Quality control of leucodepleted products.
A comparative analysis through interleukin assays and residual leucocyte count

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Abstract

Background. Leucodepletion of blood components ensures high-quality transfusion products and prevents or reduces some of the adverse effects of transfusions in recipients. The aim of this study was to evaluate the characteristics of various in-line and laboratory filters, focusing particularly on the release of interleukins (IL), which seem to be the main causes of some transfusion reactions, particularly non-haemolytic febrile reactions.

Materials and methods. The performance of 8 different leucodepletion filters (2 for laboratory use and 6 in-line filters) was examined. Each type of filter was tested using 8 units of blood from the total of 64 different donations of whole blood. The parameters evaluated were: red cell yield, residual white cell count and cytokine concentrations. The cytokines assayed were: IL-1β, IL-6, IL-8, IL-10, IL-12, and tumour necrosis factor (TNF-α).

Results. As far as concerns red cell yield, residual haemoglobin (Hb) and ease of use, there were no relevant differences. The times of filtration did, however, vary considerably (from a minimum of 13.3 to a maximum of 34.1 minutes). The residual white cell count also varied substantially, depending on the type of filter used, but always remained well within the limits of "safety". The concentrations of IL (particularly IL-1β, IL-6, IL-10 and IL-12) differed significantly according to the filter used.

Introduzione

Nel XX congresso della Società Internazionale della Trasfusione del Sangue, tenutosi a Londra nel 1988, fu organizzato il primo workshop sul ruolo della leucodeplezione. La problematica suscitò tanto interesse che nel luglio del 1994, ad Amsterdam, sull’argomento fu dedicata una intera sessione. I leucociti sono responsabili di diversi effetti negativi secondari alla trasfusione di sangue, taluni anche di notevole gravità clinica (GvHD, infezioni, immunomodulazione ecc.). Sono state rilevate precise correlazioni tra livelli di leucoriduzione e relative complicanze1-4. I leucociti, inoltre, interferiscono con la vitalità delle emazie e delle piastrine, sia per competizione per il glucosio presente nella soluzione conservante e negli additivi, sia a causa della lisi cellulare leucocitaria, responsabile della liberazione di sostanze che interferiscono col metabolismo cellulare e che causano alterazioni della membrana dei globuli rossi e delle piastrine.

La filtrazione costituisce certamente la più usata tecnica di leucodeplezione oggi disponibile; essa consente la rimozione leucocitaria, sia attraverso azioni fisico-mecaniche (setacciamento) legate alle dimensioni cellulari (screen filtration), sia attraverso la capacità dei leucociti di aderire alle fibre sintetiche, indipendentemente dalle loro dimensioni, per effetto di fenomeni di natura biologica, legati alla presenza di molecole proteiche dotate di proprietà adesive, presenti sulla superficie esterna delle loro membrane cellulari (integrine, selectine, immunoglobulin-like)3,6. L’adesione leucocitaria alle fibre (depth filtration) può verificarsi sia in forma diretta che indirettamente. In questo caso, risulta fondamentale il ruolo svolto dalle
Conclusions. Although no differences of note were found between the filters for many characteristics, the IL concentrations demonstrated that filters with an electrically charged surface (whether positive or negative) tend to cause greater release of inflammatory cytokines, albeit rarely to the extent of being able to induce adverse reactions in the recipients of the filtered units.

Key words: interleukins, leucodepletion, quality control, filtered products

Introduction

The first workshop on the role of leucodepletion was organized at the XX Congress of the International Society of Blood Transfusion held in London in 1988. The issue raised such interest that in July 1994, in Amsterdam, a whole session was devoted to leucodepletion. White blood cells are responsible for various negative side effects of blood transfusion (graft-versus-host disease, infections, immunomodulation, etc.), which can sometimes be of very considerable clinical severity. Precise correlations have been found between the levels of leucoreduction and the related complications. Furthermore, white blood cells influence the viability of the red blood cells and platelets, both by competing for the glucose present in the storage solution and additives, and because of leucocyte lysis, which causes the release of substances that affect cell metabolism and provoke changes in the membranes of red blood cells and platelets.

Filtration is certainly the most widely used technique currently available for leucodepletion; this procedure removes the white cells either by physico-mechanical mechanisms (sieving) based on the size of the cells (screen filtration) or by exploiting the capacity of white cells, independently of their size, to adhere to synthetic fibres through natural biological phenomena involving proteins with adhesive properties on the external surface of their cell membranes (integrins, selectins, immunoglobulin-like proteins). Leucocyte adhesion to fibres (depth filtration) can occur both directly and indirectly. In the latter case the role played by the platelets is fundamental, since these bind to the fibres through their own adhesions molecules and subsequently piastrine, le quali si legano alle fibre mediante loro molecole di adesione di membrana e, successivamente, diventano substrato per la adesione dei leucociti. A tale proposito, sono state rilevate differenze di comportamento delle varie popolazioni cellulari leucocitarie: i linfociti vengono rimossi principalmente per setacciamento, i monociti per adesione diretta, i granulociti per adesione mediata dalle piastrine.

Oggi sono disponibili filtri per leucodeplezione composti da fibre con diversa carica elettrica di superficie. In alcuni di questi è stata rilevata la presenza di attività enzimatiche serin-proteasiche, probabilmente secondarie alla attivazione della cascata coagulativa della cosiddetta fase di contatto. Questo sembra essere alla base della comparsa, a distanza di tempo non superiore a una ora dalla trasfusione, di tachicardia e ipotensione, in qualche caso anche di grave entità, specialmente nei pazienti in terapia con farmaci ACE-inibitori. Un ruolo sempre più importante assume oggi la filtrazione pre-storage, la quale prevede che tale metodica venga condotta entro 24 ore dal prelievo. In questo modo si impedisce il passaggio di leucociti vitali, con membrane cellulari e organuli intracellulari integri, prevenendo così la frammentazione cellulare responsabile della liberazione di mediatori pro-infiammatori e della formazione di microaggregati. Negli ultimi anni viene data sempre maggiore importanza alla liberazione delle interleuchine (IL) intracellulari, relativamente alla comparsa di reazioni post-trasfusionali non emolitiche.

Scopo di questo lavoro è esaminare la capacità leucodepletiva, la manualità, la resa e la velocità di filtrazione di alcuni dei filtri, sia in linea che da laboratorio, in commercio in Europa. Inoltre, si vuole verificare se la filtrazione di per sé possa determinare un incremento della concentrazione di talune citochine proinfiammatorie potenzialmente responsabili di alcuni effetti collaterali post-trasfusionali indesiderabili.

Materiali e Metodi

Sono stati utilizzate 64 donazioni di sangue, da donatori periodici idonei alla donazione al momento del prelievo, condotte secondo la prassi normale, selezionando unità il più possibile vicine agli standard medi di Ht, Hb e conta leucocitaria. Si sono testate
become the substrate for the adhesion of the leucocytes. Differences have been found in the behaviour of the various white cell populations: lymphocytes are removed mainly by sieving, monocytes by direct adhesion, granulocytes by platelet-mediated adhesion\(^7,8\).

The leucodepletion filters currently available are made of fibres with different surface electrical charges. Some of these filters have been found to cause serine-protease enzymatic activity, probably secondary to activation of the coagulation cascade of the so-called contact phase. This phenomenon seems to be at the basis of the development of tachycardia and hypotension within less than an hour of the transfusion. In some cases, especially in patients receiving ACE-inhibitor treatment, these side effects can be very severe. Pre-storage filtration is currently gaining an increasingly important role. This method of filtration is carried out within 24 hours the blood being collected. In this way the passage of viable leucocytes, with intact intracellular organelles and cell membranes, is prevented: this avoids the risk of the cellular fragmentation, that is responsible for releasing pro-inflammatory mediators and the formation of microaggregates\(^9,14\). In recent years the release of intracellular interleukins (IL) has been attributed increasing importance as being involved in the development of non-haemolytic post-transfusional reactions\(^15-18\).

The aim of this study was to examine the efficacy of leucodepletion, the ease of use, the yield and the speed of filtration of some in-line and laboratory white blood cell filters commercially available in Europe. Another aim was to determine whether filtration itself could cause an increase in the concentration of various pro-inflammatory cytokines potentially responsible for some post-transfusional side effects.

**Materials and Methods**

Sixty-four units of donated blood were used. These units had been collected from periodic donors who were fit for donation at the time of the collection, which was carried out according to normal practices. The units were selected for having values of haematocrit, haemoglobin and white cell count as close as possible to the standard mean. Eight units were used to test each of the following filters commercially available in Europe: the laboratory
filters Imugard III RC (Terumo Italia, Rome, Italy) and Leucolab LCG 2 (Maco Pharma Italia, Rho, MI, Italy) and the in-line filters R7546 (Baxter Fenwal, Rome, Italy) Leucored RC/PL SK (Grifols Italia, Ghezzano, PI, Italy), RCT 434CL (Pall Italia, Milan, Italy) T3945 (Fresenius HemoCare, Midolla, MO, Italy), LPT6265LR (MacoPharma) and BB*WGQ45613 (Terumo). In all cases the filtration procedures was carried out according to the instructions provided by the manufacturers of the filters and the bags with in-line filters. Furthermore, all the filtrations were carried out in uniform conditions of temperature (18-22 °C), time and handling. Pre-filtration and post-filtration white cell counts were measured in all the bags and cytokine concentrations were assayed post-filtration. The filtration with laboratory filters was done within five days, where as the in-line filtration was done within 24 hours of the collection. In all cases the pre-filtration white cell count was determined within 6 hours of the donation, while the post-filtration count was evaluated within 24 hours of the filtration. Other measurements were the weight of the filtered bag, its full blood count and the weight of the filter following filtration. The time required for the filtration was calculated from the moment of opening the valves, that allow the blood to flow towards the filter, until the moment that there was no longer any visible blood del Kit LeucoCOUNT System e dell'Human Inflammation Kit. I risultati ottenuti sono stati analizzati statisticamente mediante il calcolo della media, come indice di localizzazione, e della deviazione standard, come indice di dispersione.

### Risultati

La tabella I mostra la trascodifica dei filtri utilizzati in questo studio.

L’analisi di alcuni parametri quali il residuo, la resa in emazie concentrate, il contenuto in grammi di emoglobina delle sacche filtrate e il tempo necessario per la filtrazione, pur con alcune minime variazioni, non ha riscontrato differenze sostanziali tra i filtri esaminati: la resa varia dai 222 grammi del filtro H ai 251 grammi del filtro B. Il residuo dai 19,4 grammi del filtro H ai 28,4 grammi del filtro A. La quantità di emoglobina dai 44,7 grammi del filtro H ai 52,4 grammi del filtro B.

Il tempo di filtrazione varia dai 13,3 minuti del filtro G ai 34,1 minuti del filtro A (Tabella II). Nell’uso corrente dei filtri in esame non si sono rilevate differenze di rilievo nella manualità; le differenze sono piuttosto dovute alla diversa conformazione delle sacche.

L’analisi della conta leucocitaria residua dimostra come con tutti i filtri testati si sono ottenute conte
flow from the filter to the collection bag. The filtration was carried out on red cell concentrates without the buffy-coat, except for the Grifols, Terumo and Fresenius bags whose systems allow filtration of whole blood before fractionation into red cells, buffy-coat and platelet-rich plasma. The control assays for IL-1β, IL-6, IL-8, IL-10, IL-12 and TNF-α were carried out on bags from twelve periodic donors, fit for donation at the moment of the collection; the assays were performed on plasma obtained from bags of red cell concentrate from which the buffy-coat had been removed, at 3, 6, and 24 hours and 3, 10, and 20 days after the collection. In the filtered products, the abovementioned cytokine assays were performed on samples of plasma obtained immediately after filtration. Both the white cell count and the cytokine concentrations were determined by flow cytometry, with a FACSCalibur (Becton Dickinson Italia, Milan, Italy), according to the manufacturers’ instructions for the use of the Kit LeucoCOUNT System and the Human Inflammation Kit, respectively.

The statistical analysis of the results was performed by calculating the mean, as the index of localisation, and the standard deviation, as the index of dispersion of the data.

Results

Table I shows the codes used for the filters investigated in this study. The analysis of some of the leukocitarie post-filtrazione decisamente inferiori ai parametri considerati “di sicurezza” (pari a 1x10^6): in tre casi di filtri in linea si sono ottenuti valori medi oscillanti tra 4 e 2x10^5, in tutti gli altri inferiori a 1.5x10^5 (Figura 1).

Considerando che le unità di partenza avevano un contenuto medio di leucociti pari a circa 1,5x10^9 (peso medio per contenuto medio di leucociti) e considerando che la conta leucocitaria residua media era pari a 2,0x10^5, la leucodeplezione media calcolata è risultata pari a 0,75x10^4, ovvero oltre quattro logaritmi.

Per quanto riguarda il dosaggio delle IL nei controlli, si è evidenziato che per il TNF-α e per la IL-10 non si sono trovati valori quantitativamente rilevanti. Per la IL-1β, si è registrata una progressiva riduzione dei valori, dal prelievo al terzo giorno di conservazione; in seguito, fino al decimo giorno, si è verificato un netto decremento (Figura 2).

Per IL-6, IL-8, IL-12 si nota un lieve incremento dal momento del prelievo fino alla 24^a ora, per poi rapidamente ridursi entro il 10 giorno di conservazione. I dosaggi delle IL nei prodotti di filtrazione hanno dato i risultati riportati di seguito:

IL-1β. La filtrazione con i filtri A, B, E, G non ha determinato aumento della sua concentrazione, rispetto ai controlli; nel caso dei filtri C, D, F, H, invece si sono verificati netti aumenti di concentrazione nei prodotti filtrati (Figura 3).

IL-6. Nelle sacche filtrate con i filtri A, B, G e H non si sono trovate quantità di IL-6 dosabili col nostro
<table>
<thead>
<tr>
<th>Time</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IL-8</th>
<th>IL-10</th>
<th>IL-12</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 hours</td>
<td>44.05 ± 36.56</td>
<td>0.00</td>
<td>4.95 ± 0.49</td>
<td>4.15 ± 0.07</td>
<td>6.35 ± 1.48</td>
<td>2.85 ± 0.35</td>
</tr>
<tr>
<td>6 hours</td>
<td>31.90 ± 5.24</td>
<td>11.00 ± 1.12</td>
<td>9.30 ± 0.57</td>
<td>4.10 ± 0.94</td>
<td>11.60 ± 8.20</td>
<td>2.00 ± 0.36</td>
</tr>
<tr>
<td>24 hours</td>
<td>26.65 ± 14.35</td>
<td>10.60 ± 2.57</td>
<td>10.16 ± 14.31</td>
<td>4.55 ± 1.34</td>
<td>16.20 ± 23.05</td>
<td>3.70 ± 0.65</td>
</tr>
<tr>
<td>3 days</td>
<td>27.20 ± 6.19</td>
<td>1.50 ± 0.63</td>
<td>3.10 ± 1.15</td>
<td>3.80 ± 2.40</td>
<td>8.70 ± 2.7</td>
<td>2.60 ± 0.99</td>
</tr>
<tr>
<td>10 days</td>
<td>0.00</td>
<td>0.00</td>
<td>3.72 ± 0.88</td>
<td>1.80 ± 0.78</td>
<td>0.00</td>
<td>2.20 ± 1.02</td>
</tr>
<tr>
<td>20 days</td>
<td>0.00</td>
<td>1.70 ± 0.83</td>
<td>4.70 ± 1.46</td>
<td>2.05 ± 0.49</td>
<td>0.00</td>
<td>1.90 ± 0.34</td>
</tr>
</tbody>
</table>

**Figure 2** - Interleukin concentrations in controls. Values in the table are means ± SD expressed in pg/mL.

**Figure 3** - Concentrations of IL-1β; the K column represents the mean of the values measured in the control bags 24 hours after collection; the horizontal line indicates the mean level in the controls. The table shows means ± SD.

**Figure 4** - Post-filtration concentrations of IL-6 (The table shows means ± SD).
parameters, such as residue, yield of red cell concentrate, haemoglobin content (in grams) of the filtered bags and the time necessary for filtration showed that although there were some minimal variations, there were no substantial differences in these performance characteristics of the filters investigated: the yield ranged from 222 grams with filter H to 251 grams with filter B. The residue ranged from 19.4 grams with filter H to 28.4 grams for filter A. The amount of haemoglobin varied from 44.7 grams with filter H to 52.4 grams with filter B. The filtration time ranged from 13.3 minutes for filter G to 34.1 minutes for filter A (Table II). With the current use of the filters under examination there were not substantial differences in handling; rather, differences were due to the different conformations of the bags.

The analysis of the residual white cell count showed that all the filters tested produced post-filtration leucocyte counts well below the so-called level of "safety" (that is, 1x10^6): in three cases in-line filters gave mean values ranging between 4 x10^4 and 2x10^5, in all the other cases the values were below 1.5x10^5 (Figure 1). Considering that the starting units had a mean leucocyte count of about 1.5x10^9 (mean weight for mean content of leucocytes) and considering that the mean residual leucocyte count was 2.0x10^5, the mean calculated leucodepletion was 0.75x10^4, or more than four log.

As far as concerns the cytokine assays in the control samples, quantitatively relevant amounts of TNF-α and IL-10 were not found. The concentration of IL-1β decreased gradually from the time of collection until the third day of storage; from then on until the tenth day there was a very sharp drop (Figure 2). The concentrations of IL-6, IL-8 and IL-12 rose slightly during the first 24 hours after the collection and then decreased quickly within the 10 days of storage.

The results of the cytokine assays in the filtration products were as follows.

IL-1β. Filtration with filters A,B,E, and G did not cause any increase in the concentration of this IL over the concentration in control samples; in contrast, filtration through filters C, D, F, and H produced a clear increase in the concentration of IL-1β in the filtered products (Figure 3).

IL-6. Any IL-6 present in bags filtered with filters A,B, G and H was below the detection limit of our assay, whereas when filters D and E were used, higher concentrations of IL-6 were found in the filtered bags than in the control bags (Figure 4).

IL-8. Nei prodotti di filtrazione ottenuti con i filtri A,B,G si sono trovate concentrazioni decisamente minori rispetto ai controlli; invece in quelli ottenuti con i filtri E e F abbiamo trovato concentrazioni superiori (Figura 5).

IL-10 e IL-12. Per le interleuchine IL-10 e IL-12, solamente nel caso delle sacche filtrate con il filtro E si è trovato un notevole aumento della loro concentrazione (Figure 6 e 7).

TNF-α. Per il TNF-α, in nessun caso si sono rilevate concentrazioni rilevanti nelle sacche filtrate esaminate (Figura 8).

Discussione

La scelta dei campioni da sottoporre alla nostra analisi è stata guidata dalla necessità di testare unità di sangue intero il più possibile simili tra loro in termini di Ht (da un minimo di 43,8% ad un massimo di 45,2 %), contenuto emoglobinico per unità (da 65,2 a 72,2 g) e conta leucocitaria (da 5.200 a 6.300 leucociti/µL), allo scopo di ridurre al minimo le interferenze dovute alle differenze di partenza, nella valutazione della resa post-filtrazione e della capacità leucodepletiva dei singoli filtri. Nonostante le inevitabili differenze, in tutti i casi i prodotti finali si sono dimostrati del tutto rispondenti agli standard richiesti dai protocolli internazionali.14

I dosaggi delle citochine nei controlli hanno evidenziato valori superiori ai 25 pg/µL solo per la IL-1β dalla terza ora al terzo giorno di distanza dal prelievo; per tutte le altre citochine si sono sempre trovate concentrazioni inferiori ai 20 pg/µL. Dosaggi così bassi, però, possono essere dovuti a diversi meccanismi biologici capaci di interferire con la loro corretta titolazione nel comparto extracellulare: il fisiologico re-uptake molecolare, ad esempio, oppure la compartimentalizzazione intracellulare, oppure l'adsorbimento di talune citochine sulle membrane eritrocitarie. Sono state descritte, infatti, affinità di talune citochine, in particolare la IL-8, per gli antigeni eritrocitari responsabili della specificità di gruppo antigenico della classe Duffy. Dopo il decimo giorno di conservazione, e fino al ventesimo, per tutte le citochine in esame abbiamo rilevato valori inferiori ai 5 pg/µL.
Quality control of leucodepleted products

**Figure 5** - Post-filtration concentrations of IL-8 (The table shows means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>K</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL8</td>
<td>10.16 ± 14.31</td>
<td>2.76 ± 1.22</td>
<td>2.30 ± 0.49</td>
<td>9.40 ± 1.30</td>
<td>1.36 ± 5.11</td>
<td>15.10 ± 1.60</td>
<td>16.90 ± 26.35</td>
<td>2.78 ± 1.37</td>
<td>8.16 ± 6.68</td>
</tr>
</tbody>
</table>

**Figure 6**: Post-filtration concentrations of IL-10. (The table shows means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>K</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL10</td>
<td>4.55 ± 1.34</td>
<td>2.92 ± 1.18</td>
<td>2.00 ± 1.10</td>
<td>0.00</td>
<td>2.60 ± 0.30</td>
<td>16.00 ± 3.40</td>
<td>4.70 ± 1.40</td>
<td>3.95 ± 1.20</td>
<td>6.43 ± 2.50</td>
</tr>
</tbody>
</table>

**Figure 7** - Post-filtration concentrations of IL-12. (The table shows means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>K</th>
<th>A</th>
<th>B</th>
<th>C</th>
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<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL12</td>
<td>16.20 ± 23.05</td>
<td>3.67 ± 2.38</td>
<td>4.25 ± 0.35</td>
<td>17.35 ± 3.40</td>
<td>9.55 ± 1.70</td>
<td>54.30 ± 16.40</td>
<td>9.55 ± 11.62</td>
<td>3.20 ± 0.35</td>
<td>5.20 ± 2.85</td>
</tr>
</tbody>
</table>

MEAN ± S.D.
IL-8. The filtration products obtained using filters A, B, and G contained much less IL-8 than did control bags; in contrast, the bags obtained with filters E and F contained higher concentrations (Figure 5).

IL-10 and IL-12. The concentrations of IL-10 and IL-12 increased notably only in bags filtered using filter E (Figures 6 and 7).

TNF-α. Relevant concentrations of TNF-α were not detected in any of the filtered bags (Figure 8).

Discussion

Our choice of samples to test was guided by the need to evaluate units of whole blood that were as similar as possible with regards to haematocrit (from a minimum of 43.8% to a maximum of 45.2%), haemoglobin content per unit (from 65.2 to 72.2 g) and white cell count (from 5,200 to 6,300 leucocytes/μL), with the aim of minimising biases due to different starting values in the assessment of the post-filtration yield and the leucoreduction capacity of each filter. Despite the inevitable differences, in all cases the final products conformed with the standards required by international protocols.

The cytokine assays in the control samples showed levels above 25 pg/μL only for IL-1β from the third hour to the third day after collection; concentrations of all the other cytokines were always below 20 pg/μL. These low concentrations could, however, be due to various biological mechanisms that can affect correct titration of cytokines in the extracellular compartment, L’analisi quantitativa delle citochine nei prodotti filtrati non ha dimostrato differenze importanti rispetto ai controlli di riferimento; per le citochine IL-1β, IL-6, IL-10 e IL-12 abbiamo rilevato aumenti di lieve o media entità, specialmente nei prodotti ottenuti da filtri a carica negativa. In nessun caso, comunque, nei riceventi si sono verificate reazioni post-trasfusionali indesiderate clinicamente evidenti.

Ricordiamo le principali caratteristiche delle varie citochine.

IL-1β. È prodotta soprattutto dalle cellule mononucleate attivate. I suoi effetti biologici dipendono dalla quantità di molecola prodotta ed immessa in circolo; a basse concentrazioni prevale l’effetto pro-infiammatorio a livello locale, mentre a concentrazioni maggiori si manifestano azioni sistemiche di tipo endocrino, capaci di determinare la comparsa di febbre e di indurre la sintesi di molecole di fase acuta della flogosi da parte del fegato. Per quanto riguarda i dosaggi sui prodotti di filtrazione, il filtro F (a carica elettrica negativa) ha provocato un sensibile aumento di concentrazione; aumenti di minore entità si sono trovati nei casi riferibili ai filtri C, D e H.

IL-6. È prodotta dai monociti, dai fibroblasti, e dalle cellule endoteliali vasali in risposta alla IL-1β e al TNF-α. La sua azione si esplica a livello degli epatociti, che vengono inibiti nella loro capacità di produzione di varie proteine plasmatiche di fase acuta, tra le quali il fibrinogeno, nonché a livello dei linfociti B, per i quali costituisce un fattore di crescita e di differenziazione, essendo in grado di determinare la
such as physiological molecular re-uptake or intracellular compartmentalization, or absorption of some cytokines onto the red cell membrane.

In fact, it has been described that some cytokines, particularly IL-8, have an affinity for the red blood cell antigens of the Duffy blood group system. From the tenth to the twentieth day of storage the concentrations of all the cytokines assayed were below 5 pg/µL.

The concentrations of cytokines in the filtered products did not differ substantially from those in the control samples although there were slight or moderate increases for IL-1β, IL-6, IL-10 and IL-12, especially in those products obtained with negatively charged filters. However, in no case did clinically obvious post-transfusion reactions occur in the recipients.

The following is a summary of the main characteristics of the various cytokines.

**IL-1β.** This interleukin is produced predominantly by activated mononuclear cells. Its biological effects depend on the amount of the molecule produced and introduced into the circulation; at low concentrations local pro-inflammatory effects predominate, while at higher concentrations there are systemic endocrine-like reactions, which are able to cause fever and induce hepatic synthesis of acute phase inflammatory molecules. As far as concerns the assays on the filtered products, filter F (negatively charged) caused an appreciable increase in the concentration of IL-1β; less substantial increases occurred with the filters C, D and H.

**IL-6.** This is produced by monocytes, fibroblasts and vascular endothelial cells in response to IL-1β and TNF-α. It acts on hepatocytes, which are inhibited from synthesising various acute phase plasma proteins, including fibrinogen. It is also a growth and differentiation factor for B lymphocytes, being able to induce their maturation into plasma cells. It has recently been determined that IL-6 has some stimulatory effect on thymic cells and T lymphocytes. The concentration of IL-6 in the samples examined was often under the threshold of detection (2.5 pg/µL); only filter E (with a positive electrical charge) caused a significant increase in the concentration of this interleukin; a slight increase was also seen with filter D.

**IL-8.** This interleukin is produced by monocytes, vascular endothelial cells, fibroblasts and megakaryocytes. It is the best known member of the their maturation in plasmacellulare. Recently it has been identified as also participating in the stimulation of the T cells. In patients with multiple transfusions the concentration of IL-6 is often below the limit of detection (2.5 pg/µL). In particular, the filter E (with a positive electrical charge) determined a significant increase in concentration; a slight increase was also observed with filter D.

**IL-10.** This is produced by monocytes, fibroblasts and megakaryocytes. It is the best known member of the family of chemokines, a group of molecules involved in the production of acute phase proteins, including fibrinogen. It is also a growth and differentiation factor for B lymphocytes, being able to induce their maturation into plasma cells. It has recently been determined that IL-6 has some stimulatory effect on thymic cells and T lymphocytes. The concentration of IL-6 in the samples examined was often under the threshold of detection (2.5 pg/µL); only filter E (with a positive electrical charge) caused a significant increase in the concentration of this interleukin; a slight increase was also seen with filter D.

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family of chemokines, a group of molecules able to stimulate the motility of leucocytes. Slight increases in its concentration were found in products obtained using filters E and F, which are positively and negatively charged, respectively.

**IL-10.** This is produced by the Th2 subpopulation of lymphocytes and by activated B lymphocytes. It can inhibit the synthesis of IL-1β, IL-12 and TNF-α by macrophages. Furthermore, it inhibits activation of T lymphocytes by cells of the monocyte-macrophage system.

**IL-12.** This is produced by dendritic cells and by mononuclear cells. It regulates cell-mediated immune responses by acting on T and NK lymphocytes. It activates CD8+ T lymphocytes to acquire cytolytic properties and stimulates the differentiation of CD4+ lymphocytes towards a Th1 phenotype. Appreciably increased levels of both IL-10 and IL-12 were found only in the products obtained using filter E.

**TNF-α.** This cytokine is produced by monocytes that have been activated by lipopolysaccharide-endotoxin released from the cell wall of Gram-negative bacteria. Its actions vary depending on its plasma concentration: at low concentrations it stimulates endothelial cells to express surface adhesion molecules, that is, to express receptors for leucocytes, at first only for neutrophils, and then also for monocytes and lymphocytes.

This leads to an accumulation of leucocytes in the site of inflammation. TNF-α activates neutrophils, enabling them to kill bacteria directly, and stimulates the production of cytokines by mononuclear cells. It is an endogenous pyrogen capable of inducing hyperthermia by acting on the thermal regulation centre in the hypothalamus.

TNF-α stimulates the production of IL-2 and IL-6 by mononuclear and endothelial cells and the release of these interleukins into the circulation. It activates the coagulation cascade and inhibits replication of bone marrow stem cells.

At higher plasma concentrations it depresses myocardial contractility and reduces vasal smooth muscle tone, causing a reduction in systemic blood pressure and tissue perfusion to the point of causing thrombosis and disseminated intravascular coagulation. Given its potential harmfulness, we were satisfied to find that there were no significant increases in the concentration of this cytokine in any of the samples tested.

coagulativa e inibisce la replicazione delle cellule staminali midollari. A concentrazioni plasmatiche più alte provoca depressione della contrattilità miocardica, riduce il tono della muscolatura liscia vasale, determinando riduzione della pressione arteriosa e della perfusione tissutale, fino a causare trombosi e CID. Soddisfacentemente, considerata la sua potenziale lesività, in nessuno dei nostri campioni abbiamo rilevato aumenti significativi della sua concentrazione.

Dall’analisi dei risultati in esame, si evince che, pur riscontrandosi in alcuni casi aumenti non trascurabili di alcune citochine, sia nell’uso di filtri a carica elettrica positiva che negativa, in nessun caso si sono riscontrate reazioni post-trasfusionali nei pazienti (per la maggior parte microcitemici) che hanno ricevuto le unità oggetto del nostro lavoro. Per la verità nessun tipo di reazione post-trasfusionale è stata riscontrata anche nei riceventi delle unità utilizzate come controlli.

**Conclusioni**

Senza voler fare una classifica dei filtri in commercio, bisogna tener conto di alcune caratteristiche peculiari di ciascuno di essi, come la resa in grammi di emoglobina e la velocità di filtrazione. Per quel che riguarda la capacità leucodepletiva, tutti i filtri analizzati si sono rivelati efficaci nel ridurre la conta residua nettamente al di sotto del livello di sicurezza, tuttavia alcuni di essi hanno rivelato efficacia doppia o quasi tripla rispetto ad altri. I dosaggi delle interleuchine nei prodotti leucodepleti hanno evidenziato come i filtri a carica elettrica di superficie positiva, ma in particolar modo negativa, abbiano la tendenza a causare la liberazione di citochine inflamatorie, le quali solo raramente raggiungono concentrazioni tali da causare effetti collaterali clinicamente rilevanti (reazioni trasfusionali febrili non emolitiche o FNHTR).

**Riassunto**

**Premesse.** La leucodeplezione degli emocomponenti assicura prodotti trasfusionali di alta qualità e evita o riduce alcuni effetti sfavorevoli della trasfusione nei riceventi.
An analysis of the results shows that although there were appreciable increases in some cytokines in some cases, using both positively and negatively charged filters, there were no post-transfusional reactions in any of the patients (most of whom had thalassaemia) who received the units that had been studied. However, it should also be noted that no type of post-transfusion reaction was seen in the recipients of the control units.

Conclusions

Without wanting to make a classification of the commercially available filters, some particular characteristics of each of them, such as the haemoglobin yield in grams and the speed of filtration, should be taken into consideration.

As far as concerns the capacity to remove white blood cells, all the filters analysed were effective in reducing the residual count to well below the safety level, although some were two or nearly three times more effective than others.

Assays of cytokines in the leucodepleted products showed that the filters with a surface charge, whether positive or mainly negative, tended to cause release of inflammatory cytokines, although the concentrations of these rarely reach levels such as to cause clinically relevant side effects (febrile non-haemolytic transfusion reactions or FNHTR).

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We thank the technical and nursing staff of the Immunohaematology and Transfusional Medicine Service of Crotone for their collaboration.

Il lavoro ha avuto lo scopo di valutare le caratteristiche di alcuni filtri, sia in linea sia da laboratorio, soprattutto per quanto riguarda la liberazione di interleuchine (IL), che sembrano essere le principali responsabili di alcune reazioni trasfusionali, in particolare di quelle febrili non emolitiche.

Materiali e metodi. Sono state studiate le prestazioni di 8 diversi filtri per leucodeplezione (2 da laboratorio e 6 filtri in linea) per ognuno dei quali sono state testate 8 unità di sangue, provenienti, complessivamente da 64 donazioni di sangue intero. Sono stati valutati: le rese in emazie concentrate, la conta leucocitaria residua e i dosaggi delle IL. Le IL studiate sono state: IL-1β, IL-6, IL-8, IL-10, IL-12, TNF-α.

Risultati. Per quanto riguarda rese in emazie concentrate, Hb residua e manuturità d’impiego non si sono note differenze di qualche rilievo. I tempi di filtrazione variano, invece, considerevolmente (da un minimo di 13,3 a un massimo di 34,1 minuti). Anche la conta leucocitaria residua presenta oscillazioni consistenti da filtro a filtro, ma sempre entro ampi limiti "di sicurezza". Anche il dosaggio delle IL (in particolare di IL-1β, IL-6, IL-10 e IL-12) varia significativamente da filtro a filtro.

Conclusioni. Se per molti aspetti non si sono individuati differenze degne di nota fra i filtri esaminati, il dosaggio delle IL dimostra che i filtri a carica elettrica di superficie (sia positiva che negativa) hanno la tendenza a liberare citochine infiammatorie, solo raramente in grado, peraltro, di indurre reazioni avverse nel ricevente.

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References


