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PRT Mirasol System — validation experience in Poland

Walidacja systemu Mirasol w Polsce

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Blood and blood components have never been as safe as they are now because much progress has been made to improve their safety through: better donor selection, implementation of more sensitive viral tests (both serological and molecular biology), quarantine of plasma, closed-system preparation, and the establishment of quality assurance (QA) in BTS [1, 2].

However, despite all these preventive measures, blood components are still not safe enough; we need to consider emerging and re-emerging pathogens as well as pathogens for which there are no tests or for which the tests are not in routine use. It has been confirmed that factors such as migration and climatic changes (particularly caused by the green-house effect) may also contribute to higher pathogen incidence rate [3]. Moreover, statistical data for post-transfusion complications show a high risk of bacterial contamination for PCs stored at room temperature, which favours bacteria proliferation. PC transmitted bacteria-related complications have been estimated at 1:1000-2000 transfusions [4]. Consequently, there seem to be several reasons for PRT implementation: protection against emerging pathogens for which no testing is currently available, reduction of the transmission risk of currently tested pathogens, and minimizing bacterial- and some transfusion-related adverse reactions. Inactivation methods are mostly based on photosensitive agents with a high affinity to nucleic acids as well as microorganism surface structures, and they are used to reduce cell proliferation [5].

Blood Transfusion Service (BTS) in Poland is an integral part of the public Polish health service. More than one million units of blood and plasma are collected every year from voluntary, non remunerated donors, i.e. statistically over 26 donations per 1000 inhabitants in a country inhabited by 38.2 million people. The Polish blood transfusion centres are currently equipped with 27 illuminators (Mirasol® PRT; Pathogen Reduction Technology) for routine pathogen inactivation in plasma and some of them (e.g. the Regional Blood Transfusion Centre in Warsaw) have implemented pathogen inactivation also for PCs.

Pathogen inactivation procedures for both plasma and PCs were validated and evaluated at the Institute of Hematology and Transfusion Medicine (IHTM). Many methods were tested but the best experience has been with the Mirasol® PRT System. The Mirasol® PRT (Pathogen Reduction Technology) System is a new method for pathogen inactivation in plasma and platelet concentrates. It offers a nucleic acid-targeted pathogen reduction technique based on UV light and riboflavin. Riboflavin molecules form complexes with the pathogen nucleic acid. Exposure to UV light activates riboflavin to cause a chemical alteration to functional groups of the nucleic acid (primary guanine bases) rendering the pathogen unable to replicate. Riboflavin is most effective for lipid-enveloped viruses. Reductions were also reported for the West Nile virus and the non-enveloped parvovirus B19 as well as for some bacteria and protozoa. Multicentre trials by Cardo et al. have shown that the riboflavin-method is effective for killing *Leishmania* and other emerging plasma and platelet pathogens (5–7 log10 reduction) [6, 7]. Numerous studies have also confirmed this method to be effective for white blood cell inactivation [8, 9]. Clinical trials on Mirasol®

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PRT System effectiveness have proven that neoantigen formation is not a critical side effect of this system [10]. The aim of the validation study was to: 1. evaluate the effect of the Mirasol®PRT System on the functions of PCs during 5 days of storage, 2. compare the quality of plasma inactivated before freezing to that of plasma inactivated after thawing, and 3. determine if the Mirasol®PRT System can be used as an alternative to irradiation of blood components.

Evaluation of the effect of the Mirasol® PRT System on the functions of PCs during 5 days of storage

The purpose of the first study was to evaluate the effect of PRT treatment with riboflavin and UV light (Mirasol® PRT System) on biochemical and functional characteristics of PCs obtained from buffy coats [11]. The study comprised 15 PCs (control group) and 15 PCs inactivated with the Mirasol® PRT System. PCs from 10 (ABO identical) buffy coats were suspended in 2 plasma units, divided into 2 equal-weight parts, and placed in bags. After adding 35 ml of riboflavin solution, the PCs were illuminated in the Mirasol® PRT System; in the control PC we added saline solution to the same volume. Both types of PCs were stored at 22°C with gentle agitation. Samples for analysis were collected on days 1, 3, and 5. We measured: glucose and beta thromboglobulin levels, pH, pCO_2, pO_2, hypotonic shock response (HSR), MPV, platelet and leukocyte counts, and CD62 and CD42b antigen expression and aggregation. Results and conclusions: no significant differences in HSR (%) or CD42 b expression were observed between riboflavin-treated and control PC groups. The pH values for all PCs were stable during the whole storage period (7.1 – 7.5). On storage day 1 the CD62 expression in control PCs was significantly higher (10.8%±10.0%) as compared to riboflavin-treated PCs (7.4%±6.3%). On all storage days a significantly higher glucose consumption was noted in the inactivated PCs. On day 5, a 2-3-fold increase of beta thromboglobulin concentration was observed in both riboflavin-treated and control PCs as compared to day 1; beta thromboglobulin concentration was 32% higher in the riboflavin-treated PCs as compared to control PCs on day 5. On all storage days, the pCO_2 value was comparable for both PC groups; lower pO_2 values were reported for inactivated PCs. In both PC groups the *in vitro* values of pH, HSR, aggregation, CD42b antigen expression, MPV, and platelet count were comparable. The quality of Mirasol® PRT-inactivated PCs is comparable to that of the control group.

Comparison of the quality of plasma inactivated before freezing to that of plasma inactivated after thawing

In the second part of our study we evaluated the effect of pathogen reduction technology with riboflavin and UV light (Mirasol® PRT System) on the quality of plasma inactivated immediately prior to freezing and after thawing. The study comprised 15 plasma units (control) and 15 plasma units inactivated with the Mirasol® PRT System immediately prior to freezing and 15 plasma units inactivated after thawing. A bag with plasma and saline solution added up to the volume of 35 ml was the control, the second one with 35 ml of riboflavin solution was illuminated in the Mirasol® PRT System, and the third was simultaneously frozen, stored for 1 week, then thawed, inactivated, and frozen again. After 1 month of storage, samples of the three types of plasma were analysed for: total protein, factors: VIII, IX, XIII, fibrinogen, vWF, and APTT. The results (i.e. markedly higher content of labile coagulation factors such as VIII, IX, XIII, and vWF in plasma inactivated prior to freezing) confirm that the Mirasol® PRT System must be applied before freezing.

Lymphocyte survival and activation in stored PCs after gamma-irradiation or Mirasol® PRT System treatment

The aim of this part of the study was to compare the effect of irradiation and PRT treatment (Mirasol® PRT System) on lymphocyte survival and inactivation in non-leukoreduced PCs. Pathogen reduction technology (PRT) targets nucleic acids after exposure to riboflavin and UV-light. We analysed 7 untreated PCs (control), 7 PCs treated in a Mirasol® PRT System, and 7 irradiated PCs. The PCs were prepared by pooling 15 (ABO identical) buffy coats suspended in 3 plasma units; the PC pool was then divided into 3 equal-weight units and poured in bags made of the same material. Mirasol units were illuminated after adding 35 ml of riboflavin solution. Saline solution at a volume of 35 ml was added to the control group and the irradiated units. All PCs were then stored at 22°C with agitation for 6 days. Samples for analysis were collected on days 1, 3, and 6. The lymphocyte survival rate was determined by 7AAD (7-amino-actinomycin D) staining of dead cells, and their activation by anti-CD69-APC staining antibody (Becton Dickinson, USA). Samples were also stained with anti-CD45--PE antibodies to identify and gate on lymphocytes. Samples were analysed on a Becton Dickinson

Cytometer (FACSCanto). The following results were obtained: statistically significant increase in the number of dead lymphocytes after 6 days of storage (two-fold higher in Mirasol PRT PCs than in gamma-irradiated PCs) and a decrease in lymphocyte activation during 6 days of storage (lymphocyte activation/CD69 expression), which was significantly lower in Mirasol PRT PCs than in control or irradiated PCs. Data from this study may suggest that to prevent TA-GvHD the Mirasol PRT System may be as effective as gamma irradiation.

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