide). The results obtained indicated a merely Plt dysfunction (decreased activity) in the bloodstream of ET patient with ETC.

In our earlier research studies [34, 36–38] of “unmanipulated” and cryopreserved BC-PCs, various surface antigens were analyzed by flow cytometry using

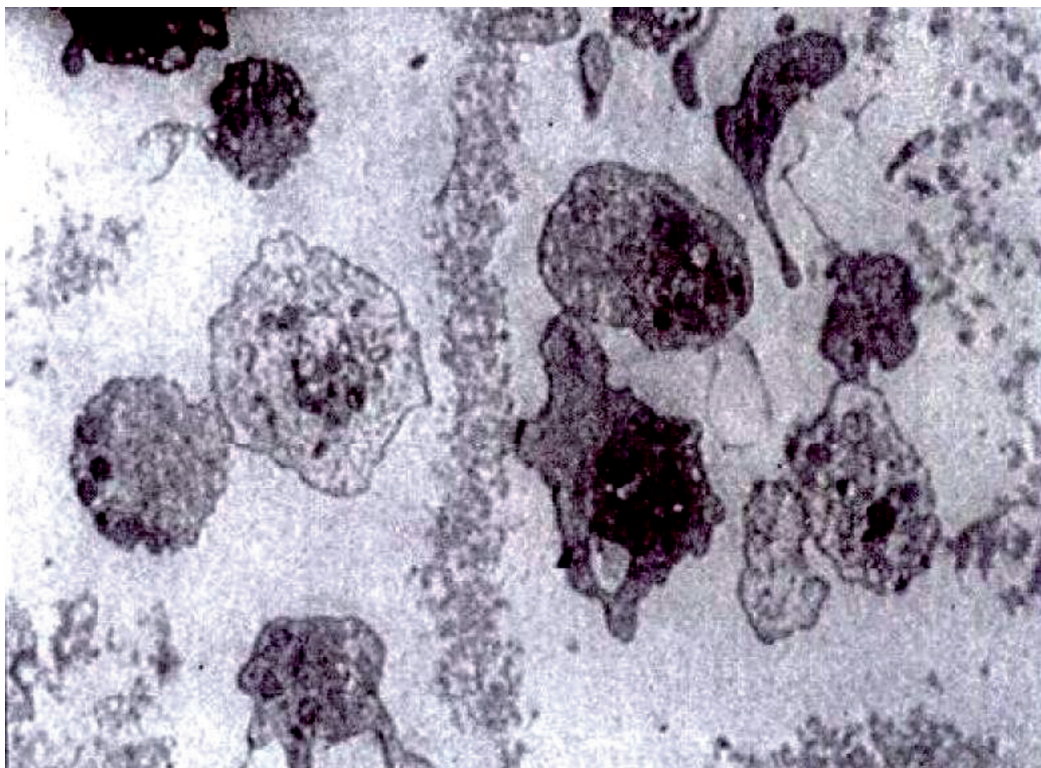


Figure 2.

Electron microscopy of Plt shapes in ET patient bloodstream (our own data). Discoid shapes had typical ultrastructural properties with intact microtubules and open canalicular system, without damage of the membrane-system integrity. Spherical shapes by peripheral location of granules/organelles in the cytoskeleton were also recognized (with resulting Plt dysfunction). A small number of dendritic shapes with cytoskeletal “rearrangement,” membrane-system damages and pseudopod formations were also recognized.

monoclonal antibodies (MoAb) anti-GPIX/CD42a, anti-GPIIb-IIIa/CD41, anti-GPIb α /CD42b, anti-GP140/P-selectin/CD62p, anti-GP53/CD63, and anti-GPIV/CD36. Plts were incubated with fluorescein isothiocyanate or phycoerythrin-conjugated MoAb and then tested by flow cytometer.

Plts were evaluated on the basis of their characteristic cell size (“linear forward scatter”) and granularity (“log side scatter”) too [34]. Anionic phospholipid expression was tested using annexin V binding assay by flow cytometer. Cell viability was assessed by the hypotonic shock response test [34–36]. In the supernatant of BC-PCs, the levels of transforming growth factor β (TGF- β), soluble P-selectin, soluble annexin, platelet factor 4 (PF4), and β -thromboglobulin (β -TG) were tested by enzyme-linked immunosorbent assays [34, 36].

These studies verified that the GPIb/CD42b expression was reduced in all cryopreserved BC-PC groups in opposition to “unmanipulated” or unfrozen (control) group [36, 37]. The expression of GP140/CD62p was in all cryopreservation groups higher than in control [36]. It was concluded that evaluation of expression of activation markers on Plt surface and flow cytometric analysis of Plt subpopulations could be a helpful approach for the quality control of liquid-stored or cryopreserved BC-PCs [34].

4. Thrombocytopenia and therapeutic thrombocytapheresis

Before evaluation of cytapheresis cyto-reductive efficacy in our studies—using different apheresis systems in critical “clinicopathological” conditions with ETC, in which apheresis is beneficial, required, or sometimes might result in rescue of patient with acute “life-threatening” hazardous situations—common thrombocytopenia-related data will be summarized.

4.1 Symptomatic thrombocytosis and thrombocytopenia

The “critical threshold” for clinical significance of increased Plt count is variable from patient to patient—but, usually, conditions with Plt $\geq 450 \times 10^9/L$ is designated as thrombocytosis. If thrombocytosis—typically with Plts $\geq 1500 \times 10^9/L$ —appears within different myeloproliferative disorders, regularly we would talk about essential thrombocytopenia (ET) [43–47].

Therefore, conditions with thrombocytosis and/or ETC can be classified into (1) primary disorder or ET, which is the consequence of clonal disease of the pluripotent hematopoietic stem cell, and (2) reactive or secondary thrombocytoses, which might evolve within patients with malignancies, chronic inflammatory processes, after splenectomy, etc. The existence of ETC induces an acquired “thrombo-hemorrhagic” diathesis, occasionally with potentially fatal vascular consequences [23–29, 43–50].

The entity and nature of ET is like polycythemia vera (PV), primary myelofibrosis (PMF), and myeloproliferative neoplasm (MPN) [46]. Due to the disease course, some patients with ET or PV might progress into a PMF-like post-ET or post-PV myelofibrosis [47]. Namely, all three MPN share three commonly exclusive “driver” mutations: JAK2, CALR, and MPL3. The most frequent is JAK2V617F, which originates in approximately 99% of patients with PV, 55% with ET, and 65% with PMF [48]. Concerning other driver mutations, 15–30% patients are CALR mutated, and 4–8% are MPL mutated, while 10–20% of the patients might not express any one of the three mutations, so they can be triple-negative [49].

Hemostatic mechanisms, by which thrombocytosis lead to “thrombo-hemorrhagic” events, are not completely explained. Different hemostatic defects and

cell abnormalities are described—such as altered Plt aggregability, intracellular accumulation of specific active substances, decreased activity of cofactors von Willebrand ristocetin and multimers of vWF with high molecular mass, etc. Diagnosis of thrombocythemia is established on the basis of the existence of the following criteria of the World Health Organization (WHO): (1) persistent Plt count $\geq 450 \times 10^9/L$ in the blood; (2) the presence of one of the three mentioned driver mutations or in their absence the exclusion of other causes of thrombocytosis (reactive and clonal); and (3) bone marrow morphologic evaluation, especially for discriminating ET from pre-fibrotic PMF and “masked” PV [50].

In ET or with difficult cases of secondary or reactive thrombocytosis (some carcinomas or asplenia), characteristic manifestations are headaches, vertigo, transitory visual disturbances, modest or intense chest or abdomen pain, as well as acrocyanosis, paresthesia, and other disturbances (e.g., priapism). In these patients, it is common manifestation of “thrombo-hemorrhagic” syndrome—thrombosis, with successive hemorrhages (epistaxis and other minor bleedings), as well as increased occurrence of first trimester miscarriage. However, in thrombocytosis or thrombocythemia, it is not undoubtedly proven that there is a correlation between the degree of increased Plt count and complexity of patients’ clinical condition.

In therapy of ET patients, the main reason for treatment is to prevent “thrombo-hemorrhagic” episodes, and from this point of view, none of the most recent drugs have been shown to be superior among the traditional drugs, such as hydroxyurea. Three major risk factors for thrombosis are history of thrombotic events, JAK2/MPL mutations, and advanced age. In order to classify of ET patients—according to risk factors—there are four categories: “very-low-risk” (absence of all risk factors); “low-risk” (presence of JAK2/MPL mutations); “intermediate-risk” (presence of advanced age ≥ 60 years); and “high-risk” (presence of thrombotic events or occurrence of both JAK2/MPL mutations and advanced age) [48].

Patients with “very low risk” can be only observed or can take once-daily aspirin, while patients with “low-risk” disease take once- or twice-daily aspirin. In intermediate-risk setting, hydroxyurea and once-daily aspirin is therapy of choice, while in “high-risk” disease with arterial thrombosis, hydroxyurea and twice-daily aspirin is standard therapy. High-risk patients with venous thrombosis take hydroxyurea plus systemic anticoagulation, and when the JAK2/MPL mutation or cardiovascular risk factors are present, addition of aspirin is needed. Thus, therapy of these disorders includes the application of chemotherapy, antiaggregation, and other medications, as well as the use of TP procedures, when rapid reduction in Plt count is urgently needed (apheresis Plt depletion). Hydroxyurea is considered the drug of the first line for “medicamentous cytoreductive therapy.” Second-line therapeutic drugs are pegylated interferon- α (IFN- α), busulfan, anagrelide, and pipobroman [48].

4.2 Therapeutic thrombocytapheresis

Generally, in ET patients by ETC (Plt $\geq 1500 \times 10^9/L$) and with altered Plt morphology/aggregability (Plt dysfunction) and immature reticulated Plts, the risk of “thrombo-hemorrhagic” event incidence is enlarged up to 50–60% [18, 22].

In the treatment of symptomatic ET—when low-dose aspirin or other antiplatelet and high-dose chemotherapies are without adequate response or contraindicated, as in pregnancy—cytoreduction by TP is beneficial or even essential [1, 2, 51]. The evidence-based clinical guideline for therapy of asymptomatic ET (e.g., exact cytapheresis threshold, initial and target Plt count, etc.) is not yet established. The treatment of ET in pregnancy is still mainly individualized [51]. The first TC procedure in our apheresis center was performed in 1971 for treatment of pregnant women with “hyperleukocytosis-leukostasis” syndrome [17].

Generally, the most critical step in TC therapy of patients with different types of “cythemia” (blood cell overproduction) is to define the optimal timing, frequency, as well as the intensity and duration of treatment. The use of TC procedures requires determination of excessive cell category and total cell quantity and to resolve also the predominant cell divisions (including intravascular vs. extravascular portions), as well as to establish and compensate for “peri-apheresis” intensity of novel blood cell production [15–17].

The goal of TP (in combination with described medication) in therapy of ET patients with clinical symptoms is to minimize of consequences of ETC. TC procedures performed using various devices (blood cell separators) operate in a wholly automatic manner—thus allowing “intra-apheresis” cell recruitment or refreshment and also minimizing the manual or individual (physician) variations and differences during treatment [16–18].

Therefore, TP procedures are used to obtain an effective and rapid cytoreduction in patients with ETC, in order to prevent or reduce “thrombo-hemorrhagic” events. The application of TP could be a life-saving procedure for selected ET patients with “life-threatening” clinical situation—due to acute episode of ETC. This treatment results in a dramatic Plt count fall and following clinical improvement [2, 22, 43–45]. As well, TC procedures are helpful in the treatment of patients with symptomatic reactive or secondary thrombocytosis (all together with correction of the cause of ETC).

However, the definitive decision to apply of TP treatment should be “individualized” on the basis of clinical scenario and intensity of ETC, as well as patients’ risk profile [29]. In this context, the efficacy of TP is dependent on the initial Plt count, kinetics of cell production, and the volume of whole blood processed, but also on the efficacy of different apheresis devices.

In our clinical settings [8, 16–18], typically antecubital (seldom subclavian or jugular) veins were used for vascular access. During TP procedures patients were anticoagulated by acid-citrate-dextrose formula B solution (ACD-B; USP; with 1.8% citrate concentration) or ACD-A solution (ACD-A; USP; with 2.2% citrate concentration). A systemic heparinization was performed only in singular TP procedures. All patients tolerated intensive TP treatments well without any adverse effects.

The aim of our latest clinical study [18] was to evaluate the cytoreductive potential of the Spectra Optia/IDL System, based upon *ex vivo* Plt removal and *in vivo* Plt depletion (Plt removal/depletion) efficacy in the treatment of a 68-year-old female patient suffering from symptomatic ET (with headaches, vertigo, visual disturbances, and paresthesia). Modifications of manufacturer’s original apheresis protocol included the collection preference and inlet flow correction (altered collection speed), as well as an increase of the “target cell suspension” volume [18]. Plt removal/depletion efficacy obtained in this study was compared to our earlier results (historical database) [8, 17] and the recent literature data [23–25] for different devices.

To the best of our knowledge, our clinical study [18] was only the second published clinical evaluation of the efficacy and safety of TP treatment using Spectra Optia/IDL System.

In earlier studies [8, 17], using apheresis devices of the first and the second generations (Haemonetics M-30, IBM 2997, and Cobe Spectra), TP procedures were performed typically every second day (rarely every day)—combined with standard immunochemotherapy and other medications. Namely, in the treatment of our comparable ET patients ($n = 20$; procedures = 126; historical database), applying mentioned apheresis devices, by one single TP procedure, it was possible to remove approximately 3×10^{12} Plt/L in cell suspension approximately

800–1300 mL or total $7\text{--}10 \times 10^{12}$ Plts by one “whole therapeutic cure” (typically 5 single TPs; range = 3–11).

Opposite, in the treatment of recent ET patient with ETC-associated clinical emergency—using apheresis device of the newest generation and an intensive single TP treatment—the quantity of removed Plts was 7.5×10^{12} in the cell suspension (volume = 1150 mL) [18]. As replacement fluid, albumin in saline was used. There were no side effects due to intensive TP. Consequently, by Spectra Optia/IDL System using one TP, it was possible to achieve similar therapeutic effect to that one which was realized after the application of “whole therapeutic cure” with devices of earlier generations.

In Cobe Spectra group (historical database) of our patients [17], the *in vivo* Plt depletion was approximately threefold lower. Only the use of “whole therapeutic cure” resulted in a satisfactory *in vivo* Plt fall (mean = $68 \pm 14\%$; range = 55–85%). Recent literature data [28] are comparable—precisely, a 67% decrease in circulating Plt count ($1553 \times 10^9\text{--}513 \times 10^9/\text{L}$) was reported, but after two TP procedures. Contrary to our recent study [18], a significant Plt count depletion (from 2330×10^9 to $633 \times 10^9/\text{L}$) and excellent *in vivo* Plt fall (72.8%) were realized by one single TP procedure—followed by clinical advances and prevention of potential “thrombo-hemorrhagic” events (e.g., cerebrovascular accident—stroke).

The *in vivo* Plt depletion intensity in our current study (72.8%) was also superior among the most recent literature data [23–25]. Exactly, using a single TP by different apheresis devices, such as Haemonetics-MCS+ (therapy of low-body-mass child; Plts = $3072 \times 10^9/\text{L}$) [52], CS-3000 Plus and Cobe Spectra (management of hemato-oncological patients) [25], as well as Spectra Optia apheresis system (treatment of ET patient; manufacturer’s protocol used) [24], the post-apheresis reductions of *in vivo* Plt depletion averaged only 37, 38, and 56%, respectively.

5. Conclusion

The purpose of TAph treatment is to reduce the “patient’s load” of specific substances, responsible for the development/progress of disease, to the levels that will allow clinical recovery. Using TC procedures, the excessive cellular quantity is removed from the patient’s bloodstream with the intention to reduce total blood cell count in the body and to prevent the symptoms caused by various “cytemias.” TAph is an aggressive method; therefore it might be followed by side effects and complications. Thus, the use of TAph procedures is not possible without personal education for treatment of acute “life-threatening” conditions, as well as clinical experience related to working with extracorporeal circulation and cardiopulmonary reanimation.

After activation, Plts change their morphology—from discoid into spherical (seldom to dendritic) shapes—as a consequence of reorganization of the membrane-system and cytoskeleton network (“shape change reaction”). Specific granules are centralized intracellularly, and their contents are discharged into the open canalicular system and then to the outside of the cell (“releasing reaction”). The alteration in shape represents the beginning of a series of reactions during Plt response to the action of agonists. Investigation of Plt functions is important for diagnosis of pathological conditions with qualitative Plt disorders—e.g., hemorrhage tendency or risk of interventional bleeding, especially in patients treated by antiplatelet drugs—and also for determination of “storage lesions” (liquid-state conserved or cryopreserved cells), as well as for the establishment of the “critical threshold” for Plt supportive therapy. The evaluation of membrane antigens and

flow cytometric analysis of Plt subpopulations could be helpful for the quality control of stored BC-PCs.

Based on the literature data and our own results, it is possible to conclude that intensive TP is an effective and safe procedure—even in long-term application (repeated or intermittent procedures) over a period of a few weeks/months—for patients with ET or secondary thrombocytoses and “life-threatening” ETC.

The application of a single TP procedure in the treatment of recent ET patient by Spectra Optia/IDL System resulted in undoubtedly superior Plt removal/depletion efficacy (for both normal and altered cells) when compared to our earlier study (Cobe Spectra) and literature data for CS-3000 Plus or Cobe Spectra, even for the Spectra Optia (when the manufacturer’s original protocol was used). Although TPs represent an effective cytoreductive therapy with high level of Plt removal/depletion potential, it cannot influence clinical remission.

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