Update on Leucocyte Depletion of Blood Components by Filtration

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INTRODUCTION

Leucocytes in homologous blood components are rarely of therapeutic value but may induce serious transfusion reactions such as non-haemolytic febrile transfusion reactions [NHFTR] \(^1,2\), alloimmunization against HLA \(^3\text{--}7\) and immunosuppression \(^8\). Further, they may harbour bacteria or pathogenic viruses such as Cytomegalo Virus [CMV], Human T-cell Lymphotropic Virus [HTLV types I and II], and Epstein Barr Virus [EBV] \(^8\text{--}12\). Recently B-lymphocytes have been named as vectors of prions of the new variant Creutzfeldt Jakob disease [nvCJD] \(^13\). The presence of leucocytes is associated with accelerated cellular damage during storage.\(^1\)

Therefore, leucocyte removal from blood components has become the standard to diminish the potential on towards effects and to reduce transmission or reactivation of the above viruses and cellular damage. However, one should keep in mind that different leucocytes are responsible for the various side effects. Granulocytes and granulocyte fragments are causes for NHFTR, whereas antigen presenting cells [APC] presenting both MHC classes I and II antigens appear to induce alloimmunization; granulocytes may be the vector for CMV and mononuclear cells for other viruses. Because leucocytes cannot selectively be removed from a blood component an upper limit of residual leucocytes is proposed to prevent these
side effects, a maximum of $5 \times 10^6$ and preferably $1 \times 10^6$ per blood component.

However, with the available methods such low numbers of residual leucocytes after filtration are very difficult to determine. At present the accuracy of various leucocyte counting methods is limited to 0.01–0.1 cell per $\mu l$. This means that when one leucocyte is detected with the best counting method, $<10^4$ to $<10^5$ leucocytes are present in a blood component of about 300 ml. It would be inappropriate to give more precise numbers since the error of counting will be 100%.

Better understanding of the filtration mechanisms and the properties of blood components will allow to selectively choose a filter for leuco-depletion of a specific blood component with the aim to have optimal leuco-depletion together with minimal losses of other cells than leucocytes and the least detrimental side effects due to the filtration procedure. To assure reproducible leuco-depleted blood components the leucocyte removal filters should be reliable in leucocyte-depleting capacity and performance whereas the blood components and filtration procedure should meet specifications.

In this paper filtration mechanisms and properties of blood components and filtration procedure will be reviewed.

**FILTRATION MECHANISMS**

**Materials and type of filtration**

Following cotton wool and cellulose acetate, layers of non-woven webs of polyester fibres and more recently microporous polyurethane are used as materials for leuco-depletion filters. The removal of leucocytes from cellular blood components occurs by screen or depth filtration. In screen filtration particles larger than a given size cannot pass through the pores of the filter surface, thus the particles are separated from the fluid. Depth filters in general have an open porous sponge-like structure with a wide distribution of pore sizes throughout the filter. Retention of particles happens at any place in the filter matrix. As shown by Steneker et al. removal of leucocytes from red cell concentrates (RCC) in filters with non-woven webs of small (1–2 $\mu m$) diameter fibres of melt blown polyester depend on depth filtration. Three mechanisms of entrapment could be recognized by electron microscopy:

1. *Indirect adhesion*: adhesion and spreading of activated platelets to the filter material followed by attachment of (viable) granulocytes to the platelets.
2. *Direct adhesion* of granulocytes and monocytes/macrophages, from small pseudopods to surrounding of the fibres by cytoplasmic material probably based on their phagocytic activity.
3. **Mechanical sieving**: leucocytes, mononuclear cells but also less viable granulocytes, and deformed red cells (‘echinocytes’) were caught in the pores formed by the fibres, blocked by each other when reaching a pore or intercepted in dead ends.

Usually the polyester filters consist of layers with coarse pores at the inlet of the filter, layers with middle coarse pores in between and layers with fine pores at the outlet of the filter. Granulocyte capture occurred in the first layers whereas the mononuclear cells were predominantly present in the last layers with fine pores. To enhance the filtration mechanisms physical and chemical modifications can be applied by coating or chemical treatment of the fibres which will affect surface charge, hydrophilicity (‘wettability’), chemistry and free energy and thus the filter performance. In this way a filter can be adapted for the intended use i.e. for filtration of RCC platelets may be activated to enhance indirect adhesion of leucocytes whereas for filtration of platelet concentrates (PC) the material
should not retain platelets. Such modifications may on the one hand improve leucocyte removal but on the other hand may also cause cell disintegration or activation of platelets, complement or clotting factors. Adverse effects have for example been described following transfusion of blood components through negatively charged filters. The number of the layers, the type of fibres and the time the cellular component is in contact with the fibres will influence the effectiveness of the leucocyte removal by depth filtration.

Screen filtration is obtained in the more recently developed biocompatible microporous polyurethane filters. Electron microscopically cells are caught in the pores or dead ends of the material and neither remarkable deformation indicating adhesion could be observed nor were platelets activated. It was suggested that the flexibility of the cell membranes are of high importance in this kind of filtration. The more rigid the membrane the better the filter will act as a barrier. Granulocytes and monocytes might ‘escape’ by moving through the pores but energy is necessary for this process and the temperature at filtration plays a role. A larger depth of the material can also contribute to prevent detachment. The physical and chemical properties of these polyurethane filters can be influenced by coating or the choice of the polymer ratio and/or chain lengths. The biocompatibility of the material is very promising for filtration of whole blood from which only the leucocytes should be removed and the subsequently prepared leuco-depleted plasma, red cells and platelets can be transfused.

PROPERTIES OF BLOOD PRODUCTS AND FILTRATION PROCEDURE

Composition and age of blood component

For both depth and screen filtration the composition of the blood component i.e. the number of red cells, platelets and leucocytes, and the presence of plasma or additive solution is of importance. Red cells in a capillary have a marginating effect i.e. they push the platelets and leucocytes towards the walls of the vessel. In filtration of RCC the same effect might influence the leucocyte removal. This effect will be absent in filtration of PC. Platelets play a role in indirect adhesion of granulocytes and differences in leucocyte depleting capacity of a filter may occur with RCC containing different numbers of platelets. However, when PC are leuco-depleted platelet activation by the filter fibres may lead to high losses of platelets and adverse transfusion reactions. Therefore, platelet friendly material such as polyurethane seems to be very promising for future leuco-depletion of PC or whole blood.

The presence of plasma is of influence for the coating of the fibres and therewith the hydrophilicity (‘wettability’) of the fibres which improves the contact between leucocytes and fibres, which in turn improves adhesion. If the cells are suspended in additive protein free medium the behaviour of the cells in the filter may change as well as the ‘wettability’ of the fibres and the flow rate.

The age of the blood component reflects the viability of especially the granulocytes. These cells disintegrate after 24 h and then might dispose their contents such as ingested bacteria, intracellular viruses and detrimental contents of their lysosomes and lose ability to adhere to foreign material. Granulocyte fragments may pass the filter and still induce FNHTR and contribute to secondary alloimmunization.

Effect of leuco-depletion on storage of RCC

Comparing storage of buffy coat depleted RCC in saline–adenine–glucose–mannitol (SAGM) with leuco-depleted RCC in SAGM revealed significantly
higher glucose consumption, lactate dehydrogenase release, rate of haemolysis and lower pH in the buffy coat depleted RCC\textsuperscript{37–39}. These findings are in accordance with early studies of Högman who showed that increased haemolysis was induced by leucocytes if RCC were stored in protein free additive solution\textsuperscript{40}. For storage of RCC early leuco-depletion creates better storage conditions. Another option will be to first leuco-deplete whole blood and subsequently separate it into plasma, red cells and platelets\textsuperscript{29}. However, this concept needs further study.

**Effect of leuco-depletion on storage of PC**

The literature is indecisive whether leucocytes in PC can exert negative effects on the platelets during storage\textsuperscript{27,28,41,42}. However, formation of cytokines in PC due to the presence of leucocytes and inducing NHFTR have been described\textsuperscript{43,44}. Therefore, it is recommended that if filtration is to be performed prior to storage of PC this should be done within 24–48 h of collection\textsuperscript{43,44}. As mentioned before, especially for platelets the filtration procedure should have no harmful side effects.

**Temperature of blood component at filtration**

The temperature of the blood component at filtration and during filtration is another important factor\textsuperscript{45}. The temperature of blood cells greatly affects their membrane flexibility\textsuperscript{46}. At 4°C the cells are rigid\textsuperscript{46} and will more easily be caught in pores, whereas at ambient temperature granulocytes and monocytes can better adhere to foreign surfaces\textsuperscript{47}. RCC that have been stored at 4°C easily warm up at ambient temperature, in about an hour the temperature will have increased to 10°C or higher. So to create uniform procedures a filtration intended to be performed at 4°C should be done in a refrigerated room, which is not very attractive. However, a filter can substantially differ in leucocyte depleting capacity if used at either 20°C or 4°C. Recently six different filters were investigated in our institute, five filters containing polyester layers and one filter made of polyurethane\textsuperscript{48}. To omit interdonor differences a large pool of RCC was made and divided. Some filters were tested at only 4°C, some at 20°C and some at both temperatures. All filters removed leucocytes to below 5 × 10\textsuperscript{6}. From the polyester filters tested at both temperatures the leucocyte removal was significantly better when performed at 4°C, which was in accordance with earlier reports\textsuperscript{45}. However the polyurethane filter performed equally well at 20°C: 12/12 (100%) below 1 × 10\textsuperscript{6} \textsuperscript{48}.

From a logistical point of view RCC which have been prepared within 24 h of collection, should be cooled thoroughly to 4°C if filtration is to be performed at that temperature. Normally this will take another night and therefore filtration will take place between 24 and 48 h of collection which might induce detrimental effects. If in the future whole blood is filtered this will probably be done at ambient temperature requiring filters which have a good leuco-depleting capacity at that temperature.

For PC, filtration will always be done at 20°C but the time of filtration after processing, especially for apheresis platelets is of importance. Freshly prepared platelets might be activated inducing larger platelet losses in the filter\textsuperscript{24}.

**Flow rate**

The flow rate will influence the contact time of blood component and filter. At the bedside the flow rate will be much slower than in the laboratory i.e. for RCC about 2 h versus 8–10 min. It was shown by Ledent and Berlin\textsuperscript{49,50} that a
slow flow decreases filter performance of polyester filters.

Quality monitoring standards

While leucocyte reduction to below 10° remains the essential criterion of the filtration process filters differ from each other in terms of generation and or retention of detrimental ingredients which may appear in the filtrate. Filtration may also induce changes in storage stability of the filtered cell suspension. More recently several sensitive and practical tests have been introduced which are well suited both for the screening and quantitative assay of filtration induced changes in final products. These include the use of kallikrein substrants as a marker of contact activation, dMPV/dPLT assay, quantitative index of changes in cellular integrity/aggregation/micro vesiculation, and Annexin V as an essential marker of cellular injury are now becoming the standard procedures of validation program on leucocyte filtration process in the UK.

DISCUSSION

Removal of leucocytes by filtration occurs by direct or indirect adhesion to filter fibres or by mechanical entrapment in the pores or dead ends of the filter material. The filter material can be modified to enhance these effects. The modifications should be adapted to the blood components to be filtered. The composition, age, temperature, and temperature of the blood component play a role in the adhesive capacity and deformability of the leucocytes. Flow rate and temperature at filtration will also affect the filter performance.

Increasing evidence exists that leuco-depletion before storage creates better storage conditions for RCC and will prevent adverse reactions following transfusion of PC. To prevent platelet loss in the filtration procedure biocompatible filters which do not harm the platelets are preferred for filtration of PC and whole blood if later platelets are harvested.

For the future the following recommendations should warrant reproducible and reliably leuco-depleted blood components. Filter manufacturers have the difficult task to provide the customers with filters which are designed for a specific blood component and meet the following criteria:

1. have a leucocyte removal capacity which is in accordance with the usual number of leucocytes present in the blood component;
2. remove all types of leucocytes to below 0.1 or 1 cell/μl;
3. preserve the majority of specific cells of the component i.e. red cells in RCC, platelets in PC, or red cells and platelets in whole blood;
4. do not damage blood cells or induce harmful activation of platelets, complement or clotting factors;
5. are user friendly requiring a minimum of labour.

The customers will first have to specify and standardize that composition of the blood component, then investigate filters according to the information from manufacturers and choices for filtration methods. Next a selection can be made, the standard procedures written and the method validated with the selected filter before implementing it for routine use.

Guidelines according to Good Manufacturing and Laboratory Practice and state of the art current practices need to be continuously updated to include leucocyte counting and acceptable loss/recovery of some essential active ingredients. Moreover it should embody simple and practical function tests that reflect the functional integrity and cellular damage. In this respect the assessment of kallikrein-like enzymes by
chromogenic substrate, the changes in cellular indices by automated cell counter and the release of Annexin V have proven to be useful markers of filtration-induced changes in the quality of PC.

REFERENCES


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