Pros And Cons Of Different Methods Of Leucoreduction And Its Scope Of Implementation In The Cost Constrained Settings

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ABSTRACT

Half a century ago, most of the blood transfused was whole blood. However, since the 1960s, whole blood has been separated into its various components such as RBCs, platelets and plasma. Rapidly growing size of patients in our country requiring multiple transfusion of blood and components pose a great challenge to transfusion services to provide them red cell and platelet antigen matched products in alloimmunized subjects. However, it has been shown that the removal of leucocytes from various blood products can minimize the risks[1–5] associated with these, which are: Nonhemolytic febrile transfusion reactions, Human leukocyte antigen (HLA) alloimmunization, Platelet refractoriness observed in multi-transfused patients and Transmission of leucotropic viruses such as EBV and CMV. Thus removal of leucocytes below a certain threshold, ≤ 5 × 10⁶ in a blood component certainly helps in prevention of associated risks in these patients. Currently, the best Leucoreduction can be achieved with the help of 3rd generation leucofilters, both in laboratory and patient bed side and apheresis devices. We did a 6 month comparison study of transfusing leucodepleted RCC (Red Cell Concentrate) in Thalassaemia patients prepared by both the methods of leucodepletion (manual buffy coat removal and leucofiltration) in P.D.U. Medical College and Hospital, Rajkot, Gujarat (India) and assessed the effectiveness of both in preventing NHFTRs in multiply transfused patients, especially in cost contrained settings.

Although the terms, leucoreduction and leucodepletion are used interchangeably in literature, Leucoreduction technically implies removal of leukocytes by gross removal method, whereas, Leucodepletion connotes removal of leukocytes with the help of certain specific filters or devices.

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Introduction
Half a century ago, most of the blood transfused was whole blood. However, since the 1960s, whole blood has been separated into its various components such as RBCs, platelets and plasma. Rapidly growing size of patients in our country requiring multiple transfusion of blood and components pose a great challenge to transfusion services to provide them red cell and platelet antigen matched products in alloimmunized subjects. However, it has been shown that the removal of leucocytes from various blood products can minimize the risks[1–5] associated with these, which are: Nonhemolytic febrile transfusion reactions, Human leukocyte antigen (HLA) alloimmunization, Platelet refractoriness observed in multi-transfused patients and Transmission of leucotropic viruses such as EBV and CMV. Thus removal of leucocytes below a certain threshold, ≤ 5 × 10^9 in a blood component certainly helps in prevention of associated risks in these patients. Currently, the best Leucoreduction can be achieved with the help of 3rd generation leucofilters, both in laboratory and patient bed side and apheresis devices. We did a 6 month comparison study of transfusing leucodepleted RCC (Red Cell Concentrate) in Thalassaemia patients prepared by both the methods of leucodepletion (manual buffy coat removal and leucofiltration) in P.D.U. Medical College and Hospital, Rajkot, Gujarat (India) and assessed the effectiveness of both in preventing NHFTRs in multiply transfused patients, especially in cost contrained settings.

Although the terms, leucoreduction and leucodepletion are used interchangeably in literature, Leucoreduction technically implies removal of leucocytes by gross removal method, whereas, Leucodepletion connotes removal of leucocytes with the help of certain specific filters or devices.

Materials and Methods
The original leucocyte depletion filter contained sterile cotton wool as a filtering agent and was designed by Diepenhorst who published his work in 1972.[6] Subsequently cellulose acetate filters were discovered and found to be more suitable. Other methods included red cell washing, centrifugation and buffy coat removal, freezing and deglycerolization of red cells and blood component collection through apheresis technology.[7] Of all these methods, leucoreduction by leucofilters (third generation) and components collected through apheresis devices meet the current standards of leucocyte depletion, that is < 5 × 10^9 WBC/unit of blood component.[8] whereas, other methods achieve leucocyte depletion to a variable extent, as follows:

i. Centrifugation and buffy coat removal — 1-2 log leucodepletion

ii. Filtration- 3-4 log leucodepletion

iii. Washed red cell concentrate — 1-2 log leucodepletion

iv. Frozen deglycerolized red cells — 2-3 log leucodepletion

Centrifugation and Buffy Coat Removal
This is one of the easiest and least expensive methods and it can be done in closed system. This causes 1-2 log reduction, but sacrifices RBCs upto 20%.

Leucofiltration
Current generation of leucofilters (third and fourth) have excellent leucocyte removal efficiency (99.99%) as compared to the first and second generation filters (90-96%). Presently we have depth and screen-type filters. Depth filter (non woven) has filter material in the form of compressed wool fibers arranged in an irregular fashion, whereas, screen filters (woven type) have fibers arranged in multiple layers in a regular fashion.

The primary mechanism [9] of leukocyte removal is the charge-based adhesion of negatively charged leucocytes to the filter material by Vander Waals and electrostatic forces.

Timing of leucofiltration
Leucofiltration of blood components can be done either at the time of collection and processing, post processing (within the blood bank), or by the side of the patient (post storage). Each of them has their own advantages and disadvantages. However, pre-storage leukoreduction is currently the most widely accepted mode. The advantages of pre-storage over post-storage leucoreduction are as follows:

- It eliminates the scope of inflammatory cytokine (interleukin-1, interleukin-6, tumor necrosis factor) accumulation due to leucocytes, during storage, and hence, is quite efficient in the prevention of febrile non-hemolytic transfusion reactions.[10-12] It also minimizes the risk of HLA-alloimmunization in multitransfused patients, as it removes the intact leucocytes.[13,14]

- Pre-storage leucofiltration can also minimize the risk of leucotropic virus transmission as leucocytes disintegrate and release the intracellular organisms after 72 hours of storage in blood components.[15,16]

- It is always easier to perform leucocyte quality control in the laboratory rather than by the patient’s bedside. Hence during pre-storage leucoreduction, blood components can be thoroughly studied and evaluated for quality control, and various factors affecting the process of leucofiltration modified accordingly.[17,18]

At present these factors favour pre-storage leucoreduction, either in the form of universal leucoreduction for all the
patients or as a selective protocol for a special group of patients. The major drawback with universal leucoreduction is the cost involved; however, selective leucoreduction has its inherent issues of inventory management, as it is quite difficult to predict the requirement of leucoreduced blood components at the time of component preparation.

**Washing red cells**
This will achieve a 2 log removal, provided the Buffy coat is removed before or during the washing procedure. This product is generally not recommended as it is more expensive and less effective than LDF(Leucodepletion by Filters) and the lifespan of the product is very short.

**Freezing and deglycerolizing red cells**
The level of leucocyte depletion approximates that of LDF(Leucodepletion by Filters). This process is very expensive and is not recommended for the routine preparation of a leucocyte depleted product. Use of this product should be reserved for patients for whom long term storage is required either because they have a rare blood type or have multiple antibodies.

**Leucacytapheresis**
Blood components collected with the help of the current generation low leucacytapheresis devices are generally 3 logs reduced and hence require no further filtration to meet the standards of leucoreduced blood components.

### Currently Accepted Clinical Indications for Leucoreduced Blood Components[19]

<table>
<thead>
<tr>
<th>Proven benefits</th>
<th>Probably clinically relevant</th>
<th>Unproven clinically</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced frequency and severity of FNHTRs</td>
<td>Reduced infectious risk associated with immunomodulation (TRIM)</td>
<td>Avoidance of vCJD transmission</td>
</tr>
<tr>
<td>Reduced risk of CMV transmission</td>
<td>Reduced organ dysfunction and mortality</td>
<td>Avoidance of HTLV I/II, EBV etc.</td>
</tr>
</tbody>
</table>

**Current accepted standards for leucodepleted blood components**

<table>
<thead>
<tr>
<th>RED CELL CONCENTRATE</th>
<th>American Association of Blood Banks (USA)</th>
<th>European Council criteria</th>
<th>Director General of Health Services (India) criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;5 × 10⁶ WBC/Unit (red cell loss not more than 15%)</td>
<td>&lt;1 × 10⁶ WBC/Unit</td>
<td>&lt;5 × 10⁶ WBC/unit (red cell loss not more than 10%)</td>
</tr>
</tbody>
</table>

**Result**
We collected month-wise data of transfusion of RCC( Red Cell Concentrate) to Thalassaemia patients [ TABLE-1] and the number of blood transfusion reactions noted among them [TABLE-2] at P.D.U. Medical College and Hospital, Rajkot ,Gujarat ( India). 92.5% of the RCCs issued were prepared by Manual and Buffy Coat Removal following standard protocols and 7.5%RCC were prepared by using 3rd generation leucofilters[ TABLE-1]. Total 3 Blood Transfusion Reactions were noted in the period of 6 months , out of that 2 (66.7%) reactions occurred in thalassaemia patients who were given RCC prepared by Centrifugation and Buffy Coat Removal method of leucodepletion and 1 (33.3%) reaction was noted in patient who was transfused with RCC prepared by leucofiltration method of leucodepletion[TABLE-2].

**Discussion**
Though, today many new more efficient techniques have arrived , but if this method of leucoreduction ( i.e. centrifugation and manual buffy coat removal ) if performed under standard protocols and with expertise , reduction of leucocytes by approximately 2 log can be

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**TABLE 1: Month-wise Data of RCC(Red Cell Concentrate) Issued to Thalassaemia Patients**

<table>
<thead>
<tr>
<th></th>
<th>Leucodepleted RCC prepared by centrifugation and manual buffy coat removal</th>
<th>Leucodepleted RCC prepared by using 3rd generation filters</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEPTEMBER 2013</td>
<td>232</td>
<td>12</td>
</tr>
<tr>
<td>OCTOBER 2013</td>
<td>252</td>
<td>20</td>
</tr>
<tr>
<td>NOVEMBER 2013</td>
<td>223</td>
<td>15</td>
</tr>
<tr>
<td>DECEMBER 2013</td>
<td>220</td>
<td>29</td>
</tr>
<tr>
<td>JANUARY 2014</td>
<td>264</td>
<td>26</td>
</tr>
<tr>
<td>FEBRUARY 2014</td>
<td>377</td>
<td>24</td>
</tr>
<tr>
<td>TOTAL</td>
<td><strong>1568 (92.5%)</strong></td>
<td><strong>126 (7.5%)</strong></td>
</tr>
</tbody>
</table>
achieved easily and may be sufficient to prevent most febrile nonhaemolytic transfusion reactions (NHFTRs) in patients who have previously experienced these reactions (20). Rather, it will be cost effective also especially for the government set ups, where it is practically difficult to do leucodepletion by using filters for masses.

My institute (P.D.U. Medical College and Hospital, Rajkot, Gujarat), follow standard protocols regarding component separation, through which we have been able to achieve up to 2 log reduction of leucocytes. These protocols are as follows,

- All components are prepared with in 24 hrs. of blood collection, even if it is plasma too.
- FFP (Fresh Frozen Plasma) and PC (Platelet Concentrate) is prepared within 6hrs of blood collection.
- Plasma is prepared within 24hrs of blood collection.
- All the procedures are done under strict aseptic conditions and optimal temperature control.

So, RCC prepared is free from WBC fragments and cytokines release which have been mentioned as major culprits for NHFTRs (Non Hemolytic Febrile Transfusion Reactions) in multiple transfused patients.

**Conclusion**

Removing the buffy coat from red cells at source results in approximately a 2 log removal of leucocytes and may be sufficient to prevent most febrile nonhaemolytic transfusion reactions in multiply transfused patients and who have previously experienced these reactions and specially in cost constrained settings.

**Acknowledgements**

I am highly indebted to all my wonderful teachers, colleagues and last but not the least my Head of the Department for their guidance, constant supervision and immense support in completing the project.

### TABLE 2: No. of Blood Transfusion Reactions Occurred by Different Methods of Leucodepletion

<table>
<thead>
<tr>
<th></th>
<th>No. of Blood Transfusion Reactions occurred by Leucodepleted RCC prepared by centrifugation and manual buffy coat removal</th>
<th>No. of Blood Transfusion Reactions occurred by leucodepleted RCC prepared by using 3rd generation filters</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEPTEMBER 2013</td>
<td>01</td>
<td>00</td>
</tr>
<tr>
<td>OCTOBER 2013</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>NOVEMBER 2013</td>
<td>01</td>
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</tr>
<tr>
<td>DECEMBER 2013</td>
<td>00</td>
<td>01</td>
</tr>
<tr>
<td>JANUARY 2014</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>FEBRUARY 2014</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>TOTAL</td>
<td>02(66.7%)</td>
<td>01(33.3%)</td>
</tr>
</tbody>
</table>

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None

**Competing Interests**

Not declared

**REFERENCE**


http://www.pacificejournals.com/aabs


11. Muylle L, Joos M, Wouters E, De Bock R, Peetermans ME. Increased tumor necrosis factor alpha (TNF alpha), interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: Relationship between TNF alpha and IL-6 levels and febrile transfusion reactions. Transfusion. 1993;33:195–9.


