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HEMODIAFILTRATION WITH SELF-CLEANING SYSTEM

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Abstract: *Hemodiafiltration (HDF) is considered the primary method for replacing kidney and liver function through blood filtration. Strong convective flows have been linked to improved clinical outcomes, but achieving high blood flows for effective convective therapies can be challenging due to technical obstacles and the risk of defense cell blockages in the filtration system. Despite optimized procedures, issues such as increased transmembrane pressure, blood viscosity, and filter clogging may still arise. To address these challenges, two potential solutions are proposed: automatic predilution and automatic backflush. Predilution involves diverting a portion of filtered dialysate in the opposite direction from filtration at regular intervals to achieve hemodilution and improve filter performance. Backflush, triggered by TMP control, involves infusing ultrapure dialysate into the filtration system to improve hemodilution and clean membrane pores. These innovations aim to overcome technical barriers and ensure effective convective dose delivery in HDF treatment.*

Keywords: *hemodiafiltration, convection, ultrafiltration, predilution, back filtration.*

1. INTRODUCTION

Hemodiafiltration (HDF) is a renal replacement method that utilizes both diffusion and convection to improve the elimination of solutes across a wide range of molecular weights.[1] Within HDF, ultrafiltration (UF) surpasses the intended fluid extraction, necessitating the provision of replacement fluid to uphold the targeted fluid equilibrium. The significance of convection in eliminating solutes amplifies with the escalation of molecular weight. Advances in membrane, machine, and fluid technologies have made HDF a reliable and efficient method. Thin synthetic membranes now enable a blend of diffusive and convective techniques.[1] Enhanced UF monitoring mechanisms in dialysis equipment have reduced the likelihood of fluid balance discrepancies. The availability of sterile, pyrogen-free solutions has facilitated the safe delivery of large fluid volumes, making high-volume HDF a straightforward and secure procedure. [1-2]

2. THE HDF TECHNICAL PROCEDURE (METHODS AND TOOLS)

HDF can be implemented using various technical methods. [1-3] The traditional HDF approach involved reinfusing an average of 8.5–9.2 liters per session in post-dilution mode. To achieve sufficient ultrafiltration rates while keeping transmembrane pressure (TMP) differentials within acceptable limits, a blood flow rate exceeding 280 mL/min was required. Vital medical equipment included a system for controlling ultrafiltration (UF), a centrifugal pump for re-infusion, and a scale for automatically measuring the weight of re-infusion bags. A distinct form of HDF known as acetate-free biofiltration has successfully eliminated even minute traces of acetate from both the dialysate and replacement fluid, leading to significant improvements in the hemodynamics of unstable patients. Another version of HDF, referred to as “high-volume HDF,” involved reintroducing 14 liters or more per session.[1]

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The high cost associated with commercially available replacement fluid bags has led to the development of a new method called online HDF (OL-HDF). In this approach, freshly prepared ultrapure dialysate undergoes filtration through multiple stages and is then used as replacement fluid. This process results in the generation of large quantities of cost-effective replacement solutions, enabling HDF to be conducted with a significantly high fluid turnover rate (up to 22-30 liters per session). The fluid can be administered in varying proportions either before or after dilution, or a combination of both. Additionally, alternative methods such as HDF internal filtration, paired filtration dialysis, low-to-medium dilution HDF, high-flux dual HDF, and push-pull HDF have been suggested to effectively integrate convection and diffusion. [1-3]

A crucial factor in ensuring the effective and safe implementation of HDF is the careful selection of a suitable membrane and hemofilter. The efficiency of the diffusion process may be compromised if there is an imbalance in the flow distribution of blood and dialysate within the dialyzer. Hence, it is crucial to ensure consistent flow gradient velocities between the central and peripheral blood and dialysate within the filter. The flow velocity of each fiber should be uniform throughout both the center and periphery of the capillary bundle. Furthermore, the dialysate flow rates in both the central and peripheral areas of the dialyzer should be equal. This alignment facilitates the optimal countercurrent configuration of blood flow to dialysate, thereby enhancing the efficiency of the diffusion process.[1]

Efforts have been undertaken to enhance flow within the blood compartment through the creation of specialized blood ports. Various alternatives have been suggested for the dialysate compartment, including spacer threads (filaments that act as barriers to prevent fiber contact) and the moiré structure (modified fiber shape to prevent contact between neighboring fibers). [1-4-5].

The critical factor in selecting a dialyzer is the technological efficacy of the membrane in terms of solute removal and biocompatibility. Innovations in nanotechnology have resulted in enhanced performance through advancements in membrane design, chemical composition, and sterilization techniques. The membrane and dialyzer play a central role in extracorporeal treatment, making the choice of membrane and dialyzer from the extensive range on the market crucial for meeting specific clinical requirements for blood purification. Criteria for selection may include membrane type, surface area, sterilization method, permeability, and molecular size cut-off point.

The membrane expands the range of uremic toxins that can be eliminated based on their chemical and physical properties. However, it is vital to take into account the particular use of each membrane in a filter and the integration of each filter into the extracorporeal circuit, as these elements can have a substantial effect on performance.

Membranes can be categorized based on their chemical composition, with the polymer constituting the membrane playing a key role in determining its chemical and physical characteristics and suitability for use in extracorporeal techniques. Cellulose-derived natural polymers have been gradually substituted by synthetic polymers and nano polymers, with recent advancements in nano controlled spinning techniques enhancing filtration efficiency.[1]

An ideal membrane should demonstrate biocompatibility, strong physical characteristics, outstanding diffusive and convective abilities, and resistance to chemical and physical sterilization techniques. An ideal permeability profile would enable high sieving coefficients for large solutes without significant albumin loss. Certain membranes may possess a high adsorption capacity, enhancing solute removal. The membrane should have a thin structure to enhance diffusivity coefficients, with pores of uniform size and quantity per unit area, tailored for optimal efficiency. [6-7-8]

The inner surface of the inner membrane filter should be smooth in design to prevent interactions with blood components, especially platelets. Minimizing thrombogenicity is crucial for decreasing the need for heparin and preventing platelet activation. Selecting the hemofilter requires careful consideration and adherence to the manufacturer's specific criteria, including factors like membrane type,

sterilization method, surface properties, and design.

The ideal membrane – filter for HDF- should demonstrate compatibility with biological systems, strong physical characteristics, superior diffusive and convective abilities, and durability against various sterilization methods. An ideal permeability profile would enable the effective elimination of large solutes while minimizing albumin loss. Some membranes may have a high capacity for adsorption, further improving solute elimination. The membrane's structure should be thin to promote effective diffusivity, with pores of consistent size and quantity per unit area, designed for maximum efficiency.[1]

The contemporary casing housing the package is typically lightweight and thoughtfully designed to minimize dead spaces. The basic structure of the fiber bundle is essential, as the number and length of fibers influence the dialyzer's cross-sectional area and its capacity to endure flow resistance. Hence, in each dialyzer, the size and layout of the fiber bundle are critical factors that determine its effectiveness.

It is essential to minimize the priming volume and ensure that each fiber is enveloped by a consistent dialysate flow during the dialysis process. The quantity of fibers and the compactness of the fiber bundle are crucial factors in determining the filter size for a specific area. To optimize efficiency and minimize activation of the humoral and cellular blood systems, it is vital to utilize an entirely inert potting compound and carefully cut the ends to create a smooth surface. The two ends of these surfaces are sealed by end caps that contain the blood inlet and outlet ports. The composition of the potting material has progressed over time to minimize the risks associated with hazardous substances that could be generated during the sterilization process, such as those produced by beta or gamma radiation.[1]

3. THEORETICAL CONSIDERATION

The choice of filter for HDF is contingent upon the chosen technique. While certain methods necessitate a specific type of dialyzer, the selection process typically relies on straightforward and explicit criteria. The dialyzer must provide sufficient surface area to achieve the targeted Kt/V per session, necessitating a minimum filter KoA of 1,000 or greater. In cases where elevated filtration rates are anticipated, such as in high-volume HDF (involving a convection volume exceeding 18 liters per session), a membrane with a minimum permeability of 28-32 ml/h/mm Hg/m2 should be evaluated.[1]

Important factors to consider are high resistance to increased TMP levels and minimal vulnerability to fouling and clotting. Initially, optimizing blood flow is crucial, followed by a thorough assessment of both Kt/V and convection volume per session. If the outcomes are unsatisfactory, adjustments should be made promptly during the treatment by modifying dialysate and blood flow rates. If the desired objectives remain unattained despite these interventions, an alternative dialyzer with a distinct membrane or increased surface area can be chosen.[9]

At times, technical obstacles may hinder the attainment of the desired convective clearance level. In cases where TMP and end-to-end pressure drop exceed a certain threshold despite optimizing blood flow, a positive response has been observed with a filter rinse in predilution mode using 180-250 ml of saline over 30 seconds when the UF pump is stopped (on-demand predilution). This sudden hemodilution achieved through this action can lead to a restoration of parameters to acceptable levels. The initial technological challenges in HDF implementation have been surmounted thanks to notable technological advancements.[10]

Specifically, the issue of replenishing the required large volumes of solution was resolved through the creation of microbiologically safe fluid, suitable for infusion. Advanced machinery and custom software have been effectively integrated to enhance the safety and ease of HDF with the innovative online technique (OL-HDF). The subsequent phase involved validating the notion that a more efficient dialysis method, like OL-HDF, serves as the foundation for notable enhancements in morbidity and mortality rates among dialysis patients.[1-11]

In the past decade, numerous studies have highlighted significant enhancements in inflammation, cardiovascular health, complications linked to β2-microglobulins, and various clinical outcomes, including mortality rates. Notably, recent and meticulously conducted analyses have shown that substantial advantages can be realized in dialysis patients when a greater volume of fluid exchange is accomplished in postdilution HDF. [6] By examining individual research findings and assessing the outcomes to the level of convective clearance achieved, a correlation with survival rates can be established based on the concept of "convective dose" (Figure 2)[1-12-13-14]

4. THE BARRIERS TO ACHIEVING HIGH VOLUME -HDF- AT LOW TMP PRESSURE

Several biophysical factors influence membrane solute transport in HDF. The clearance of small solutes like urea is significantly influenced by blood flow, while the clearance of larger solutes such as inulin is primarily affected by the UF rate.[1]
Convection requires a direct flow of fluid driven by a TMP gradient. Therefore, the generation of a convective solute flux will depend on factors like the UF rate, solute concentration in the plasma water, and the sieving coefficient of the solution, ideally represented as $S = 1 - \sigma$ under optimal circumstances, where σ denotes the membrane reflection coefficient. While these descriptions delineate convection and diffusion as distinct processes, pinpointing the precise contribution of each mechanism to solve elimination is challenging due to their ongoing interactions. Additionally, particularly in therapies that incorporate a combination of diffusion and convection, there is continual overlap between the two transport mechanisms

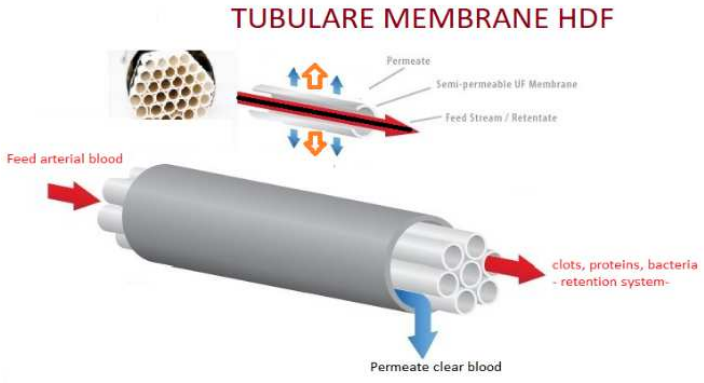

Fig. 1. The membrane HDF tube.

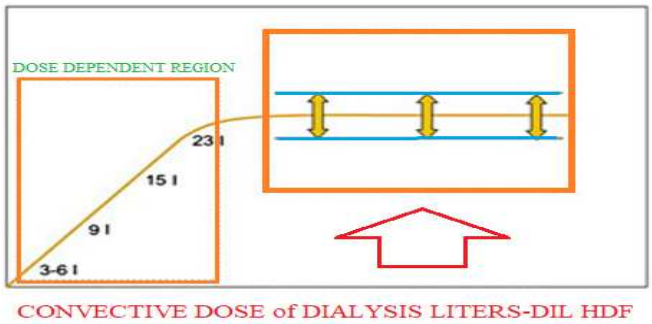

Fig. 2. Convective dose**.**

Ultrafiltration (UF) rates are determined by the mechanical porosity and hydraulic permeability of the membrane for fluid flow. However, they are also significantly influenced by the system's operating conditions and its interaction with plasma proteins. There are two possible scenarios: when ultrafiltration rates are low, a thin protein layer forms on the inner

surface of the fiber due to an electrochemical bond. This protein layer indicates the biocompatibility of the membrane, as it enables blood to flow over a surface resembling typical autologous material once the protein layer is absorbed. Simultaneously, the absorption by the adhesive substance causes a slight decrease in the membrane sieving coefficient, with a relatively consistent trend. [1-15]

At higher ultrafiltration rates, particularly with elevated filtration fractions, the membrane experiences a buildup of thick protein due to the heightened polarization effect. This gradual buildup diminishes the permeability of the filter capillaries (membrane), causing the sieving of the solution to be influenced by a new reflection coefficient (σ1) specific to the membrane. The development of these deposits is affected by multiple factors, with the "shear rate" at the filter wall being especially important. As blood flows into the hollow fiber, the shear stress initiates the creation of distinct blood layers at different velocities throughout the capillary, ranging from the bulk phase to the membrane interface. The standard relationship between the changes in fluid strand velocities within the fiber and the distance from the fiber's center (referred to as 'shear rate' and measured in liters per minute) is influenced by blood viscosity and the typical shear stress of the membrane. The shear rate indicates the relationship with the blood flow on a single fiber. The thickness of the protein layer at the blood-membrane interface depends on the wall's shear rate value and is crucial for the membrane's filtration effectiveness. The standard shear rate value exhibits a direct relationship with shear stress in the instance of Newtonian fluids, resulting in a consistently parabolic velocity profile. Blood displays Newtonian characteristics solely at shear rates surpassing 190/s. The UF and solution screening coefficients are significantly impacted by the wall shear rate as it aid in maintaining a thin polarization layer. This factor is particularly crucial for mid to high-range solutes. Additionally, diffusion is influenced by the shear rate value, as elevated shear rates assist in minimizing the diffusion distance between blood and dialysate. This occurs due to concentration polarization and the development of a secondary protein layer, resulting in the creation of a pseudo membrane that contributes to the overall thickness along with the original membrane. In practical medical settings, elevated wall shear rates are attained through increased blood flows and suitable device design, leading to enhanced UF rates and solution clearances.

Through experiments involving dye injection into the blood compartment of different hollow fiber dialyzers, we showed that blood flows and shear rates are notably lower in the peripheral fibers of the bundle compared to the central fibers, unless high blood flows are specified and flow distribution is effectively managed by advanced hemodialyzers. These results should guide the decision-making process concerning operational parameters and the selection of hemodialyzers in Hemodiafiltration (HDF).[16]

Additionally, the location of reinfusion (predilution versus post dilution) can impact the overall efficiency of the HDF system. The initial physicochemical effect leads to a decline in membrane permeability, necessitating higher TMPs to sustain designated filtration rates. The latter effect results in heightened blood viscosity within the dialyzer, leading to a gradual rise in end-to-end pressure drop and pre dialyzer pressure (Figure 3).[1]

The relatively high cost associated with commercially prepared sterile bag fluids, along with advancements in dialysate preparation technology and online fluid filtration, has led to the emergence of a technique known as OL-HDF in recent years. This method entails extracting a specific volume of freshly prepared ultrapure dialysate from the dialysate inlet line, passing it through multiple filtration stages, and subsequently utilizing it as replacement fluid. This method allows for the generation of substantial quantities of economical replacement solutions, making it easier to conduct.[1-17] Hemodiafiltration (HDF) involving a substantial fluid turnover of up to 30-40 liters per session. This can be accomplished by using pre- or post-dilution sites, or a combination of both in varying proportions to attain different outcomes.

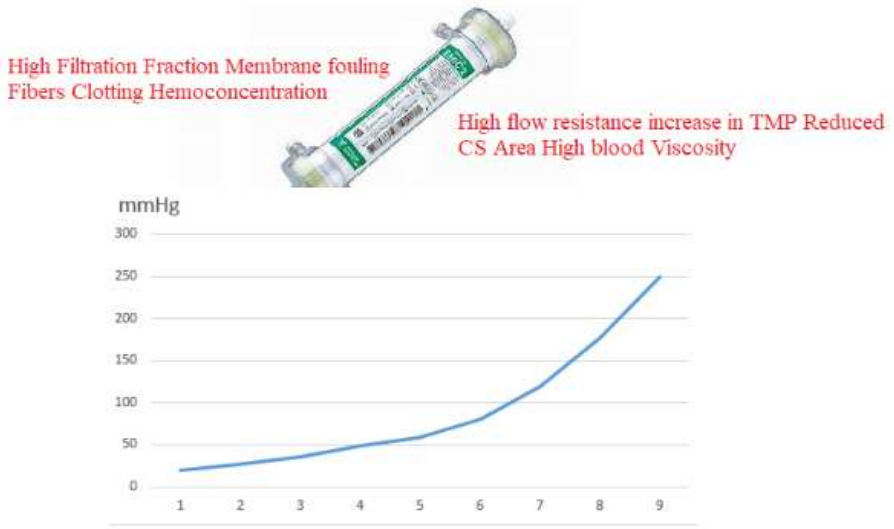

Fig. 3. The relationship between Pressure and High Filtration / High flow resistance

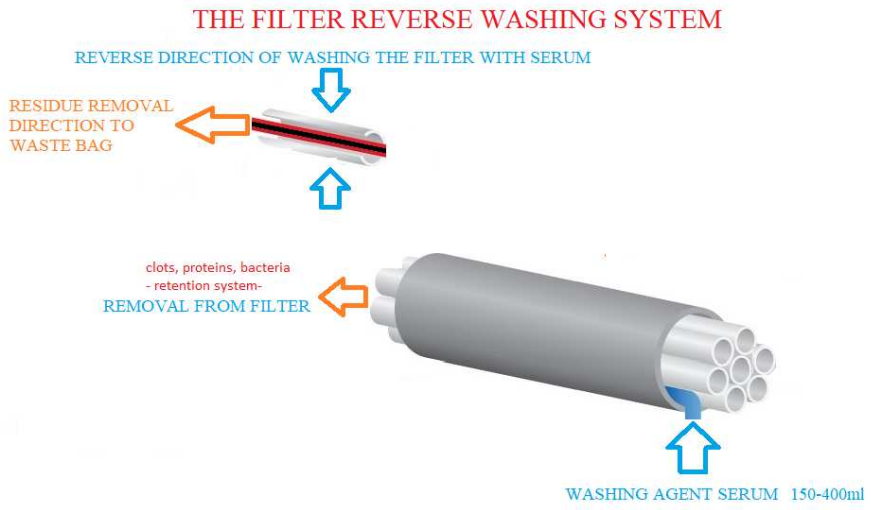

Fig. 4. The filter reverse washing system

5. AUTOMATIC BACKFLUSH OR ON DEMAND

Automated backflushing or on-demand, in alignment with the phenomenon noted in the study, involves employing ultrafiltration through an automatic feedback mechanism activated by the autosub plus. This process generates positive pressure in the dialysate compartment by halting filtration and swiftly infusing a minimum of 200 ml of clean dialysate into the fiber void in the opposite pumping direction. This approach not only induces notable hemodilution but also cleanses the membrane pores by dislodging protein layers, leading to a substantial enhancement in membrane permeability (Figure 4).

Backfiltration on demand or automatic is utilized in post-dilution HDF. Throughout the session, intermittent cleansing of the empty fibers occurs through a ultrafiltration mechanism triggered by a sudden alteration of pressure and flow direction within the dialyzer.

This process causes the dialysate to push proteins away from the inner surface of the hollow fiber, thus reinstating membrane permeability and disrupting the protein layer

formed due to concentration polarization. The procedure can be automated based on a specific algorithm selected by the software or manually initiated as needed. Laboratory assessments have shown positive outcomes regarding the hydraulic permeability performance of the filter. E-E ΔP refers to the end-to-end pressure drop detected using two sensors positioned downstream and upstream. The result is further represented by (Figure 5), by TMP pressure graph.

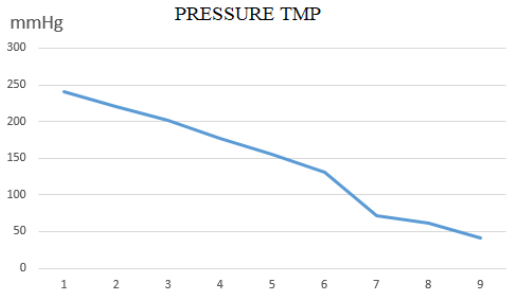

Fig. 5. The filter reverse washing system

6. CONCLUSIONS

Automatic cleaning through the reverse washing system increases the performance of the filtration system, helps to decrease the transmembrane pressures and implicitly reduce the consumption of heparin. As advancements in hemofiltration technology slowed down, the necessity for a more effective, milder, and cost-effective dialysis method to enhance outcomes became apparent. To attain this objective, the preferred treatment approach should satisfy various requirements: consistent utilization of dialyzers equipped with a highly permeable synthetic membrane, enhanced diffusion dialysis dosage, and optimized convective dosage components to support the elimination of small, medium, and larger uremic toxins (optimal blood flow and dialysate flow are crucial for maximizing solute mass transfer). Furthermore, regular use of ultrapure dialysis fluid, a dependable and adaptable hemodialysis machine that can efficiently handle fluid volume exchange balance, and offering a range of treatment customization options are essential considerations.[1] The main problem that must be developed in the future consists in the association of the hemofiltration system with the destruction of the body's defense system.

7. REFERENCES

[1] Henderson LW: Biophysics of ultrafiltration and hemofiltration; in Maher JF (ed): Replacement of Renal Function by Dialysis. Dordrecht, Kluwer Academic, 1989, pp 300-326. Available at https://karger.com/bpu/article/40/Suppl.%201/2/48636/Hemodiafiltration-Technical-and-Clinical-Issues [accessed at 30 March 2024]

[2] Ledebo I: On-line preparation of solutions for dialysis: practical aspects versus safety and regulations. J Am Soc Nephrol 2002;13(suppl 1):S78-S83.

[3] Ronco C, Cruz D: Hemodiafiltration history, technology, and clinical results. Adv Chronic Kidney Dis 2007;14:231-243.

[4] Ronco C, Heifetz A, Fox K, Curtin C, Brendolan A, Gastaldon F, et al: Beta 2-microglobulin removal by synthetic dialysis membranes. Mechanisms and kinetics of the molecule. Int J Artif Organs 1997;20:136-143.

[5] Vienken J, Ronco C: New developments in hemodialyzers. Contrib Nephrol 2001;133:105-118.

[6] Canaud B, Barbieri C, Marcelli D, Bellocchio F, Bowry S, Mari F, Amato C, Gatti E: Optimal convection volume for improving patient outcomes in an international incident dialysis cohort treated with online hemodiafiltration. Kidney Int 2015, Epub ahead of print.

[7] Lévesque R, Marcelli D, Cardinal H, Caron ML, Grooteman MP, Bots ML, Blankestijn PJ, Nubé MJ, Grassmann A, Canaud B, Gandjour A: Cost-effectiveness analysis of high-efficiency hemodiafiltration versus low-flux hemodialysis based on the Canadian arm of the CONTRAST study. Appl Health Econ Health Policy 2015, Epub ahead of print.

[8] Kim JC, Garzotto F, Cruz DN, Goh CY, Nalesso F, Kim JH, Kang E, Kim HC, RoncoC: Enhancement of solute removal in a hollow-fiber hemodialyzer by mechanical vibration. Blood Purif2011;31:227-234.

[9] Kim JC, Kim JH, Sung J, Kim HC, Kang E, Lee SH, Kim JK, Kim HC, Min BG, Ronco C: Effects of arterial port design on blood flow distribution in hemodialyzers. Blood Purif2009;28:260-267.

[10] Kim JC, Kim JH, Kim HC, Kang E, Kim KG, Kim HC, Min BG, Ronco C: Effect of fiber structure on dialysate flow profile and hollow-fiber hemodialyzer reliability: CT perfusion study. Int J Artif Organs 2008;31:944-950.

[11] Martín-Malo A, Aljama P: On-line hemodiafiltration reduces the proinflammatory CD14+CD16+ monocyte-derived dendritic cells: a prospective, crossover study. J Am Soc Nephrol 2006;17:2315-2321.
[12] Henderson LW, Beans E: Successful production of sterile pyrogen-free electrolyte solution by ultrafiltration. Kidney Int 1978;14:522-525.
[13] Henderson LW, Sanfelippo ML, Beans E: 'On line' preparation of sterile pyrogen-free electrolyte solution. Trans Am Soc Artif Intern Organs 1978;24:465-467.
[14] Canaud B: Online hemodiafiltration. Technical options and best clinical practices. Contrib Nephrol 2007;158:110-122.
[15] Roy T: Technical and microbiological safety of online hemodiafiltration: a European perspective. Semin Dialysis 1999;12:S81-S87.
[16] Canaud B, Mion C: Water treatment for contemporary hemodialysis; in Jacobs C, Kjellstrand CM, Koch KM, Winchester JF (eds): Replacement of Renal Function by Dialysis, ed 4. Dordrecht, Kluwer Academic Publishers, 1996, vol 8, pp 232-255.
[17] Draghici, D. A., Pantea, I., Roman, N., Druguș, D., & Repanovici, A. (2023). In Vitro Mechanism Whit Pulsative Laser on Thrombolysis. Acta Technica Napocensis-Series: Applied Mathematics, Mechanics, and Engineering, 65(3S).

Hemodiafiltrare cu sistem de autocuratare

Hemodiafiltrarea (HDF) pare a fi principala cale în domeniul înlocuirii funcției renale și hepatice prin filtrarea sângelui. Fluxurile convective de volum mare au fost corelate cu rezultate clinice mai bune. Uneori, însă, există bariere tehnice în calea atingerii fluxurilor sanguine mari adecvate pentru a efectua terapii convective eficiente, precum și probleme reale de blocare a celulelor de apărare în sistemul de filtrare. În ciuda procedurilor optimizate, creșterile progresive ale presiunii transmembranare (TMP), vâscozitatea sângelui datorită hemoconcentrației și rezistența la calea sângelui devin uneori inevitabile prin înfundarea filtrului. De aceea, propunem două soluții posibile care pot fi operate manual sau automat prin intermediul unui software din aparatul de dializă: prediluție automată și spălare automată. Prediluția constă în feedbackul automat al dispozitivului la un anumit interval, deturnând o parte din dializatul filtrat într-un mod de prediluție cu o perfuzie de 450 ml în 30 s în sens invers sensului de filtrare, acordând atenție deschiderii supapă de cale în timp ce pompa de ultrafiltrare se oprește. Aceasta produce hemodiluție cu clearance bruscă, parametrii revenind la valori acceptabile. Înlocuitorul cu resturile de depozite este infuzat în punga de deșeuri dializate. Performanța filtrului se îmbunătățește, iar schimbările de presiune sunt mult atenuate. Backflush la cerere constă într-un feedback automat al mașinii declanșat de controlul TMP, producând o presiune pozitivă în compartimentul de dializat datorită opririi filtrării și infuzând rapid cel puțin 250 ml de dializat ultrapur în fibra goală. Aceasta nu produce doar o hemodiluție semnificativă, ci și spălarea inversă a porilor membranei, desprinderea straturilor de proteine, cheaguri și îmbunătățirea permeabilității membranei într-un mod deosebit prin testele efectuate. Acestea sunt două exemple ale modului în care tehnologia va permite depășirea barierelor tehnice în calea implementării pe scară largă a HDF și a administrării adecvate a dozei convective.

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